

Spring 1-1-2016

Towards Defining Healthy Buildings: Investigating the Effect of Building Characteristics and Interventions on Indoor Air Microbial Exposures and Energy Efficiency

Julia Cristina Luongo

University of Colorado Boulder, jluongo1@gmail.com

Follow this and additional works at: https://scholar.colorado.edu/mcen_gradetds

 Part of the [Mechanical Engineering Commons](#), [Population Biology Commons](#), and the [Systems Biology Commons](#)

Recommended Citation

Luongo, Julia Cristina, "Towards Defining Healthy Buildings: Investigating the Effect of Building Characteristics and Interventions on Indoor Air Microbial Exposures and Energy Efficiency" (2016). *Mechanical Engineering Graduate Theses & Dissertations*. 120.
https://scholar.colorado.edu/mcen_gradetds/120

This Dissertation is brought to you for free and open access by Mechanical Engineering at CU Scholar. It has been accepted for inclusion in Mechanical Engineering Graduate Theses & Dissertations by an authorized administrator of CU Scholar. For more information, please contact cuscholaradmin@colorado.edu.

**Towards Defining Healthy Buildings: Investigating the
Effect of Building Characteristics and Interventions on
Indoor Air Microbial Exposures and Energy Efficiency**

by

Julia Cristina Luongo

B.S., Engineering, Swarthmore College, 2010

M.S., Mechanical Engineering, University of Colorado, Boulder, 2012

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Mechanical Engineering

2016

This thesis entitled:
Towards Defining Healthy Buildings: Investigating the Effect of Building Characteristics and
Interventions on Indoor Air Microbial Exposures and Energy Efficiency
written by Julia Cristina Luongo
has been approved for the Department of Mechanical Engineering

Shelly L. Miller

Prof. Michael Hannigan

Prof. John Daily

Prof. Noah Fierer

Prof. Michael Brandemuehl

Date _____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Luongo, Julia Cristina (Ph.D., Mechanical Engineering)

Towards Defining Healthy Buildings: Investigating the Effect of Building Characteristics and Interventions on Indoor Air Microbial Exposures and Energy Efficiency

Thesis directed by Prof. Shelly L. Miller

It is estimated that on average people spend more than 90 percent of their time indoors. And yet, when we think about our health and how the environment affects it, most are unaware that indoor environments may have a larger impact on our health and well-being than even the outdoor environment. Given the amount of time we spend indoors, what are we exposed to indoors? Can we design indoor environments that are not only not harmful to us, but actually good for us? And can we do this in an energy efficient manner? What does a “healthy building” look like?

To start answering these questions, we focused on studying how various building characteristics and interventions affected indoor air microbiology and energy efficiency, two qualities that are critical when designing a healthy building. This work is split into three parts: (1) Investigating a healthy building intervention: cooling coil ultraviolet germicidal irradiation (UVG-CC), (2) investigating the microbiology of indoor air quality in a university dormitory and its effect on student health, and (3) a review of the role of mechanical ventilation in the airborne transmission of infectious agents in buildings.

Part (1) (Chapter 2) investigates the effect of ultraviolet germicidal coil cleaning (UVG-CC) technology on building energy efficiency and indoor air microbiology. Cooling coil surfaces within building ventilation systems are ideal sites for biofilm formation due to the presence of adequate nutrients (i.e. deposited particles) and moisture (i.e. condensate). Biofouling of cooling coils can contribute to decreased heat transfer efficiency and possible contamination of indoor air by releasing toxins or allergens into the air entering the building. We found that, in mild condensing conditions, UVG-CC increased heat transfer effectiveness by 3–6.4%, with an uncertainty of $\pm 2.7\%$ resulting from the accuracy of our instrumentation. Microbial results showed increased airborne cell counts

downstream of the coil one month after UVG-CC installation. This increase coincided with drastic (80–90%) decreases in surface cell counts, which suggests that UVGI inactivated biofilms from the surface of the coil and these clusters were then re-entrained into the airstream. Overall this study suggests that UVG-CC is most effective at reducing microbial contamination and increasing heat transfer effectiveness in humid climates with high latent loads but care must be taken one month after installation, especially in the case of retrofits, as inactive biological material may re-entrain into the air. Installation of this technology should be carefully considered depending on the climatic region, and may not need to be operated during non-condensing states. Future studies of UVG-CC should pay careful attention to the sensitivity and detection limits of their instrumentation, and would benefit from studying environments prone to excessive biological fouling so that differences between UV and non-UV coils are more pronounced.

Part (2) (Chapter 3) of this dissertation investigates the microbiota in indoor air in a student dormitory. We have long known that human occupants are a major source of microbes in the built environment. What remains undetermined is what, if anything, we can learn about the occupants of a building by analyzing the microbial communities found in indoor air. We investigated bacterial and fungal diversity found in settled dust samples and dust collected onto HVAC air filters from 91 rooms within a university dormitory in Boulder, CO. The sex of the room occupants had the most significant effect on the bacterial communities found in both the settled dust and air filter samples, while the room occupants had no significant effect on fungal communities. By examining the abundances of taxa at the genus level, we can predict the sex of room occupants with 79% accuracy, a finding that demonstrates the potential forensic applications of studying indoor air microbiology. We also identified which taxa at the OTU level were most different in abundance and frequency of occurrence between female and male rooms, and found that taxa often identified as members of the vaginal microbiome were more common in female-occupied rooms while taxa associated with human skin or the male urogenital microbiota were more common in male-occupied rooms. Measurement methods used to characterize the dormitory HVAC system and methods of health data collection are also described.

Part (3) (Chapter 4) is a comprehensive literature review of the role of mechanical ventilation in the transmission of infectious agents in buildings. Infectious disease outbreaks and epidemics such as those due to SARS, influenza, measles, or tuberculosis have raised concern about the airborne transmission of pathogens in indoor environments. There are insufficient data to quantify how various parameters controlled by HVAC systems may affect the airborne transmission of infectious agents. To improve our understanding and design of HVAC systems to promote better infection control, our review reveals a strong need for more epidemiologic studies and meta-analyses. Specifically, we call for well designed prospective observational or intervention studies in buildings to establish causal relationships between airborne exposures and outcomes and between building factors and exposures. Future studies will benefit greatly from improved experimental design, standardized measurement methods, and better collaboration between epidemiologists and HVAC engineers.

The work presented here provides a glimpse into the complex and interdisciplinary nature of indoor air and building science and makes connections across building energy systems, HVAC science, and microbiology to demonstrate the nuances of how building characteristics or design decisions can affect indoor exposures.

Dedication

Para mi abuelito Julio.

Acknowledgements

I'd like to thank everyone who supported me and kept me going throughout my journey of graduate school. Thank you to my family for the unwavering support and advice for the last 27 years. I love you Mami, Papi, and Francisco. Gracias to my Tia Vero for always having supporting words when I needed them most. Thanks to my Colorado family (aka roommates) Daniela Molina Piper, Kaleen Adami, and Megan Monroig, the coolest kids in town, for keeping me sane, keeping me company, feeding me as I wrote this dissertation, and knowing when it's time to crack open a beer. A huge thank you to Jason Brownstein, who helped enormously with this research but more importantly has been an irreplaceable friend to me over the past 3 years. Thank you to all the friends who have made my time in Colorado so memorable and to the friends far and wide who are always a phone call away (I'm looking at you, Pam Costello and Melissa Grigsby). A special thank you to Alexandra Chakeres, Karen Barnes, and Amy Fletcher for their support and friendship (and thanks, Amy, for the amazing care package!). I'm also grateful for the unconditional love and support from the sister I never had, Susanna Zorn.

I would like to thank my defense committee for their guidance and for volunteering their time to be part of my doctoral defense. A huge thank you to Sharon Anderson for her help and for always keeping an eye out for me. Most importantly, a very special thank you to my advisor, Shelly Miller, for being an amazing mentor and always supporting me even when times were tough. I have learned so much from her guidance and help, I have grown as an individual and researcher, and am immensely grateful for the opportunities she has afforded me.

Contents

Chapter

1	Introduction	1
1.1	Research Motivation	1
1.1.1	History of Minimum Ventilation	1
1.1.2	Energy Efficiency	3
1.1.3	Ultraviolet Germicidal Coil Cleaning	5
1.1.4	The Microbiology of Indoor Air Quality	5
1.2	Research Objectives	7
2	Investigating a Healthy Building Intervention: Ultraviolet Germicidal Coil Cleaning	8
2.1	Part 1 - Impact on heat transfer effectiveness and static pressure drop	9
2.1.1	Abstract	10
2.1.2	Introduction	11
2.1.3	Materials and Methods	12
2.1.4	Results	19
2.1.5	Discussion	24
2.1.6	Acknowledgements	25
2.2	Part 2 - Decreased surface microbial fouling and resuspension of cell clusters	26
2.2.1	Abstract	27
2.2.2	Introduction	28

2.2.3	Experimental Methods	29
2.2.4	Results	32
2.2.5	Discussion	37
2.2.6	Acknowledgements	40
2.3	Future work	41
2.3.1	Real building UVG-CC study	41
3	The Microbiology of Indoor Air Quality in a University Dormitory and its Effect on Student Health	44
3.1	Microbial analyses of airborne dust collected from dormitory rooms predict the sex of occupants	45
3.1.1	Abstract	46
3.1.2	Practical Implications	46
3.1.3	Introduction	47
3.1.4	Methods	48
3.1.5	Results and Discussion	50
3.1.6	Acknowledgements	62
3.2	HVAC system characterization measurements and documentation of occupant health symptoms	63
3.2.1	Abstract	63
3.2.2	Introduction	64
3.2.3	Measurement Methods	64
3.2.4	Statistical Analyses	71
3.2.5	Conclusions	72
4	Role of Mechanical Ventilation in the Airborne Transmission of Infectious Agents in Buildings	73
4.1	Abstract	74

4.1.1	Practical Implications	74
4.2	Introduction	75
4.3	Methods	77
4.4	Epidemiologic Studies	78
4.5	Building Studies before 2000	81
4.6	Building Studies from 2000 to Present	83
4.7	Discussion	93
4.8	Conclusions	98
4.9	Acknowledgements	100
4.10	Supplementary Information	100
5	Summary and Concluding Remarks	101
	Bibliography	104
	Appendix	
A	Recommended Measurement Protocols for Epidemiologic Studies of Mechanically Ventilated Buildings	114
A.1	Introduction	114
A.2	Recommended Measurements	119
A.2.1	Level 0 – Space Description	120
A.2.2	Level 1 – HVAC System Measurements	123
A.2.3	Level 2 – Biological Exposure Measurements and Clinical Outcome Measurements	134
A.3	Review – List of measurements useful to meta-analyses	136

Tables

Table

2.1	Sampling subsets used for statistical analysis.	18
2.2	Mean p-values from bootstrapped T-tests and bootstrapped one-way analysis of variance for heat transfer effectiveness and system curve coefficient differences between UV-treated and control cooling coils.	21
2.3	Sample configurations with statistically unequal means between treatment and control cooling coils ($p < 0.01$).	35
3.1	Top OTUs that most effectively distinguished between male and female-occupied rooms, as determined by the indicator value analyses. OTUs with high indicator values indicate that they were more common and more abundant in rooms occupied by either men or women. For details on the proportional abundances of these OTUs in male versus female-occupied rooms, see Figure 3.3.	55
3.2	Bacterial taxa, or chloroplasts, were used as indicators of the potential source environments to identify the relative importance of each source environment in each of the collected samples. Details on this approach and how these taxa were selected are provided in [10]. For the determination of those bacteria taxa indicative of the vaginal microbiome, we used data from [103, 73] to identify taxa abundant in vaginal samples, but uncommon in other source environment types.	58
3.3	Results from multiple measurement methods of outdoor air dilution in dorm rooms.	67

4.1	Summary of Study Designs, Reported Measure, and Findings in Building-Related Epidemiologic studies	93
-----	---	----

Figures

Figure

1.1	Minimum Ventilation Rate History	3
1.2	Number of LEED Buildings	4
2.1	Schematic of HVAC test apparatus.	14
2.2	Quadratic curve fit of experimental data to equation 2.3, demonstrating how the coil system curves were generated.	16
2.3	Time series of calculated effectiveness for UV-treated and control coils over the course of 18 months.	19
2.4	Sampling subsets used for statistical analyses. (a) Baseline Region both coils had been running for 10 undisturbed months to build up fouling, UV lamp had not yet been turned on. (b) Humid Region 1 immediately after UV lamp was turned on for the treatment coil, still in condensing conditions. (c) Dry Region period of time in the winter when coil surfaces were dry due to very low humidity level of inlet air. (d) Humid Region 2 the second period of time when condensing conditions were achieved the following summer.	20
2.5	Results from Tukey's Honestly Significant Difference procedure on the difference in heat transfer effectiveness between UV-treated and control coils (a). Results from bootstrapped T-tests for each sampling subset is shown in (b).	22

2.6	Results from Tukey's Honestly Significant Difference procedure on the difference in system curve coefficient between UV-treated and control coils (a). Results from bootstrapped T-tests for each sampling subset is shown in (b).	23
2.7	Schematic of HVAC test apparatus.	30
2.8	Total cell counts per field of view from surface samples upstream and downstream of the treatment and control cooling coils by sampling date. Areas shaded in blue correspond to condensing mode of operation, areas in orange correspond to transitional mode of operation, and the red shaded area corresponds to dry operating condition.	33
2.9	Total cell counts per field of view from air samples upstream and downstream of the treatment and control cooling coils by sampling date. Areas shaded in blue correspond to condensing mode of operation, areas in orange correspond to transitional mode of operation, and the red shaded area corresponds to dry operating condition.	33
2.10	Comparison of downstream air samples with (a) and without (b) cell clusters.	34
2.11	Average microbial loading downstream for both coils is influenced by the coil operating conditions. When actively condensing, the presence of water promotes bioaerosol deposition and growth. When dry, microbial loading decreases on downstream surfaces and airborne concentrations increase as cell clusters are likely re-entrained into the airstream from the coil surface.	36
2.12	Schematic of the NIOSH air handler.	41
2.13	Time series of the calculated airside heat transfer effectiveness for both NIOSH cooling coils. The UV-irradiated coil is labeled <i>AB</i> and the control coil is labeled <i>CD</i>	42
3.1	Results from quantitative PCR analyses of airborne dust samples showing differences in amounts of bacteria (a) and fungi (b) collected onto settling plates over 2.5 months of sampling.	52

3.2	Proportional abundances of bacterial genera from the HVAC filter samples listed in descending order of their contribution to the boosted regression tree model (model contributions are listed in the x-axis labels).	56
3.3	Proportional abundances of top OTUs identified using indicator value analysis (listed in Table 1) comparing female and male rooms from passive samplers (a) and HVAC filters (b).	57
3.4	Differences in the relative abundances of bacterial taxa indicative of potential source environments (listed in Table 3.2) between female and male rooms. Results are based on analyses of either the passive samplers (a) or the HVAC filters (b).	59
3.5	Proportional abundances of the most common bacterial (a) and fungal (b) taxa between the two sample collection strategies.	61
3.6	An example of one week of continuous CO ₂ monitoring in a dormitory room. The flat peaks overnight were used for estimating outdoor air ventilation with an equilibrium analysis.	66
3.7	Monitoring of pressure differences over 30 minutes between rooms and the hallway (reference pressure). Rooms with stepped-up positive pressure have the fan coil operating and rooms near neutral pressure do not.	68
3.8	Trended FCU fan status data for two rooms.	69
3.9	Trended window switch data for two rooms.	69
4.1	Types of epidemiologic studies (adapted from [63]).	79
A.1	HVAC transport and air mixing.	121

Chapter 1

Introduction

1.1 Research Motivation

To begin defining a healthy building, we must understand what indoor exposures may cause adverse health effects. As engineers, we wish to design and operate buildings to reduce or eliminate harmful indoor exposures while consuming the least amount of energy doing so. This balancing act has persisted for decades, but with increasing recognition of global climate change coupled with awareness of how our indoor environments may be affecting our health and well-being in both the short and long-term, there is a need for better understanding of our indoor exposures, what controls them, and if there are energy-efficient solutions or technologies that promote healthy indoor environments.

1.1.1 History of Minimum Ventilation

The concept of ventilation was first introduced when man brought fire into the home and discovered the need to let smoke out and supply air to keep the fire burning. In the mid to late 1800s the first estimate of a minimum ventilation rate was published based on calculations of the amount of fresh air needed to purge CO₂ from the lungs. This estimate was based off of metabolic needs but proved to be too little ventilation for comfort's sake [52]. Subsequent efforts to estimate an acceptable minimum ventilation birthed a contradiction in ventilation design that still rings true to this day: should ventilation be based on physiological needs, on comfort, or on health?

The most authoritative American work on minimum ventilation estimates came from a physi-

cian named J. Billings in the late 1800s. He argued for higher ventilation rates because he was concerned about the spread of disease, particularly tuberculosis. In 1895, ASHVE (American Society of Ventilation Engineers) “adopted the view that engineers were ready to accept the ideas of hygienists and physiologists [62]”. They recommended 30 cfm (14 L/s) per person as a minimum and placed responsibility of design and construction on engineers.

For centuries a ventilation dichotomy existed between engineers and physicians. Engineers were concerned with providing comfort and reducing odors and CO₂ accumulation while physicians were concerned with minimizing spread of disease. After much research on comfort, odors, and recirculation throughout the early 1900s, ASHVE (now ASHRAE - American Society of Heating, Refrigerating, and Air Conditioning Engineers) published a guide in 1925 setting the minimum ventilation rate to 10 cfm (4.7 L/s) per person. This remained until the 1980s, when multiple studies found that 15 cfm (7.5 L/s) of outdoor air was sufficient to reduce concentrations of tobacco smoke to an odor level acceptable by 80% of the population (graph on the right in Figure 1.1). The graph on the left in Figure 1.1 shows the history of minimum ventilation rates in the United States, peaking right after the Civil War when disease transmission was the primary cause of death. Minimum ventilation rates were reduced again after many research efforts on odor and comfort as well as advances in heating, ventilation, and air conditioning (HVAC) technology. The current ASHRAE Standard 62 determines the minimum outdoor air intake rates from various parameters such as space type, space application, occupancy level, and floor area. Intake rates are also highly dependent on outdoor air quality.

An extensive literature review published in 2011 judged 27 papers that provided sufficient information on both ventilation rates and health effects to inform the relationship [115]. Higher ventilation rates of 25 L/s per person (53 cfm/person) were associated with reduced prevalence of sick building syndrome symptoms, and inflammation, respiratory infections, asthma symptoms, and short-term sick leave increased with lower ventilations rates. The authors note that while the articles they identified support a positive benefit with ventilation rates above the current ASHRAE standards, the need remains for more studies of the relationship between ventilation rates and

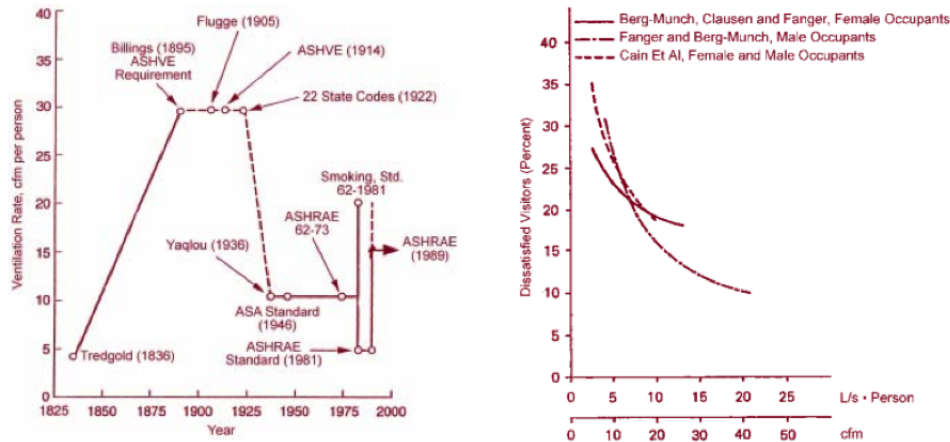


Figure 1.1: Minimum ventilation rate history (left) and odor acceptance studies in the early 1980s (right) [52].

health (specifically those that tackle the difficult methodological challenges of measuring ventilation properly).

1.1.2 Energy Efficiency

Historically the argument for a better indoor environment, whether it was a solution for odors (Figure 1.1) or disease transmission, was to increase the amount of outdoor air supplied to the space. We have now reached a point where a third factor is heavily influencing standards for mechanical ventilation: energy usage. With increasing concerns regarding depletion of fossil fuels, global climate change, and CO₂ emissions, it is important to note that 41% of U.S. energy consumption can be attributed to buildings [20]. Particularly, approximately 50% of a building's total energy consumption is used to operate the HVAC systems of that building [26]. There is now an increasing push for more energy efficient, tight, and “green” buildings. The number of LEED (Leadership in Energy and Environmental Design) Certified buildings has been steadily increasing over the past 10 years, as shown in Figure 1.2. LEED is a green building certification program run by the U.S. Green Building Council (USGBC). LEED concentrates its efforts on improving building performance across five key areas of environmental and human health: energy efficiency, indoor environmental quality, materials selection, sustainable site development, and water savings.

The USGBC describes its LEED program as “a nationally accepted benchmark for the design, construction and operation of high-performance green buildings” and “provides building owners and operators with the tools they need to have an immediate and measurable impact on their buildings’ performance.”

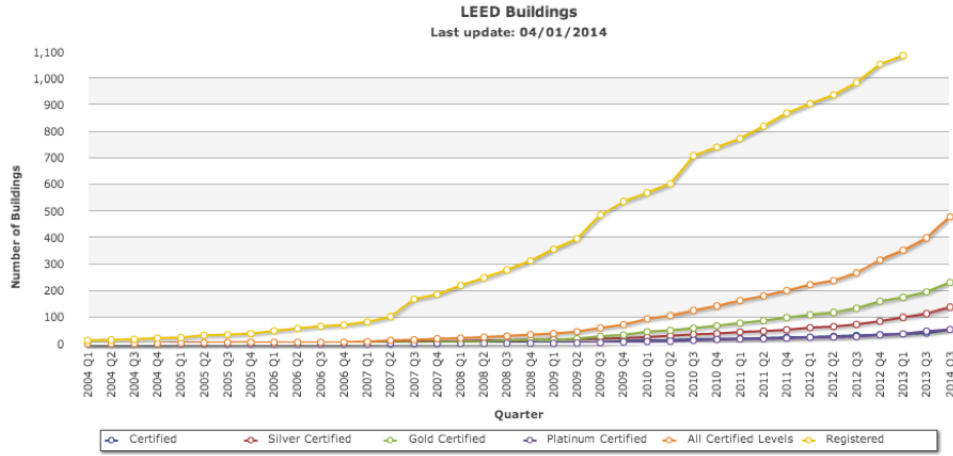


Figure 1.2: Number of LEED buildings over the past 10 years [39].

In January of 2014, mayors from ten major American cities announced that they will participate in a united effort (called the City Energy Project) to significantly boost energy efficiency in their buildings [96]. Engineers designing and maintaining building ventilation systems are encountering a new ventilation dichotomy: How do we move towards more energy efficient buildings without compromising indoor air quality and its subsequent health effects? Providing the minimum required ventilation results in less energy use by the HVAC system, but is this standard sufficient to reduce risk of disease transmission and promote a healthy and productive space? Are there more effective or energy efficient options for promoting a healthy and productive environment besides increasing the amount of outdoor air supplied? Or are there other options that can be used in a building to decrease the energy use while maintaining adequate ventilation for health? One such sustainability intervention that may promote a healthier, more productive environment at a smaller cost than increasing outdoor air ventilation is ultraviolet germicidal coil cleaning (UVG-CC) technology.

1.1.3 Ultraviolet Germicidal Coil Cleaning

Ultraviolet germicidal irradiation (UVGI) is an established means of disinfection and can be used to prevent the spread of certain infectious diseases. The two primary applications of UVGI are upper room systems and in-duct systems [104]. Efficacy of these two applications is well-documented in the literature [120, 64, 84] but the emergence of a third application, ultraviolet germicidal coil cleaning (UVG-CC) technology, calls for research to better characterize its efficacy. While air disinfection may still occur as air passes by the UVG-CC system, the primary focus of UVG-CC is surface disinfection and, in turn, energy savings, maintenance cost savings, and increased or prolonged system capacity. Anecdotal evidence exists reporting energy savings from UVG-CC [56, 1]. An increase in energy efficiency of 10-15% from coil cleaning has also been reported, but not specifically using UVG-CC [87]. The consequences of altering the microbiology inherent in these systems is poorly understood. We sought to determine if we could replicate these energy savings in a controlled laboratory experiment and, if anecdotal evidence suggests a reduction in biofouling, whether we could quantify changes in microbial loading and the fate of microbial contamination after irradiation as it may effect the quality of the air entering the indoor environment.

1.1.4 The Microbiology of Indoor Air Quality

We spend 90% of our lives indoors [54] and the quality of the air we breathe indoors is critical to human health and well-being [29]. Indoor air quality (IAQ) can significantly influence occupant health and worker performance [36]. While a myriad of air pollutants are found indoors, the microbes found in indoor air represent another key determinant of IAQ. Bacteria, fungi, and viruses are ubiquitous in indoor air with concentrations that typically exceed 100,000 cells or viral particles per cubic meter of air [102]. While many of these microbes are likely to be harmless, some are human pathogens (including cold and flu viruses, [122]) or potential allergens and triggers of allergenic asthma.

We have a surprisingly limited understanding of the microbial diversity found in indoor air. This knowledge gap persists because the methods long used by microbiologists to characterize the bacteria, fungi, and viruses found in indoor air are woefully limited. Most microbes cannot be identified using standard techniques [6] and only recently have researchers begun using molecular methods to comprehensively assess the amounts and types of bacteria and fungi found in indoor air [74, 30]. The viral diversity found in indoor air remains essentially unknown. Although many studies have focused on specific viral pathogens (including cold and flu viruses), there are likely thousands of different viral types in indoor air [123] and this viral diversity can only be quantified using DNA or RNA sequencing-based approaches.

It is critical that we characterize the microbial contributions to indoor air if we want to understand how efforts to improve the energy efficiency of buildings will impact human health and well-being. As mentioned in Section 1.1.2, the U.S. buildings sector accounts for 41% of primary energy consumption and HVAC systems often consume over half of a buildings total energy consumption. As a result, there are many efforts to improve the design or operation of buildings to reduce energy consumption associated with HVAC systems. One key strategy to reduce energy consumption is to reduce the rate of ventilation, either by reducing the leakiness of buildings in new construction or by reducing the amount of air exchanged between indoors and outdoors in existing buildings. Doing so may have unintended consequences for IAQ as reduced rates of ventilation have been associated with increased prevalence of sick building syndrome, respiratory infections, asthma symptoms, and short-term sick leave [115]. Moreover, the amounts and types of microbes found in indoor air appear to be strongly influenced by ventilation rates [59] with reduced ventilation rates expected to increase the relative abundances of potential pathogens while decreasing the relative abundances of many fungal allergens [108]. Unfortunately, the details of how ventilation rates influence the microbial diversity found in indoor air remain undetermined nor do we know if efforts to improve the energy efficiency of buildings will have unintended effects on microbial exposures indoors.

1.2 Research Objectives

This work will investigate the importance of various HVAC system and building characteristics on both energy efficiency and indoor air quality/microbial exposures. In particular, this work focuses on answering the following questions:

- (1) How does ultraviolet germicidal coil cleaning (UVG-CC) technology affect heat transfer effectiveness and pressure drop of HVAC heat exchangers?
- (2) In turn, how does the application of UVG-CC on cooling coils affect microbial loading in air and on surfaces within HVAC systems?
- (3) Do differences in mechanical ventilation rates in buildings alter the amounts and types of bacteria and fungi found in indoor air?
- (4) Do other building characteristics, such as occupancy, alter the amounts and types of bacteria and fungi found in indoor air?
- (5) Are there relationships between IAQ (both abiotic and microbial aspects) and the health of building occupants?
- (6) What do we know from current literature about how mechanical ventilation affects disease transmission and health, and what do future studies need to consider during experimental design?

The results presented here represent one of the first studies to comprehensively investigate the microbes found in indoor air in a unique building setting and with a unique sustainability intervention. Results will be directly relevant to understanding how ongoing efforts to improve the sustainability of buildings by altering properties of the mechanical systems may alter microbial exposures inside buildings, including exposures to potential pathogens and allergens.

Chapter 2

Investigating a Healthy Building Intervention: Ultraviolet Germicidal Coil Cleaning

2.1 Part 1 - Impact on heat transfer effectiveness and static pressure drop

Julia C. Luongo¹, Jason M. Brownstein¹, Shelly L. Miller^{1,*}

¹Department of Mechanical Engineering, University of Colorado, Boulder, CO, USA

* *Corresponding author: shelly.miller[at]colorado.edu*

⁰ This section is in preparation for the following journal:
Luongo, J. C., Brownstein, J. M., & Miller, S. L. (2016). Ultraviolet Germicidal Irradiation of HVAC Cooling Coils - Part 1: Impact on Heat Transfer Effectiveness and Static Pressure Drop. *In preparation for Building & Environment*.

2.1.1 Abstract

Cooling coil surfaces are ideal sites for biofilm formation due to the presence of adequate nutrients (deposited particles) and moisture (condensate), causing adverse impacts on heating, ventilation and air-conditioning (HVAC) energy usage and performance. In this study, an HVAC test apparatus was built to investigate the hypothesis that ultraviolet germicidal coil cleaning (UVG-CC) of heat exchanger surfaces improves heat transfer effectiveness and reduces the static pressure drop across the coil. The test apparatus consisted of two parallel ducts, each with its own cooling coil. One coil was treated with UVG-CC while the other was the control and left untreated. Thermodynamic properties of the air and water flowing through both heat exchangers were monitored over the course of two years with sensors and a data acquisition system. Static pressure drop across both coils was monitored as well. Differences in static pressure drop and coil effectiveness between the UV-treated and control coil were compared across multiple modes of coil operation (defined by presence of condensate). Differences across modes of operation were analyzed using a bootstrapped one-way analysis of variance (ANOVA) and differences between control and UV-treated coils were analyzed by bootstrapped t-tests. The effectiveness of UVG-CC was drastically affected by the presence of condensation on coil fins. We observed a statistically significant difference in the heat transfer effectiveness between the UV-treated and control coils in wetted conditions while no difference was observed in dry conditions. Sensor error, however, contributed to large uncertainty in our result. Heat transfer effectiveness of the UV-treated coil ranged, on average, from 3.0 to 6.4%, with an uncertainty of $\pm 2.7\%$, greater than that of the control coil in saturated conditions. UVG-CC did not significantly reduce static pressure drop.

2.1.2 Introduction

Ultraviolet germicidal irradiation (UVGI) has a long history of being used for the disinfection of air streams, primarily in environments with higher risk of airborne pathogen transmission such as healthcare facilities, schools, and prisons [104]. UVGI systems use low-pressure mercury vapor lamps that emit shortwave ultraviolet-C, peaking at 253.7 nm. Using ultraviolet germicidal coil cleaning (UVG-CC) technology in heating, ventilation, and air-conditioning (HVAC) systems has recently gained popularity [84]. While air disinfection may still occur as air passes by the UVG-CC system, the primary focus of UVG-CC is surface disinfection and, in turn, maintenance cost savings, and increased or prolonged system capacity due to cleaner heat exchanger surfaces, resulting in an overall system energy savings due to better heat transfer and reduced load on the chiller, pump, and/or fan. The buildings sector accounted for 41% of primary energy consumption in the US in 2010 [20]. More than half of the energy used in buildings is for heating, ventilating and/or air-conditioning the indoor environment [26], so energy savings for HVAC systems could have large implications for total building energy consumption.

In humid climates where the outdoor air must be dehumidified prior to entering the building space, air is cooled below the dew point to condense moisture out of the air. This moisture can linger within the densely packed fins of a cooling coil and eventually form biofilms from deposited environmental bacteria and fungi present in the air. Heat exchanger surfaces are an ideal site for biofilms due to the presence of adequate nutrients (i.e. debris inherent on coil surfaces) and moisture [88]. High bacterial and fungal concentrations have been documented within HVAC systems, specifically on cooling coils and drain pans [49, 67, 83].

Biological fouling of heat exchangers can affect HVAC system energy efficiency and usage in a variety of ways. The two direct effects are a loss in heat transfer effectiveness due to lower thermal conductivity of heat exchange surfaces and an increase in pressure drop across the heat exchanger due to increased fin thickness, both caused by biofilm covering the aluminum fins. Increased energy usage occurs when subsequent actions are taken to maintain the same system performance with

fouled equipment. One such action may be to lower the temperature of the cooling fluid to maintain the desired supply air temperature, causing the chiller to no longer perform at its optimum point (i.e. negatively impacting the coefficient of performance). Additionally, an increased pressure drop may lead to increased fan energy usage to maintain the desired air flow rate or meet the cooling load.

While health benefits of UVG-CC have been shown in the literature [83, 105], little evidence exists of the potential energy efficiency benefits of this technology. Anecdotal evidence describes “visibly cleaner” cooling coils and energy savings after the installation of a UVG-CC system [56]. An increase in energy efficiency of 10–15% from coil cleaning has also been reported, but not specifically using UVG-CC [87]. The objective of this study is to investigate the hypothesis that UVG-CC increases heat transfer effectiveness and decreases static pressure drop across the coil. We built a lab-based test apparatus consisting of two identical heat exchangers, one being irradiated with UV and the other not; thermodynamic detailed measurements were collected. This study was conducted over the course of two years and was able to discern how slight changes in inlet air properties due to outdoor air variations affected heat exchanger performance. The results presented in this study allow us to provide recommendations for effective installation and operation of UVG-CC technology for reducing coil fouling and increasing or prolonging HVAC heat exchanger effectiveness.

2.1.3 Materials and Methods

2.1.3.1 Test Facility

An HVAC test apparatus was built in the Air Quality Laboratory at the University of Colorado Boulder, consisting of two parallel ducts, each with its own cooling coil, but supplied by the same temperature and relative humidity controlled airstream (Figure 2.1). The coils were steam cleaned prior to starting the tests. The test apparatus was equipped with sensors to measure duct velocities using pitot tubes (BAPI ZPS-ACC12) connected to differential pressure sensors

(OMEGA PX2650), static pressure drops (OMEGA PX2650), entering and exiting water temperatures (OMEGA TH-44000-NPT), and entering and exiting air temperatures (OMEGA ON-405) and relative humidity (OMEGA HX71) for each branch. Voltage output from the sensors was fed into a data acquisition system (NI cDAQ-9171 with NI 9205 module) and processed with LabView in order to export data for analysis in MATLAB. Both coils were TRANE light commercial coils (Type P2) with aluminum fins (Prima-flo H) and copper tubes, one-ft² face area, 12 fins/inch, and were four rows deep. One UVC lamp (ALTRU-V V-Ray Model 23-1100, 25W) was installed ten inches away from the coil on the downstream side. The lamp was burned in for 100 hours prior to use. The lamp was shielded with mesh to achieve the desired level of surface irradiance.

The test apparatus used indoor air from the room as the inlet air. The room HVAC system supplied 100% outdoor air filtered with MERV 14 filters. Air entered each cooling coil, on average, at 75°F (24°C) and 44% relative humidity and chilled water entered at 50°F (10°C), satisfying conditions for condensation onto the coils. These conditions, however, are mild compared to the condensing conditions of cooling coils in very humid climates. The system mimicked a constant volume HVAC system, meaning the volumetric flow rate is held constant. The flow rates through each coil were held equal to one another using dampers since the static pressure drop across the coils may not be equal given equivalent flow rates. Air and water inlet temperatures, inlet relative humidity, and water flow rate were held as constant as possible. Fluctuations in outdoor air conditions slightly affected conditions within the apparatus. During summer months, both coils had water actively condensing onto fin surfaces at nearly all times and drain pans were wet. In the winter months when outdoor air became very dry (~10% RH), the apparatus was unable to humidify the air sufficiently to continue condensing water onto the cooling coils. These test periods of desiccation revealed interesting results, described in the Section 2.1.4.

The system ran undisturbed for 4 months without UVG-CC on either coil to ensure that both coils fouled at an equivalent rate and to establish a robust baseline dataset. After 4 months of operation, the UV lamp was turned on, irradiating the downstream side of one of the cooling coils (called the treatment coil). The control coil was never irradiated. The irradiance at the surface of

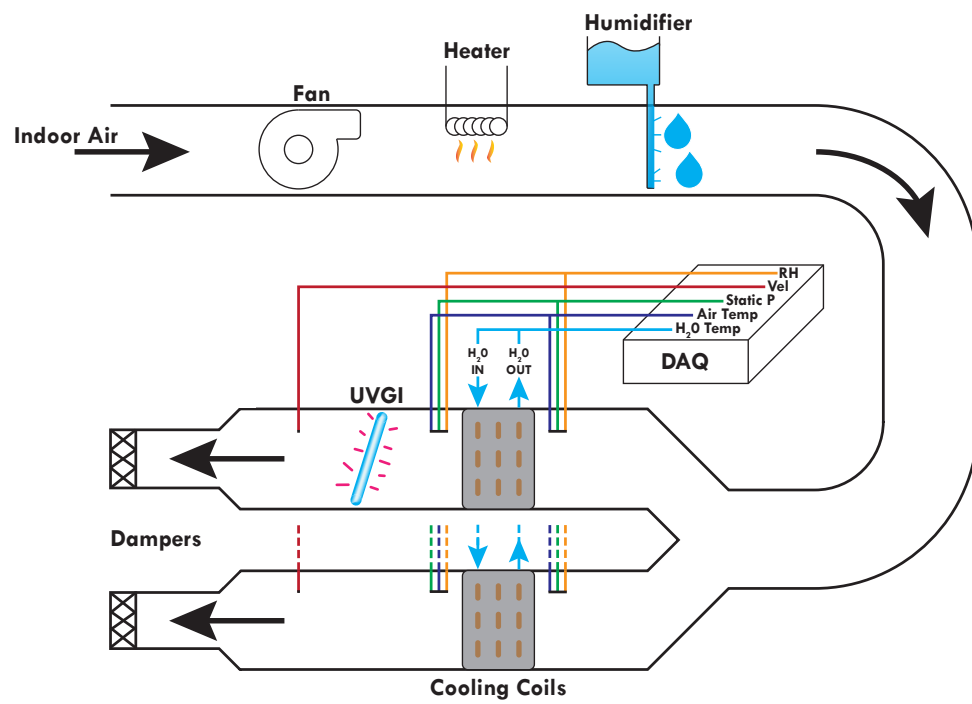


Figure 2.1: Schematic of HVAC test apparatus.

the treatment coil was on average $200 \mu\text{W cm}^{-2}$, being roughly $280 \mu\text{W cm}^{-2}$ at the center but $180 \mu\text{W cm}^{-2}$ at the corners, just above levels referenced as typical in the ASHRAE HVAC Applications Handbook [13] at 50 to $100 \mu\text{W cm}^{-2}$.

2.1.3.2 Coil Effectiveness

One of the main challenges in assessing changes in flow characteristics and effectiveness in two heat exchangers over time is that all variables affecting these qualities are never exactly the same and cannot be held completely constant. For this reason, small fluctuations in temperature, relative humidity, or flow rate will affect static pressure drop and the calculated value of heat transfer, making it difficult to compare. To remedy this, comparisons between the control and UV-treated coils were only made with dimensionless quantities, including heat exchanger effectiveness and the coefficient of an assumed quadratic relationship between static pressure drop and velocity.

Heat exchanger effectiveness is the ratio of the actual airside heat transfer to the maximum heat transfer theoretically possible. The equation for calculating heat transfer effectiveness is different for a wet coil versus a dry coil. Equation 2.1 was used to calculate the effectiveness of the heat exchangers while condensate was present in the drain pans.

$$\epsilon = \frac{q}{q_{max}} = \frac{\dot{m}_a(h_{a,i} - h_{a,o})}{\dot{m}_a(h_{a,i} - h_{sat,T_{w,i}})} \quad (2.1)$$

where $\dot{m}_a$ is the mass flow rate of air through the heat exchanger, $h_{a,i}$ is the enthalpy of the air entering the heat exchanger, $h_{a,o}$ is the enthalpy of the air leaving the heat exchanger, and $h_{sat,T_{w,i}}$ is the saturation enthalpy at the temperature that the water enters the heat exchanger. Alternatively, Equation 2.2 was used to calculate the heat transfer effectiveness when the cooling coil surfaces were dry.

$$\epsilon = \frac{(\dot{m}c_p)_{air}(T_{a,i} - T_{a,o})}{(\dot{m}c_p)_{min}(T_{a,i} - T_{w,i})} \quad (2.2)$$

Heat exchanger effectiveness was monitored throughout the entire experiment for both coils.

2.1.3.3 Coil System Curve Coefficient

To rule out changes in pressure drop from other system components, focus was placed solely on the system curve of each cooling coil. The following equation shows the relationship between static pressure drop and volumetric flow rate for the system curve of each coil:

$$\Delta P_s = aV^2 \quad (2.3)$$

where ΔP_s is the static pressure drop across the coil, V is the volumetric flow rate of air through the coil, and a is a coefficient that describes the steepness of the curve. Due to the quadratic relationship between flow and pressure drop, this coefficient, a , can be calculated for each coil for every sampling point. As the coil becomes more or less fouled, it is expected that its system curve will change, becoming steeper with increased fouling and shallower with decreased fouling. Figure 2.2 shows one of the cooling coil system curves that was derived by fitting equation 2.3 to experimental data collected when we varied the flow rate with dampers.

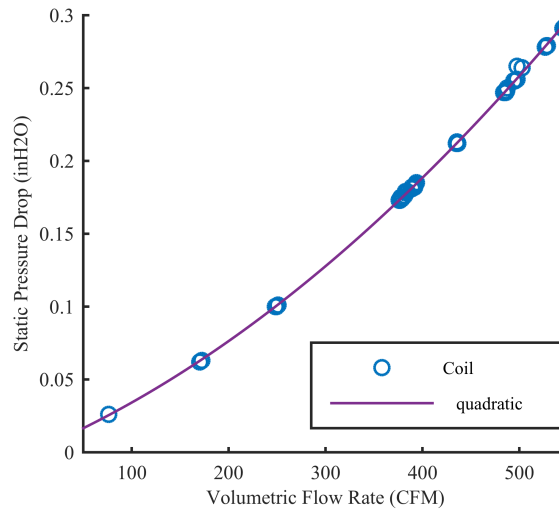


Figure 2.2: Quadratic curve fit of experimental data to equation 2.3, demonstrating how the coil system curves were generated.

2.1.3.4 Modes of Operation

Our test facility was unable to maintain constant relative humidity and temperature of the inlet air due to changes in ambient air conditions from changing seasons. This allowed us to see changes in the effectiveness of UV cleaning during various operating conditions. For our analysis, we split our time series data on a month-to-month basis and classified each month as either a *humid* month, where water was condensing out of the air for the majority of that month, or a *dry* month, where water was not condensing out of the air for the majority of the month. To simplify our analysis, we excluded months during the transition period from humid to dry months or vice versa.

2.1.3.5 Statistical Analyses

All data were smoothed using a moving average approach with a boxcar window that averaged each sampling point with the 5000 points (approximately 28 hours) to its right and left to mute short-term fluctuations due to the buildings HVAC system operation and focus on long-term UVG-CC effects.

While both treatment and control cooling coils were manufactured to the exact same specifications, they were not identical in the measured properties of heat transfer effectiveness and pressure drop. To account for this, we analyzed the difference between measured properties of each coil across discrete one month periods that fell into *humid* or *dry* month categories. One year of sampling was split into four one-month subsets described in Table 2.1.

Sampling subsets	Start date	End date	Description
Baseline Region	7/27/2014	8/27/2014	After allowing both coils to foul for four months, the month prior to turning the UV lamp on is the baseline period
Humid Region 1	8/27/2014	9/27/2014	The month immediately after UV was turned on for the treatment coil while condensation was occurring

Dry Region	12/25/2014	1/25/2015	One month during the winter while no condensation was occurring due to dry ambient air conditions
Humid Region 2	6/5/2015	7/5/2015	The following summer after returning to condensing conditions

Table 2.1: Sampling subsets used for statistical analysis.

We used a bootstrapping approach in our statistical analyses due to very large sample sizes (roughly 134,000 points per sampling subset). A one-way analysis of variance (ANOVA) statistical test was used to compare the difference in heat transfer effectiveness and system curve coefficient between treatment and control coils across sampling subsets. Student t-tests were used to test if sampling subset means were significantly different than zero. Rather than perform an ANOVA across all sampling subsets, we randomly sampled 1000 points without replacement from each subset and performed an ANOVA on those samples. This subsampling process was repeated 1000 times, generating a distribution of 1000 p-values from all ANOVAs. When the mean ANOVA p-value rejected the null hypothesis that all sampling subset means were equal, we conducted multiple comparisons across sampling subsets using Tukeys Honestly Significant Difference (HSD) procedure. Similarly, t-tests were bootstrapped by randomly sampling 1000 points from each sampling subsets and performing a t-test 1000 times to produce a distribution of t-test p-values. All analyses were performed in MATLAB.

2.1.3.6 Sensor Error Propagation

It is important to address the uncertainty in our measurements to distinguish if statistical significance is outside the range of sensor error. Each measured parameter had an associated uncertainty (obtained from the sensor manufacturer) that was propagated to determine the uncertainty of calculated values of heat transfer effectiveness and coil system curve coefficients. All results are presented with an uncertainty derived from parameter measurement error.

2.1.4 Results

2.1.4.1 Coil Effectiveness

Under the hypothesis that the UV-treated coil would have a higher effectiveness than the control coil, the difference between the coils was calculated as treatment coil effectiveness minus control coil effectiveness at every sampling point during the sampling subset. The larger the positive difference, the higher the UV-treated coil effectiveness was compared to the control coil effectiveness. Figure 2.3 shows the entire time series of calculated effectiveness for both coils and Figure 2.4 shows each sampling subset described in Table 2.1.

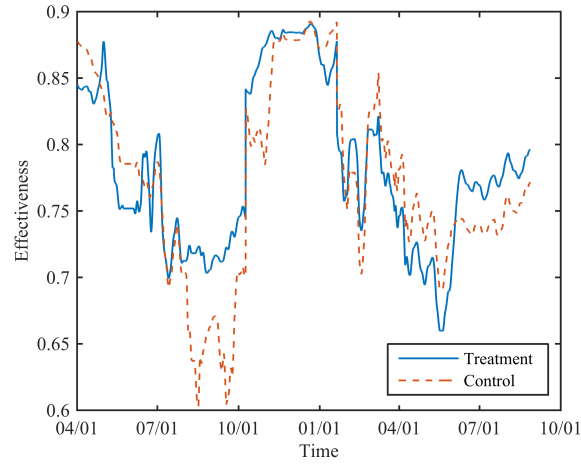


Figure 2.3: Time series of calculated effectiveness for UV-treated and control coils over the course of 18 months.

The bootstrapped ANOVA found that all sampling region means were not equal to each other (Table A1). Tukeys HSD procedure found that the Dry Region mean was not significantly different than the Baseline Region mean, indicating that UV treatment had no effect on heat transfer effectiveness during dry conditions (left panel of Figure 2.5). The two humid region means were statistically significantly greater than the Baseline Region mean. This result suggests that treatment with UV was most effective during humid operating conditions, when water was actively condensing out of the air. Between both humid regions of operation, the UV-treated coil ranged, on average, from 3–6.4% greater effectiveness than the control coil (left panel of Figure 2.5). Boot-

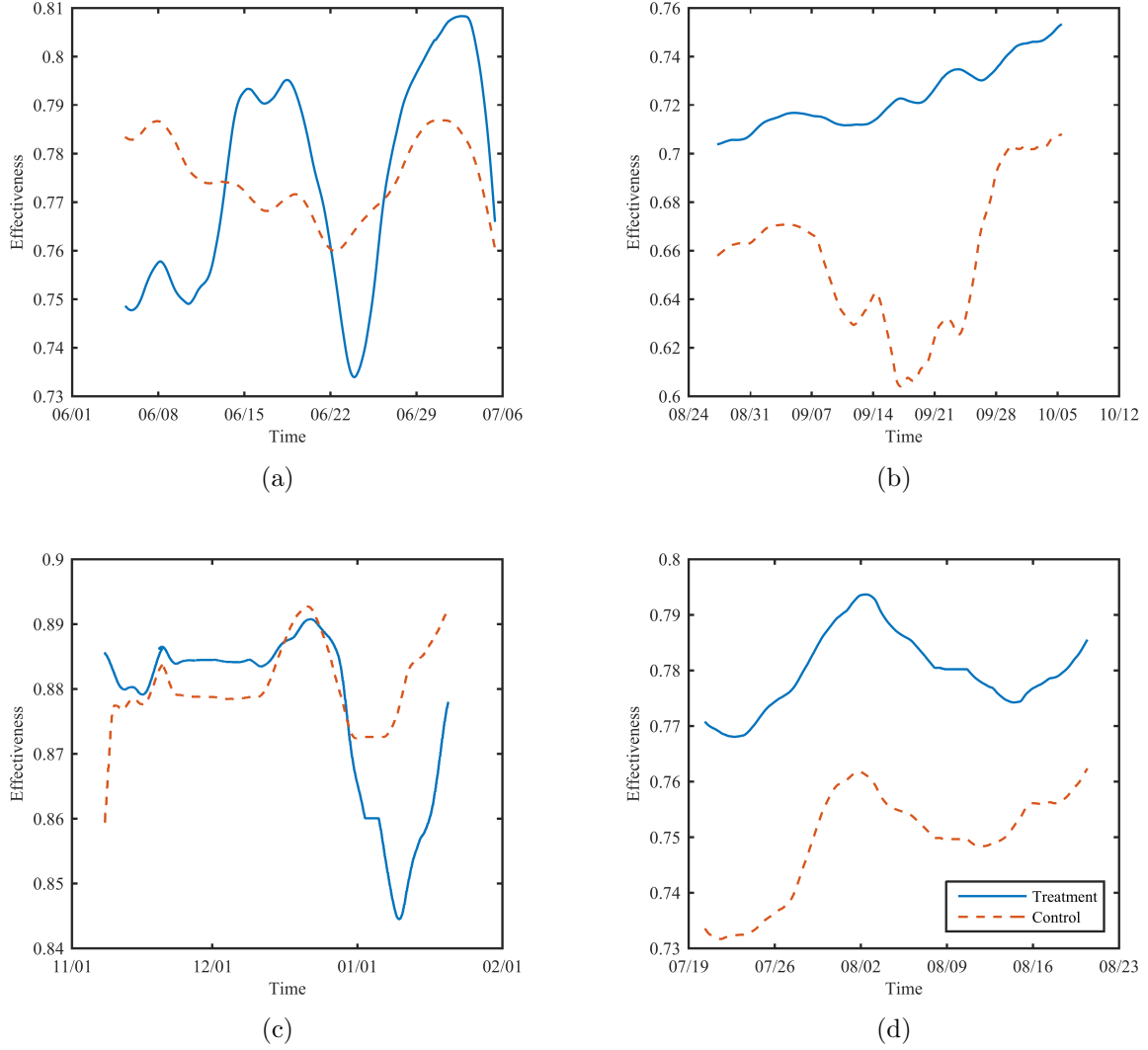


Figure 2.4: Sampling subsets used for statistical analyses. (a) Baseline Region both coils had been running for 10 undisturbed months to build up fouling, UV lamp had not yet been turned on. (b) Humid Region 1 immediately after UV lamp was turned on for the treatment coil, still in condensing conditions. (c) Dry Region period of time in the winter when coil surfaces were dry due to very low humidity level of inlet air. (d) Humid Region 2 the second period of time when condensing conditions were achieved the following summer.

strapped T-tests found that the Baseline and Dry regions were not significantly different than zero while both humid regions were significantly greater than zero (Table 2.2). Histograms of t-test p-values are shown in the right panel of Figure 2.5.

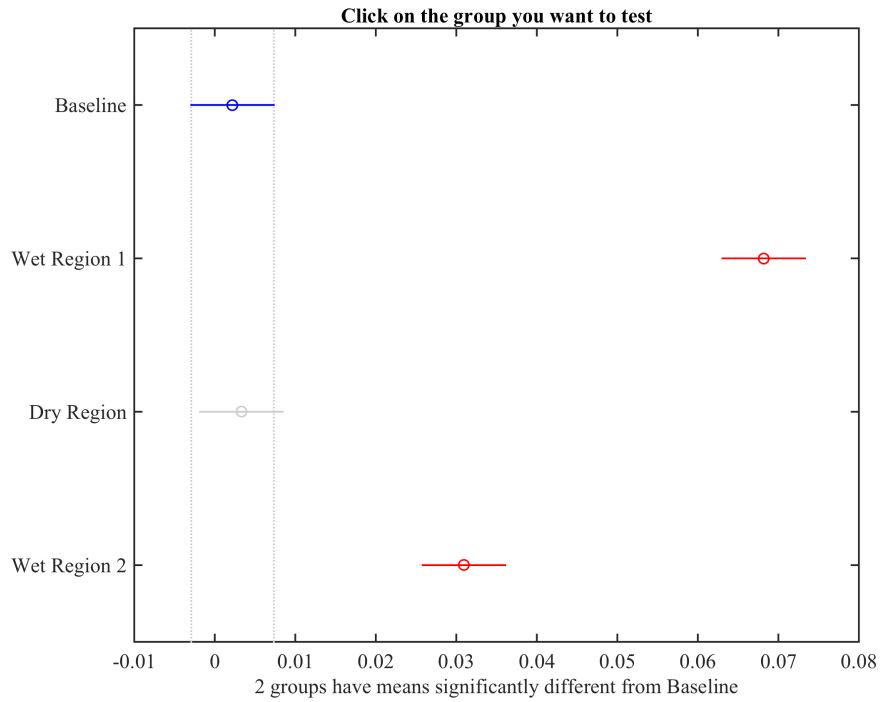
Mode of Operation	Effectiveness		System Curve Coefficient	
	T-test p-value	ANOVA p-value	T-test p-value	ANOVA p-value
Baseline Region	0.49		7.5e-05	
Humid Region 1	1.3e-43		2.1e-25	
Dry Region	0.19		1.3e-23	
Humid Region 2	1.5e-72		1.3e-08	
ANOVA of all regions		4.1e-78		6.9e-75

Table 2.2: Mean p-values from bootstrapped T-tests and bootstrapped one-way analysis of variance for heat transfer effectiveness and system curve coefficient differences between UV-treated and control cooling coils.

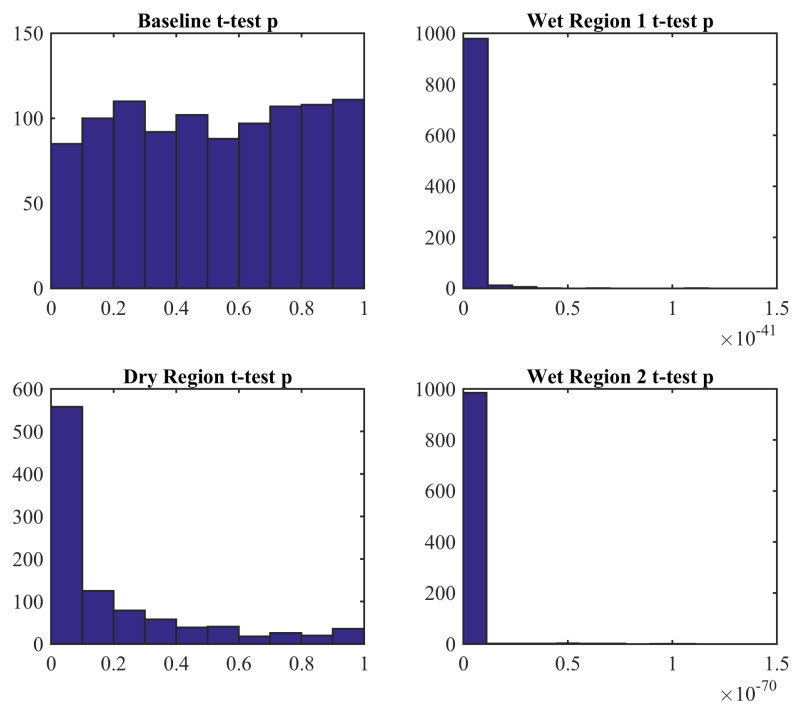
2.1.4.2 Coil System Curve Coefficient

Since the hypothesis was that UV treatment would reduce pressure drop, the difference between coils was calculated as control coil system curve coefficient minus treatment coil system curve coefficient (opposite of effectiveness calculations). The larger the positive difference, the lower the UV-treated coil pressure drop was in comparison to the control coil.

Bootstrapped t-tests revealed that all region means were significantly different than zero (right panel of Figure 2.6), meaning that the two coils did not have the same pressure drop at the beginning of the experiment during the Baseline period. The bootstrapped ANOVA showed that all four region means were not equal to each other but the Tukeys HSD procedure found that the Baseline, Humid Region 1, and Humid Region 2 subsets were not significantly different from each other (left panel of Figure 2.6). The Dry Region was significantly less than the other three regions, meaning that the pressure drop of the UV-treated coil was greater than the control during dry conditions (Table 2.2). In summary, UV treatment did not decrease the static pressure drop.

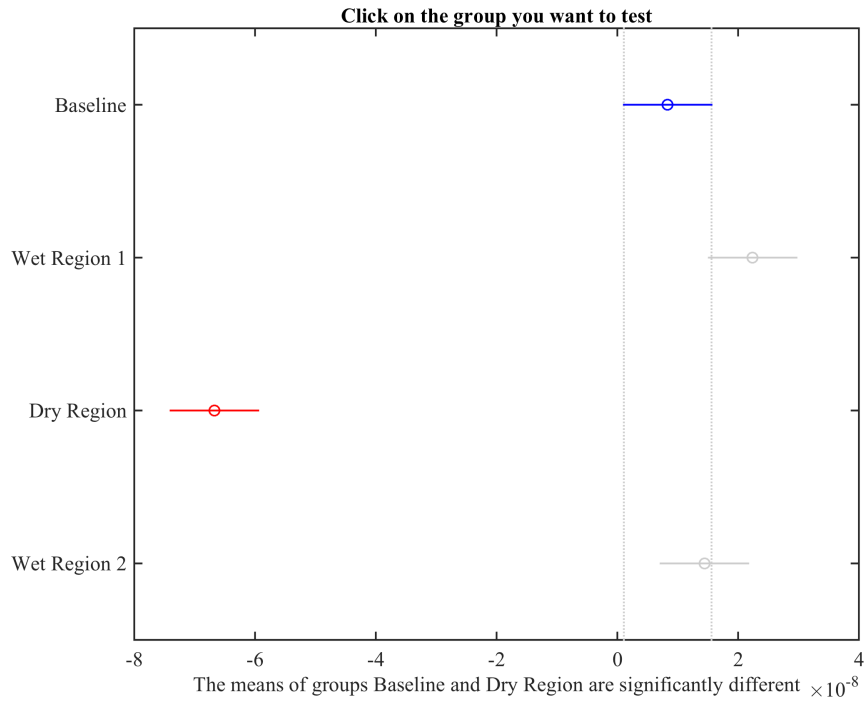


(a)

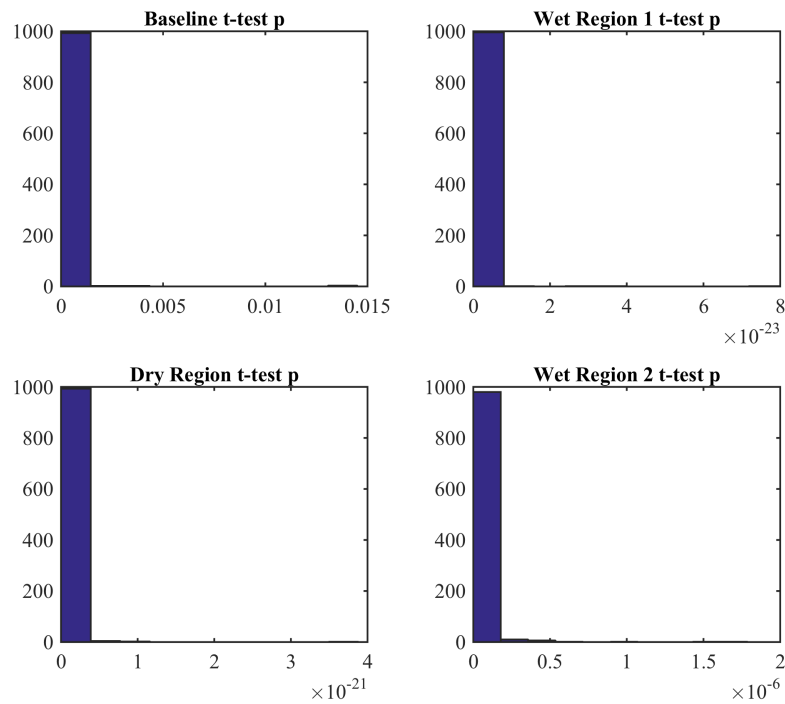


(b)

Figure 2.5: Results from Tukey's Honestly Significant Difference procedure on the difference in heat transfer effectiveness between UV-treated and control coils (a). Results from bootstrapped T-tests for each sampling subset is shown in (b).



(a)



(b)

Figure 2.6: Results from Tukey's Honestly Significant Difference procedure on the difference in system curve coefficient between UV-treated and control coils (a). Results from bootstrapped T-tests for each sampling subset is shown in (b).

2.1.5 Discussion

While our analyses show statistical significance between baseline and wet region groups for the calculated heat transfer effectiveness, sensor error accounts for a large portion of the difference detected between control and treatment coils, with error contributing from 42% up to 90% of the result. Additionally, any statistical significance between the coils for static pressure drop was not outside the range of error associated with our pressure differential sensors. We did observe that coil fin biofouling was reduced [72], thus we believe that the small effect on pressure drop went undetected due to the sensitivity and detection limits of our instrumentation.

The temperature and relative humidity of the air entering our test duct were mild compared to the condensing conditions of cooling coils in very hot, humid climates. We believe UVGI treatment is likely more effective in a region such as Southern Florida, with high cooling latent loads, and possibly more persistent fin biofilms, compared to a region such as Alaska with little to no cooling days annually [50]. Our laboratory setup was located between these two extremes, in Boulder, Colorado, with average entering conditions of 75°F (24°C) and 44% RH compared to average August conditions of 85°F (29°C) and 72% RH in Miami, FL [92]. It is possible that increases in heat transfer effectiveness may be greater than 3–6.4% in climates with higher cooling latent loads, a question we urge researchers to investigate in future UVG-CC studies.

Another possible explanation for the subtle impact of UVG-CC on coil effectiveness may be due to using indoor air that had been prefiltered (with a MERV 14) as inlet air. We were unable to use outside air in our experiment due to the location and design of the lab. The lab was intermittently occupied by personnel and was used to build and calibrate instruments, so it was not a sterile environment. In a parallel experiment in a real building air handling unit in West Virginia, consisting of a control and UV-treated coil and run for over two years, we were unable to detect any difference in performance between the UV-treated and control cooling coils (see 2.3.1). Thus it still remains to be seen if in a real building or in a lab setting treating outside air whether the effectiveness can be increased to more than 6.4%, which is the highest value we

measured.

This study investigated the effect of UV-treatment on airside heat transfer effectiveness but additional energy savings are likely due to a reduced load on the chiller supplying the cooling fluid to the cooling coil. We also believe that the effect of UV-treatment on pressure drop may be more pronounced when biofilms are more robust than our laboratory setup and when scaled up to commercial-sized cooling coils that have much more surface area than the coils in our setup, resulting in potential fan energy savings as well. A recent simulation of UVG-CC in a representative office building in Philadelphia found that eliminating biofouling led to a decrease in pump energy use between 15% and 21% as well as a decrease in fan energy use ranging between 15% and 23% [34].

In summary, UVG-CC is effective at reducing biofouling [72] and increases heat transfer effectiveness in wetted conditions. We found an effectiveness increase between 3.0–6.4% during condensing conditions in our laboratory setup under mild climate conditions, with an uncertainty of $\pm 2.7\%$ resulting from the accuracy of our instrumentation. We did not observe any differences in heat transfer effectiveness between the UV-treated and control coils during dry conditions, suggesting that installation of this technology should be carefully considered depending on the climatic region, and may not need to be operated during non-condensing states. UVG-CC also had no effect on static pressure drop in our laboratory setup with mild climatic conditions. Future studies of UVG-CC should pay careful attention to the sensitivity and detection limits of their instrumentation, and would benefit from studying environments prone to excessive biological fouling so that differences between UV and non-UV coils are more pronounced.

2.1.6 Acknowledgements

We gratefully acknowledge financial support for this project from the University of Colorado Boulder Innovative Seed Grant Program, the Department of Mechanical Engineering, University of Colorado Boulder, and an Industry Consortium consisting of four UV companies. A sincere thank you to Trane for donating the cooling coils.

2.2 Part 2 - Decreased surface microbial fouling and resuspension of cell clusters

Julia C. Luongo¹, Shelly L. Miller^{1,*}

¹Department of Mechanical Engineering, University of Colorado, Boulder, CO, USA

* *Corresponding author: shelly.miller[at]colorado.edu*

⁰ This section is in preparation for following journal:
Luongo, J. C., & Miller, S. L. (2016). Ultraviolet Germicidal Irradiation of HVAC Cooling Coils - Part 2: Decreased Surface Microbial Fouling and Resuspension of Cell Clusters. *Submitted to Building & Environment*.

2.2.1 Abstract

Cooling coil surfaces within building ventilation systems are ideal sites for biofilm formation due to the presence of adequate nutrients (deposited particles) and moisture (condensate). In this study, a heating, ventilation, and air-conditioning (HVAC) test apparatus was built consisting of two parallel ducts, each with its own cooling coil. One coil was exposed to ultraviolet germicidal coil cleaning (UVG-CC) while the other was the comparison control to investigate the impact of UVG-CC on surface microbial loading and bacterial attachment. Surface samples were collected by swabbing a uniform area of coil surface and airborne samples were collected isokinetically with sterile funnel filters. All samples were quantified via direct epifluorescent microscopy. Prior to irradiating, higher concentrations of surface microbial loading were found on the downstream side of both cooling coils under condensing conditions. Conversely, under dry surface conditions with downstream UV irradiance, surface concentrations were higher upstream. UVG-CC (at an average $200 \mu\text{W}/\text{cm}^2$ on the coil surface) reduced surface microbial loading by 55% on average during condensing conditions and inhibited bacterial attachment causing clusters of bacterial matter to become airborne downstream of the cooling coil. Additionally, it was found that desiccation also inhibited surface microbial loading and yielded cluster detachment but to a lesser degree than UVG-CC treatment.

2.2.2 Introduction

Ultraviolet germicidal irradiation (UVGI) has a long history of being used for the disinfection of air streams, primarily in environments with higher risk of airborne pathogen transmission such as healthcare facilities, schools, and prisons [104]. UVGI systems use low-pressure mercury vapor lamps that emit shortwave ultraviolet-C, peaking at 253.7 nm. Using ultraviolet germicidal coil cleaning (UVG-CC) technology in heating, ventilation, and air-conditioning (HVAC) systems has recently gained popularity [84]. While air disinfection may still occur as air passes by the UVG-CC system, the primary focus of UVG-CC is surface disinfection and, in turn, potential energy savings, maintenance cost savings, and increased or prolonged system capacity due to cleaner heat exchanger surfaces.

Heat exchanger surfaces are an ideal site for biofilm growth due to the presence of adequate nutrients (deposited particles and debris) and moisture [88]. High bacterial and fungal concentrations have been documented within HVAC systems, specifically on cooling coil surfaces and drain pans [49, 67, 83]. Previous studies investigating the microbiological makeup of HVAC cooling coils observed the desiccation-resistant species of *Methylobacterium* to be the predominant organism recovered from the aluminum fins [48, 106]. Other strains isolated from cooling coil biofilms include *Sphingomonas paucimobilis*, *Alcaligenes paradoxus*, *Bacillus cereus*, an unidentified *Sphingomonas*-like strain [48], and various members from the genera *Bacillus* and *Sphingomonas* [106]. In addition, *Methylobacterium* and *Sphingomonas* have been found to be predominant genera found in biofilms on household shower curtains [57]. The cyclical nature of these high moisture environments followed by extreme desiccation on both heat exchangers and shower curtains appears to be conducive to the formation of biofilms of *Methylobacterium* or similar bacterial members [106]. High concentrations of these and other organisms on heat exchangers have the potential to be resuspended into the airstream, possibly introducing allergens or toxins into the indoor environment.

Anecdotal evidence describes “visibly cleaner” cooling coils after the installation of a UVG-CC system but, to the authors knowledge, few peer-reviewed studies have investigated the fate

of microbial contamination after irradiation. The objective of this study is to report detailed measurements of total microbial counts on surfaces and in air for an irradiated coil versus a non-irradiated coil under various operating conditions (i.e. dry coils versus condensing coils). Biological samples were analyzed via epifluorescent microscopy. The results presented in this study allow us to provide recommendations for effective installation and operation of UVG-CC technology for mitigation of microbial contamination on HVAC heat exchangers.

2.2.3 Experimental Methods

2.2.3.1 Test Facility

An HVAC test apparatus was built in the Air Quality Laboratory at the University of Colorado Boulder, consisting of two parallel ducts (12-inch diameter), each with its own cooling coil (Trane, one-ft² face area, 12 fins/inch, copper tubes and aluminum fins), but supplied by the same temperature and relative humidity controlled airstream (Figure 2.7). The coils were steam cleaned prior to starting the tests.

One UVC lamp (ALTRU-V V-Ray Model 23-1100, 25W) was installed ten inches away from the coil on the downstream side. The lamp was burned in for 100 hours prior to use. The lamp was shielded with mesh to achieve the desired level of surface irradiance.

The test apparatus used indoor lab air from the room as the inlet air. The room HVAC system supplied 100% outdoor air filtered with MERV 14 filters. Air entered each cooling coil, on average, at 75°F and 44% relative humidity and chilled water entered at 50°F. The system mimicked a constant volume HVAC system, meaning the volumetric flow rate is maintained constant. The flow rates through each coil were kept equal to one another using dampers since the static pressure drop across the coils may not be equal given equivalent flow rates. Air and water inlet temperatures, inlet relative humidity, and water flow rate were held as constant as possible.

Fluctuations in outdoor air conditions slightly affected conditions within the apparatus. During summer months, both coils had water actively condensing onto fin surfaces at nearly all times

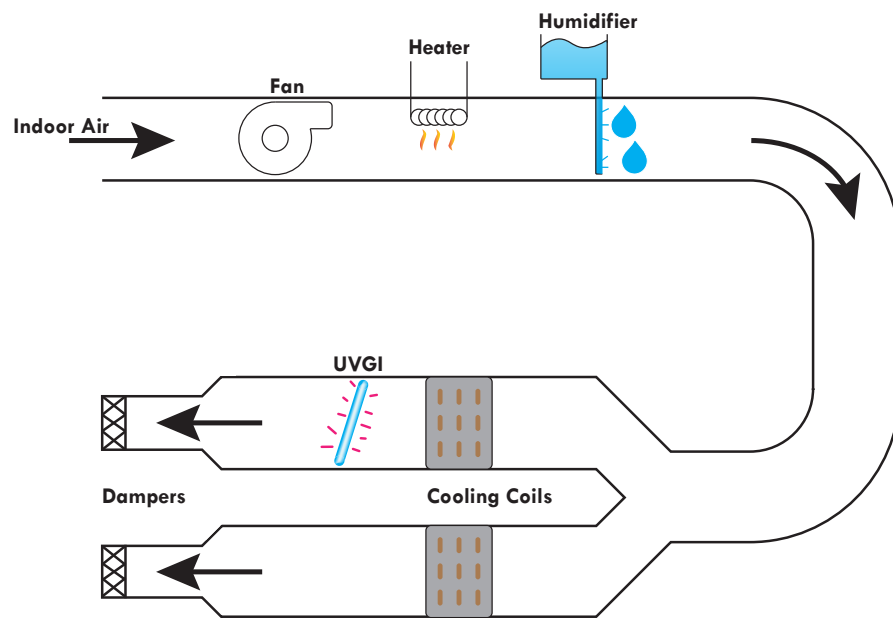


Figure 2.7: Schematic of HVAC test apparatus.

and drain pans were wet. In the winter months when outdoor air became very dry, the apparatus was unable to humidify the air sufficiently to continue condensing water onto the cooling coils. These test periods of desiccation revealed interesting results, described in Section 2.2.4.

The system ran undisturbed for 4 months without UVG-CC on either coil to ensure that both coils fouled at an equivalent rate and to establish a robust baseline dataset. After 4 months of operation, the UV lamp was turned on, irradiating the downstream side of one of the cooling coils (called the *treatment* coil). The *control* coil was never irradiated. The irradiance at the surface of the treatment coil was on average $200 \mu\text{W}/\text{cm}^2$ at the center and $150 \mu\text{W}/\text{cm}^2$ at the corners, as recommended by the ASHRAE Handbook – Systems and Equipment.

2.2.3.2 Sample Collection

Coil surface samples were taken with sterile BBL CultureSwabs (BD, Sparks, MD). A 10-cm^2 area of the coil fin-edge surface was swabbed for each sample. Both the upstream and downstream side of each coil was swabbed. Swabs were aseptically cut and placed into 7 mL of HPCL water and vortexed for 1 minute for extraction. Air samples were collected isokinetically with $0.45\text{-}\mu\text{m}$ cellulose nitrate membrane filters (Thermo Scientific, Waltham, MA) at 10 L/min for 19 hours and vortexed for 1 minute in 7 mL of HPLC water for extraction. All samples were sieved through a $40\text{-}\mu\text{m}$ cell strainer (Fisherbrand, Pittsburgh, PA) to remove large particles and debris prior to staining. Samples were stained using SYTO BC Green Fluorescence stain (Life Technologies, Carlsbad, CA) and deposited onto a $0.2\text{-}\mu\text{m}$ black polycarbonate membrane (Millipore, Billerica, MA). SYTO BC is a nucleic acid stain that penetrates both Gram-negative and Gram-positive bacteria, yielding total cell counts (both live and dead).

2.2.3.3 Enumeration by Epifluorescent Microscopy

Surface and air samples were directly counted using a fluorescence upright widefield microscope (Nikon E600) with a FITC filter. All reported counts are an average of 5 fields. In the case that the 5 fields from one sample yielded a coefficient of variance greater than 30% (non-uniform

distribution on the membrane), the sample was discarded.

Total cell counts were used as a relative comparison between the UV-irradiated treatment coil and the control coil. Methods for sample preparation were consistent between all samples and we report results in average cell counts per field instead of estimated concentrations.

2.2.4 Results

Throughout one year of sampling, the cooling coils experienced three distinct modes of operation based on the conditions of the incoming air. These modes are:

- [1] *dry* – when air exiting the coil has not reached saturation and the coil surfaces are dry,
- [2] *transitional* – when air exiting the coil has reached saturation but water is not dripping into the drain pans, and
- [3] *condensing* – when air exiting the coil has reached saturation and water is actively condensing and dripping into the drain pans.

Surface microbial loading and resuspension of cell clusters was directly influenced by the mode of operation and the transition from one mode to another.

Prior to exposing one coil to UVG-CC, both coils were operating under mode [3], actively condensing water out of the air, for four months to establish a robust biofilm on the fin surfaces. Figure 2.8 shows how microbial concentrations changed over time on surfaces and Figure 2.9 in air both downstream and upstream for treatment and control coils. The first two sets of bars on the left side of Figure 2.8 (labeled 8/26/14 and 9/9/14) represent the typical microbial loading profile for cooling coils in the summer months while actively condensing water. Due to large volumes of water present on fin surfaces, mostly located on the downstream side of the coil, microbial loading is highest on the downstream side (and in drain pan [49, 79]).

The treatment coil began being irradiated on 9/19/14. Surface concentrations were reduced by 87% (standard error, SE=4.6%) after one month of irradiation (from pre-treatment dates to 10/17/14). This effect, however, is confounded by the fact that both coils were transitioning out of

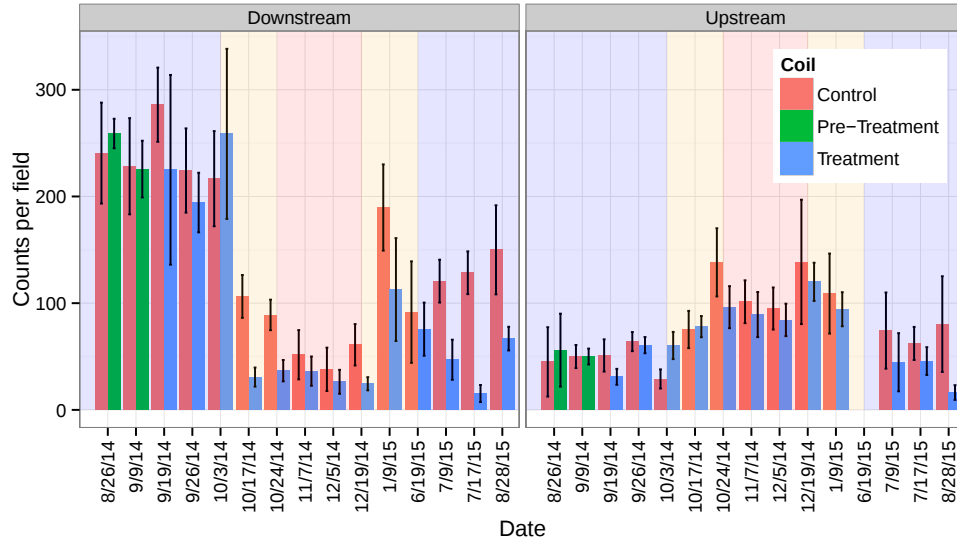


Figure 2.8: Total cell counts per field of view from surface samples upstream and downstream of the treatment and control cooling coils by sampling date. Areas shaded in blue correspond to condensing mode of operation, areas in orange correspond to transitional mode of operation, and the red shaded area corresponds to dry operating condition.

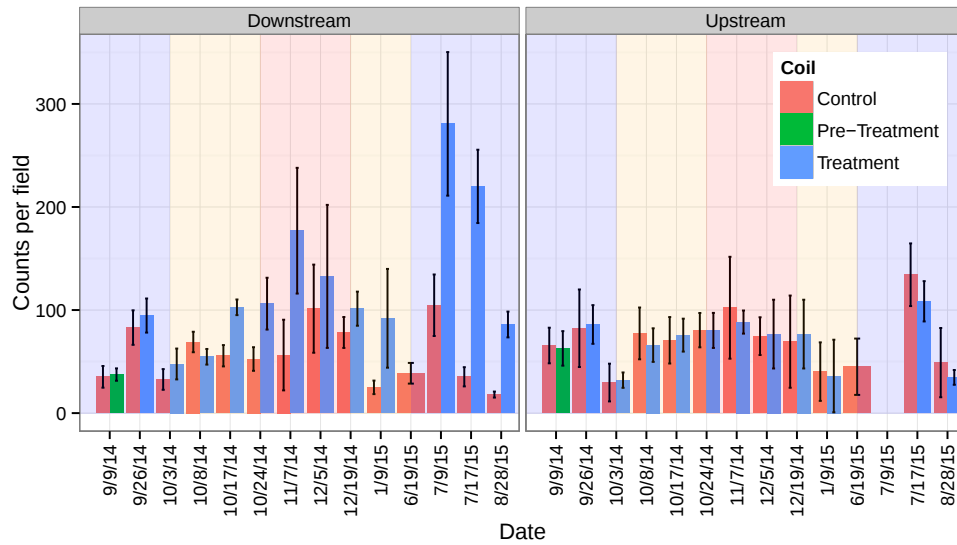


Figure 2.9: Total cell counts per field of view from air samples upstream and downstream of the treatment and control cooling coils by sampling date. Areas shaded in blue correspond to condensing mode of operation, areas in orange correspond to transitional mode of operation, and the red shaded area corresponds to dry operating condition.

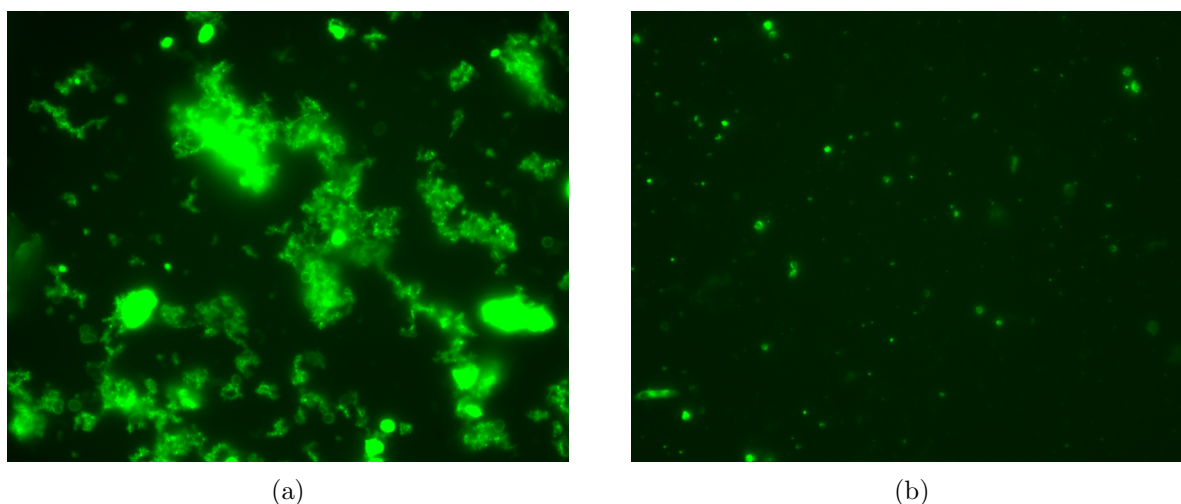


Figure 2.10: Comparison of downstream air samples with (a) and without (b) cell clusters.

the actively condensing mode of operation. Desiccation caused downstream surface concentrations on the control coil to decrease by 55% (SE=4.1%). Upon entering the summer months the following year and returning to condensing conditions, surface concentrations on the downstream side of the control coil began rising while concentrations on the treatment coil remained low. Counts were 55% (SE=10%) lower on the treatment coil in the second condensing mode region (6/19/15 to 8/28/15). Over the entire year of sampling, downstream surface cell counts were 40% (SE=13%) lower on the treatment coil than the control after the UV lamp was turned on.

Increases in airborne cell counts downstream of the coils coincides with reductions in surface counts (10/24/14 to 12/5/14), suggesting that as either UVG-CC or desiccation inactivates biofilms attached to the coil surface, clusters of cells detach and resuspend into the airstream (pictured in Figure 2.10). Airborne counts downstream of the control coil were 57% (SE=10%) lower than the treatment coil on average over all operational modes after UV was turned on.

During the condensing mode of operation, the surface conditions of the coil are favorable for microbial growth, depicted with a positive association between surface microbial loading and increasing surface wetness (or latent load), as seen in the left panels of Figure 2.11. We suspect average surface counts on the treatment coil are inflated by the lag time for inactivation. The low

level of irradiance results in long lengths of time to achieve inactivation dosage, possibly explaining the 2-week to one-month lag in decreased surface counts for the treatment coil during condensing modes of operation (Figure 2.8). Conversely, there is a negative association between airborne counts downstream and surface wetness (right panels of Figure 2.11). This suggests that biofilms are dependent on a certain amount of surface wetness to stay attached to coil surfaces or they are sloughed off of surfaces by the high air velocities through the fin channels in dry conditions.

Statistical t-tests were performed to determine if the sample means on every sampling date for the two sample types (surface and air), locations (upstream and downstream), and modes of operation (condensing, transitional, dry) were statistically different between treatment and control coils. F-tests were first performed to determine equality of variances and the appropriate t-tests was performed given that result. Table 2.3 only lists configurations that were statistically significantly different ($p < 0.01$) between control and treatment coils. Significant differences were primarily seen in downstream samples and were most common during the condensing mode of operation.

Date	Sample type	Location	Mode of operation	p-value
9/19/14	Surface	Upstream	Condensing	$p = 0.007$
10/3/14	Surface	Upstream	Condensing	$p = 0.001$
10/17/14	Surface	Downstream	Transitional	$p = 2.6e-05$
10/17/14	Air	Downstream	Transitional	$p = 9.5e-05$
10/24/14	Surface	Downstream	Transitional	$p = 7.6e-05$
10/24/14	Air	Downstream	Transitional	$p = 0.002$
11/7/14	Air	Downstream	Dry	$p = 0.002$
12/19/14	Surface	Downstream	Dry	$p = 0.001$
7/9/15	Surface	Downstream	Condensing	$p = 0.0003$
7/9/15	Air	Downstream	Condensing	$p = 0.0006$
7/17/15	Surface	Downstream	Condensing	$p = 2.5e-05$
7/17/15	Air	Downstream	Condensing	$p = 7.9e-05$
8/28/15	Surface	Downstream	Condensing	$p = 0.0003$
8/28/15	Air	Downstream	Condensing	$p = 7.2e-05$

Table 2.3: Sample configurations with statistically unequal means between treatment and control cooling coils ($p < 0.01$).

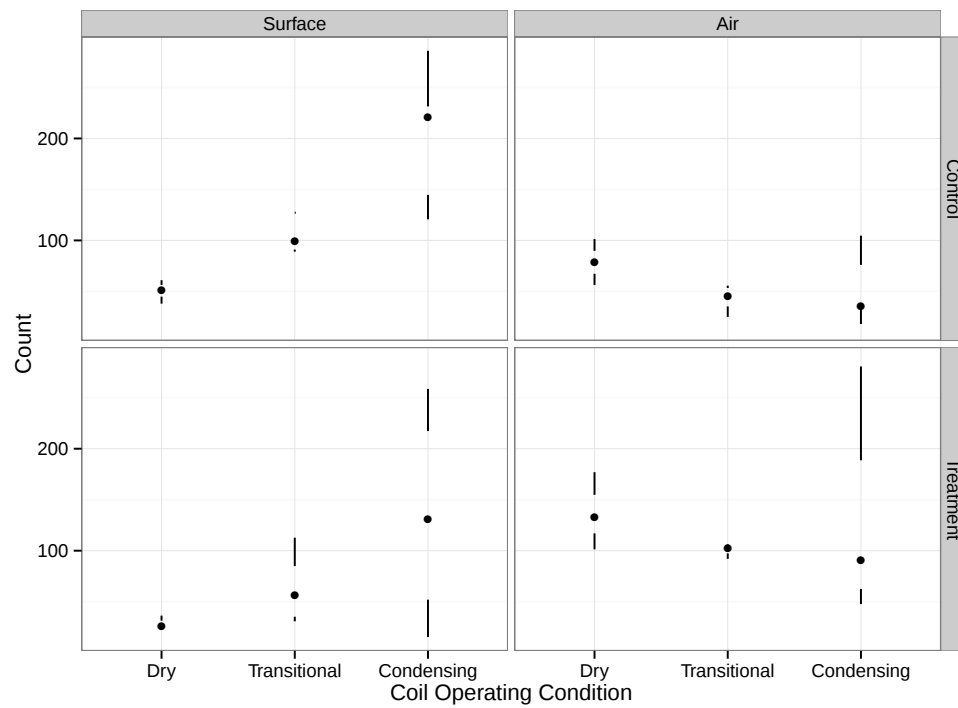


Figure 2.11: Average microbial loading downstream for both coils is influenced by the coil operating conditions. When actively condensing, the presence of water promotes bioaerosol deposition and growth. When dry, microbial loading decreases on downstream surfaces and airborne concentrations increase as cell clusters are likely re-entrained into the airstream from the coil surface.

2.2.5 Discussion

SYTO BC Green Fluorescence stain is a mixture of SYTO dyes optimized for bacterial staining and counting. It is possible that this dye also stained fungi present in our samples, as bacterial viability kits using SYTO 9 have been successfully used for fungal spore viability assays [15]. The cooling coil environment that cycles through periods of heavy moisture and desiccation is an ideal site for fungi to thrive and numerous studies have documented fungal contamination in HVAC systems [4]. HVAC system fungal contamination may also play a role in the occurrence of sick building syndrome (SBS) [112, 16]. Future studies of the efficacy of UVG-CC treatment for reducing cooling coil microbial contamination would benefit from the inclusion of fungal analyses.

The disadvantage of cell counts using a nucleic acid stain such as SYTO BC is that there is no differentiation between active and inactive cells. Methods exist for differentiating live and dead cells using microscopy techniques, one example being the LIVE/DEAD BacLight Bacterial Viability kit (Life Technologies, Carlsbad, CA). This kit consists of two stains, a green fluorescing SYTO9 and red fluorescing propidium iodide (PI). SYTO9 is able to enter any cell and stain the nucleic acid, often used for assessing total cell counts (as we did in this study). PI only enters a cell with a damaged cytoplasmic membrane to stain the nucleic acid red. The green stain is quenched in the presence of the red stain and energy is transferred to the PI stain if it is present in the cell. Low levels of UVC radiation inactivate cells by causing the formation of dimers in the DNA that prohibit replication. It is possible that an inactive cell with UV-induced DNA dimers may still stain as a live cell because its cytoplasmic membrane is not damaged. Additionally, certain dyes (such as PI) readily adhere to particles and substrate material resulting in increased non-specific binding and background fluorescence, especially with fairly “dirty” environmental samples [12]. For these reasons we were unable to assess viability using microscopy techniques.

Culturing is another form of assessing bacterial viability. We attempted to culture both surface and air samples using tryptic soy agar and Reasoners 2 agar, as these were most successful in previous culturing studies of HVAC microbial contamination [49, 106, 89], but were unable to

consistently generate enough colony forming units for accurate counting. In many environments, up to 99% of microorganisms are unculturable [6]. This statistic combined with possible low airborne microbial concentrations from indoor air passing over the heat exchangers may have contributed to unsuccessful cultivation.

The temperature and relative humidity of the air entering our test duct were mild compared to the condensing conditions of cooling coils in very hot, humid climates. We believe UVG-CC treatment is likely more effective in a region such as Southern Florida, with high cooling latent loads and possibly more robust and persistent biofilms, than a region such as Alaska with little to no cooling days annually [50]. Our laboratory setup is located between these two extremes, in Boulder, Colorado, with average entering conditions of 75°F and 44% RH compared to average August conditions of 85°F and 72% RH in Miami, FL [92].

We were only able to investigate bacterial cell counts in this study, but it can be expected that fungal cells are also present in the coil biofilms. Fungal contamination of various HVAC system components, including air filters, insulation, and cooling coils, has been reported and fungal levels have been significantly lowered in air and on insulation within an air handling unit with the use of UVGI [67]. The potential of fungal resuspension due to UVG-CC may increase the risk of allergens or toxins being introduced into the air supplied to the building.

Unfortunately, we were unable to investigate whether the biofilm clusters sloughing off of the cooling coils were completely inactive or not. We were also unsuccessful at determining what taxa were present on the fin surfaces due to unsuccessful polymerase chain reaction (PCR) amplification caused by low biomass concentrations. Regardless of activity or classification, these microbes may still serve as potential allergens or toxins for the occupants in the building. Previous studies have identified Gram-negative bacteria on cooling coils [49, 48, 106] and higher concentrations of Gram-negative bacteria in indoor air have been correlated with buildings showing evidence of sick building syndrome symptoms [117]. This suggests that microbial matter released off of cooling coil surfaces has the potential to initiate adverse health effects.

Studies of wastewater have found that detached biofilm clusters in the presence of chlorine

disinfection were able to survive and form new biofilms with relatively high viability, suggesting the possibility of perpetuating biofilm contamination due to reattachment and regrowth from the detached clusters [121]. It has also been shown that large bioclusters, particularly ones on surfaces, may shield and protect organisms within the core of the clusters from the harmful effects of UV irradiation [60]. For these reasons we recommend that UVG-CC installations consider using a higher level of surface irradiance than the recommended level for the first few months of operation in an attempt to inactivate detached microbes as much as possible. We also recommend that the final filter bank after the cooling coil use at least a MERV 13 or higher during those first few months, or during periods of desiccation, when biofilm clusters will be sloughing off of the coil. After these “sloughing” periods, surface irradiance may return to the recommended level and final filters can be returned to the designed rating.

In summary, higher microbial loading occurred on downstream cooling coil surfaces in condensing conditions and, conversely, loading was higher on upstream surfaces in dry conditions. After initial fouling, reduction in surface loading coincided with increases in air concentrations downstream of the coils, suggesting resuspension of cell clusters from inactivated surface biofilms. Both UV irradiation and desiccation reduced microbial loading on surfaces and caused resuspension of cell clusters, desiccation to a lesser degree than UVG-CC by 57%. UV was most effective at reducing surface microbial loading in condensing conditions, with 51% lower surface concentrations downstream compared to the control in the second condensing mode region. In dry conditions, however, microbial concentrations on surfaces were statistically different on the UV-treated coil versus the control on just one sampling date. This work suggests that filters downstream of cooling coils in humid climates should be carefully monitored for proper installation and possible heavy loading after UV installation or coil desiccation, particularly after periods of high latent heat transfer loads that corresponds to large amounts of condensation on coil surfaces. We recommend replacing final filters one or two months after initial UVG-CC installation due to likely microbial fouling, especially if an increase in pressure drop across final filters is observed after the first few months.

2.2.6 Acknowledgements

We thank Jason Brownstein for help building and configuring the duct apparatus. Thanks to Andrew Baugher for assistance with sample preparation, Joanne Emerson for helpful discussions on microscopy methods, and Adam Sokol for helping reconfigure the duct apparatus.

We gratefully acknowledge financial support for this project from the University of Colorado (CU) Boulder Innovative Seed Grant Program, the Department of Mechanical Engineering, University of Colorado Boulder, and an Industry Consortium consisting of four UV companies. Thanks to Trane for donating the cooling coils.

2.3 Future work

2.3.1 Real building UVG-CC study

To validate the results from our laboratory experiment of UVG-CC, we sought to replicate our experimental setup in a real building. We partnered with the National Institute of Occupational Safety and Health (NIOSH) to conduct a UVG-CC study in one of the air handlers in their headquarters building in Morgantown, West Virginia. The air handler mimicked our laboratory setup by having two identical cooling coils side-by-side which were supplied from the same outdoor air intake and had the same airflow rate. A schematic of the air handler is seen in Figure 2.12.

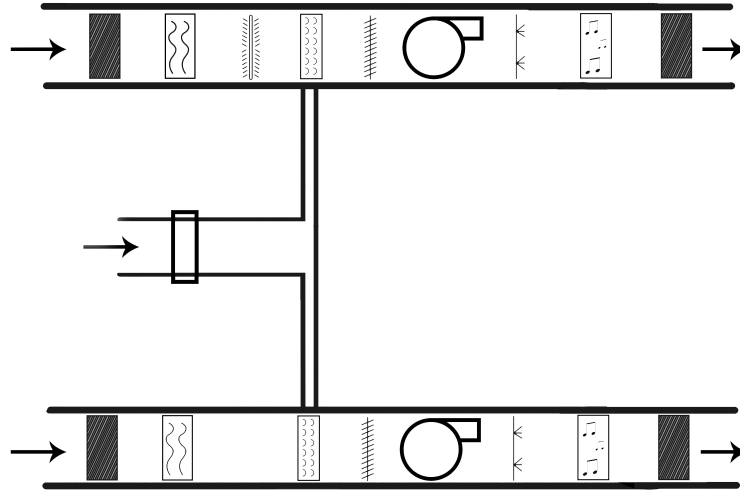


Figure 2.12: Schematic of the NIOSH air handler.

One coil was treated with UV and the other was the control. The air handler was equipped with sensors to measure duct velocities using pitot tubes connected to differential pressure sensors, static pressure drops across each coil, entering water temperature and flow rate, and entering and exiting air temperatures and relative humidities for each branch. Voltage output from the sensors was fed into a the building automation system (BAS) and exported once a week. Since the coils did not need to be in cooling mode year-round, the BAS logged a chiller switch of whether the

chiller was pumping cold water to the coils. The BAS logged one point every 30 minutes.

Preliminary analyses show no evidence of UV-effect on heat transfer effectiveness or pressure drop. Coil performance seems to modulate together between both coils but, as seen in Figure 2.13, the control and treatment coils were not identical at the beginning of the experiment, and the offset between the two coils remained over the course of one year. We attribute this to the inability to validate and calibrate the instrumentation from afar, as well as a lack of precision to sense changes in temperatures. For this type of study to be successful, the researcher needs to be able to visually inspect the cooling coils and spot-check measurements occasionally. Data of the airside heat transfer effectiveness for both coils while the chiller switch was “ON” is displayed in Figure 2.13. Note that chiller operation was infrequent during the winter months.

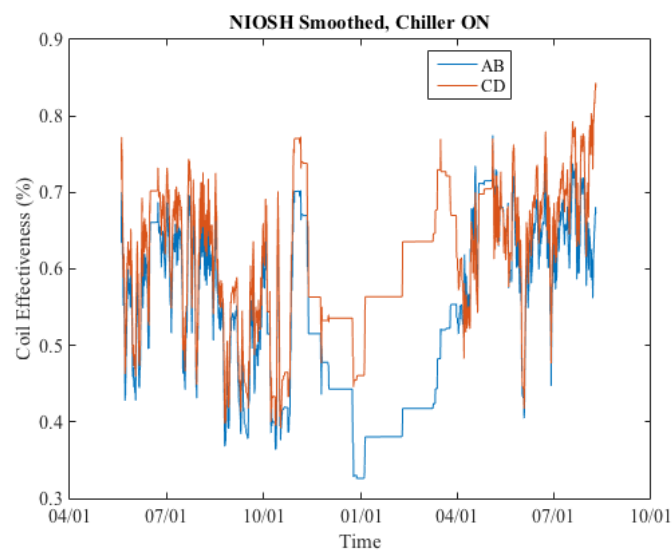


Figure 2.13: Time series of the calculated airside heat transfer effectiveness for both NIOSH cooling coils. The UV-irradiated coil is labeled *AB* and the control coil is labeled *CD*.

We hope that future work on this project will be capable of measuring differences in heat transfer and flow characteristics resulting from UVG-CC treatment. Instrumentation may need to have higher accuracy than the sensors currently installed in the air handler.

We also collected surface swab samples from both coil fin surfaces over the course of one year. In the future we hope to do 16S and ITS rRNA gene sequencing (methods described in

Section 3.1.4) on the swab samples to compare the bacterial and fungal communities present on the UV-irradiated coil compared to the control coil. This work may help to elucidate what types of microbes are thriving on cooling coil surfaces and are potentially being introduced into the indoor environment if resuspended due to inactivation from UVG-CC or dessication.

Chapter 3

The Microbiology of Indoor Air Quality in a University Dormitory and its Effect on Student Health

3.1 Microbial analyses of airborne dust collected from dormitory rooms predict the sex of occupants

Julia C. Luongo¹, Albert Barberán², Robin Hacker-Cary³, Emily E. Morgan², Shelly L. Miller¹, Noah Fierer^{2,4,*}

¹Department of Mechanical Engineering, University of Colorado, Boulder, CO, USA

²Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309

³Department of Integrative Physiology, University of Colorado, Boulder, CO 80309

⁴Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309

* *Corresponding author: noah.fierer[at]colorado.edu*

⁰ This chapter is modified from a publication in the following journal [70]:
 Luongo, J. C., Barberán, A., Hacker-Cary, R., Morgan, E. E., Miller, S. L., & Fierer, N. (2016). Microbial analyses of airborne dust collected from dormitory rooms predict the sex of occupants. *Indoor Air*. Accepted Author Manuscript. doi:10.1111/ina.12302

3.1.1 Abstract

We have long known that human occupants are a major source of microbes in the built environment, thus raising the question: how much can we learn about the occupants of a building by analyzing the microbial communities found in indoor air? We investigated bacterial and fungal diversity found in airborne dust collected onto heating, ventilation, and air-conditioning (HVAC) air filters and settling plates from 91 rooms within a university dormitory. The sex of the room occupants had the most significant effect on the bacterial communities, while the room occupants had no significant effect on fungal communities. By examining the abundances of bacterial genera, we could predict the sex of room occupants with 79% accuracy, a finding that demonstrates the potential forensic applications of studying indoor air microbiology. We also identified which bacterial taxa were indicators of female and male rooms, and found that those taxa often identified as members of the vaginal microbiome were more common in female-occupied rooms while taxa associated with human skin or the male urogenital microbiota were more common in male-occupied rooms.

3.1.2 Practical Implications

This work presents bacterial and fungal analyses of indoor air from a unique perspective comparing male-occupied versus female-occupied rooms within a university dormitory. Our study confirms the importance of human occupants in shaping bacterial communities found in indoor air and shows that the sex of the occupants can alter those communities. Results indicate that dust samples from HVAC filters can be identifying of sex-specific source environments, representing a novel forensic approach for identifying the sex of the occupants of a given room.

3.1.3 Introduction

We spend 90% of our lives indoors [54] and the quality of the air we breathe indoors is critical to human health and well-being [29]. In addition to chemical and particulate pollutants, microorganisms also impact indoor air quality. Airborne microorganisms that are allergens or pathogens are of primary concern. Bacteria, fungi, and viruses are ubiquitous in indoor air with concentrations that typically exceed 100,000 cells or viral particles per cubic meter of air [102]. Most microbes cannot be identified using standard techniques [6] and only recently have researchers begun using molecular methods to comprehensively assess the amounts and types of bacteria and fungi found in indoor air [4, 3, 2, 58, 75].

Building-related factors such as ventilation, relative humidity and temperature, and occupants can influence the diversity and composition of microbes found indoors [18, 61, 58, 75]. Although the fungi found indoors are primarily influenced by what fungi are found outside the home [4, 10], the occupants of a given building can have a significant influence on the types of bacteria found indoors. It has long been known that humans shed bacteria from their skin and other body sites into their surrounding environments [19, 37, 77, 94] as do indoor pets [25, 38, 61]. More recently, chamber experiments have detected distinct personal microbial ‘clouds’ associated with individuals, with one female subject being strongly associated with a *Lactobacillus* phylotype nearly identical to *Lactobacillus crispatus*, a bacterium commonly found in healthy vaginal samples [76]. Another recent study found that the female:male ratio of occupants living in a home influenced the types of bacteria found in the home [9]. Despite this accumulating evidence, we still lack a specific understanding of how the sex of the occupants influences the diversity, composition, and abundance of microbes found indoors. Given that we already know that humans are one of the dominant sources of bacteria in the indoor environment [116, 9], we took the next step and sought to determine if we could predict the sex of occupants from information on the amounts and types of microbes found in indoor dust samples. We used high-throughput sequencing of bacterial and fungal taxonomic marker genes along with quantitative PCR (qPCR) to study the types and abun-

dances of bacteria and fungi found in settled dust and HVAC filters in university dormitory rooms. We used multivariate statistical techniques to identify how, and to what extent, the number of occupants, sex of occupants, HVAC system operation, or other measured factors influenced microbial community composition and bioaerosol abundances.

3.1.4 Methods

We assessed the microbial communities found in the air of 91 rooms in a dormitory on the University of Colorado campus (Boulder, USA) that houses undergraduate students (85% of participants were between 18 and 20 years of age). Each room housed 1–2 individuals per room, either males or females according to the Universitys two-sex system used to assign housing. Thus, results are confined to the assumption that the majority of occupants are neither transsexual nor intersexual and cannot speak to a more nuanced sex-system [31]. Each room had a fan coil unit that provided heating or air-conditioning to recirculated room air (no outside air). The dormitory HVAC system was a constant volume system and supplied 100% outdoor air at a constant volumetric flow rate to each room. For each of the 91 rooms (65 male and 26 female), we collected airborne dust samples from fan coil unit filters (MERV 8 rating). These filters had been installed one year prior to collection and only filtered the air from each individual room, allowing us to collect a time-integrated sample from each room. For a subset of these rooms (38 rooms in total, 23 male and 15 female) we also installed two passive samplers per room, following the procedure described in Emerson et al. [28]. Passive samplers from the same room were pooled in our analyses. Passive samplers were suspended 2 to 2.5 m above the floor in each room to collect settled airborne dust from each room. The passive samplers were installed for 2.5 months, from mid-February to mid-May, 2015.

DNA was extracted from each of the 91 fan coil unit filters and the 76 passive samplers using the approach described in Emerson et al. [28]. DNA was PCR amplified using barcoded primers targeting the V4 region of the 16S rRNA gene (for bacterial analyses) or the ITS1 region of the rRNA operon (for fungal analyses). Amplicons were pooled in equimolar concentrations and sequenced on an Illumina MiSeq instrument. Details on the primers, PCR conditions, and the

sequencing approach are provided in Emerson et al. [28] and Lauber et al. [66].

Sequences were demultiplexed and forward reads were analyzed for both 16S and ITS rRNA gene sequences. All sequences were quality-filtered and singletons were removed using a combination of QIIME, UPARSE, and in-house python scripts, following the pipeline described previously in Barberán et al. [10]. All statistical analyses were performed in R (<https://www.r-project.org/>). Filter and passive samples were statistically analyzed separately. Bacterial samples were rarefied to 5,000 sequences per sample and fungal samples were rarefied to 7,500 sequences per sample. After removing potential contaminants (i.e., operational taxonomic units, OTUs, with abundances greater than 5% in the blanks and no-template controls), we generated Bray-Curtis dissimilarity community matrices after Hellinger-transformation of the rarefied OTU tables. We assessed whether variables such as sex of the room occupants, room location (floor and wing), or number of occupants affected bacterial community composition using permutational multivariate analysis of variance (PerMANOVA). Variables were tested individually; interactions were not considered. Data exploration and visualization was performed using nonmetric multidimensional scaling (NMDS) ordination plots. In total, 12 measured or reported variables were investigated including sex of occupants, number of occupants, room type, wing, floor, outdoor air delivery rate from the HVAC system (in cubic feet per minute), average overnight steady-state CO₂ concentration from one week of sampling, proportion of time the fan coil unit was operating over three months, self-reported window opening frequency, self-reported cleaning frequency, estimated skin surface area from self-reported height and weight, and self-reported dandruff diagnosis. All sequence data and the associated sample information have been made publicly available at <http://dx.doi.org/xxxxxx> (to be determined).

We used a machine learning approach to identify the relationship between the response (female-inhabited or male-inhabited room) and the predictors (relative abundance of bacterial genera). Machine learning techniques focus on algorithms to identify relationships between variables rather than starting with a given model. In particular, we applied boosted regression trees to predict the sex of the inhabitants based on the bacterial community composition. Boosted regression

tree models were trained with 70% of the samples and the remaining 30% were used to assess the predictive performance [27]. We did not have equal sample sizes between male and female rooms (the dormitory was majority male) but maintained equal ratios between sample groups, meaning that 70% of male samples and 70% of females samples were randomly chosen for model training, and the rest were used for prediction. Briefly, boosted regression trees combine the strengths of two algorithms: regression trees (models that relate a response to their predictors by recursive binary splits) and boosting (an adaptive method for combining many simple models to give improved predictive performance). One large drawback of single tree models is poor predictive performance. Boosted regression trees overcome this drawback by fitting multiple trees in a forward, stagewise fashion, leading to superior predictive performance that can give powerful ecological insight. More information on the use of boosted regression trees in an ecological context can be found in Elith et al. [27]. To identify OTUs associated with female versus male rooms, we used indicator value analyses [24], as implemented in Barberán et al. [10], to identify those taxa indicative of different sample categories from information on taxa abundances and frequencies of occurrence.

DNA extracted from the passive samplers was used for qPCR analyses following the method described in Emerson et al. [28] so we could also assess variation in the amounts of bacteria and fungi recovered in the air of the sampled dormitory rooms. For these analyses, we only focused on the passive samplers as it would have been difficult to extract DNA from the HVAC filters in a manner suitable for quantitative analyses of fungal and bacterial loads. Results for total bacterial and fungal abundances are reported in *E. coli* or *Aspergillus fumigatus* genome equivalents, respectively, but results should be interpreted as genome equivalents of bacterial or fungal cells per passive sampler.

3.1.5 Results and Discussion

The most abundant bacterial and fungal taxa observed in our samples generally matched those identified from previously published studies of the indoor microbiome [4, 2, 9]. Both passive and filter sample types were dominated by the same suite of bacterial taxa including *Streptococcus*, *Micrococcus*, *Corynebacterium*, *Lactobacillus*, *Haemophilus*, *Finnegoldia*, Staphylococcaceae, and

Oxalobacteraceae. Many of these taxa are likely associated with human skin (e.g. *Staphylococcus*, *Corynebacterium*, *Streptococcus* [41]) and the vaginal microbiota (e.g. *Lactobacillus* [103]). The most abundant fungal taxa in both sample types was *Davidiella*, a teleomorph of *Cladosporium* [45], a fungus commonly reported in house dust samples and indoor air [4, 9, 95]. Other abundant fungal taxa included common household molds such as *Aureobasidium*, *Penicillium*, *Cryptococcus*, and *Alternaria* [13, 4, 100] as well as the gastronomically relevant fungi *Pleurotus* (genus of edible mushrooms).

None of the measured or recorded room characteristics were significant predictors of the types of fungi found in either the passive or filter samples. Previous work has shown that outdoor air fungi typically dominate the fungi found indoors [4, 9] so it is not surprising that there would be minimal variation in the fungal communities identified from samples within the same building. Likewise, the amounts of fungi found in the collected dust samples, as determined via qPCR, were not significantly correlated with any of the measured variables (Kruskal-Wallis tests, $p > 0.1$). Although fungi were found to be, on average, 27% more abundant in rooms occupied by men than in rooms occupied by women, this difference was not statistically significant (Kruskal-Wallis test, $p = 0.12$) (Figure 3.1).

In contrast to the fungi, we found notable, and predictable, differences in the types of bacteria found across the sampled rooms. Sex of the room occupants was the best predictor of bacterial community composition for both sample types (PerMANOVA, passive: $p = 0.001$, $R^2 = 0.039$, filter: $p = 0.001$, $R^2 = 0.021$). The number of occupants (ranging from 1 to 2 individuals per room) was also found to be a significant predictor of the types of bacterial communities found in both sample types (PerMANOVA, passive: $p = 0.029$, $R^2 = 0.032$, filter: $p = 0.013$, $R^2 = 0.014$) with the location of the room (categorized by wing) significantly correlated with bacterial community composition across the collected filter samples (PerMANOVA, $p = 0.017$, $R^2 = 0.026$). Each wing had a separate HVAC system supplying outdoor air, which may have contributed to differences in community composition. However, we note that the wing and number of occupants was not correlated with the sex of the room occupants (Fisher test, $p > 0.05$). In other words, the

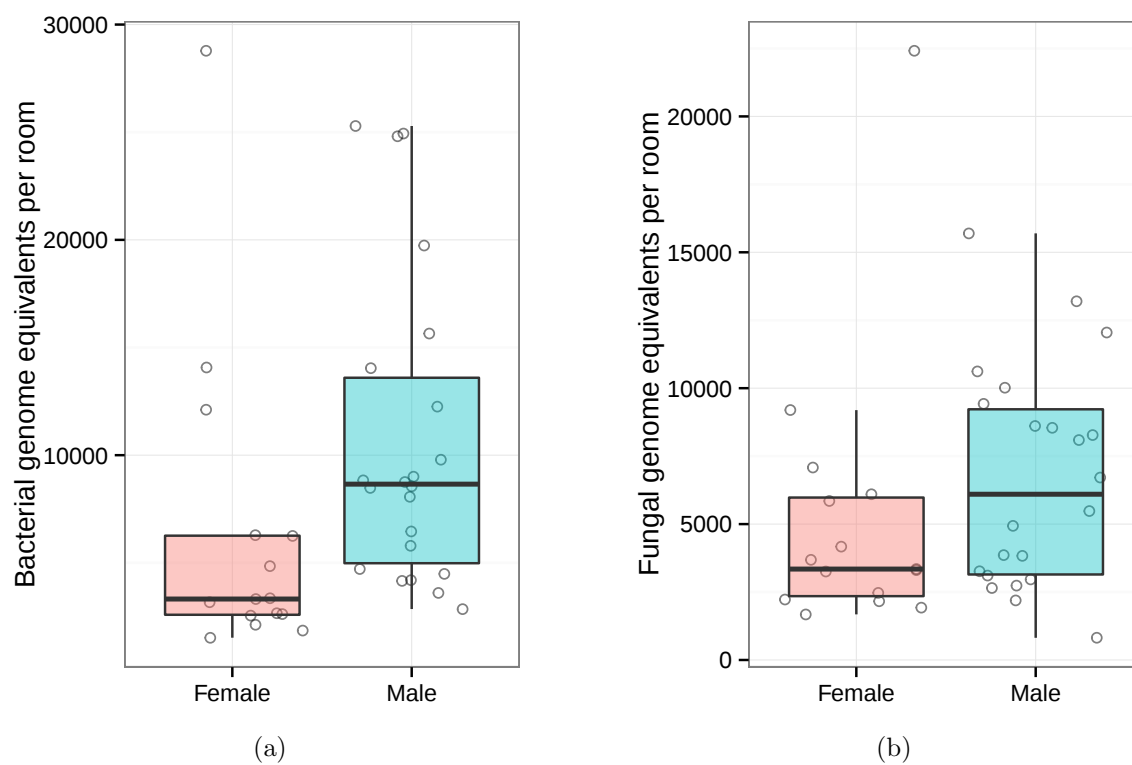


Figure 3.1: Results from quantitative PCR analyses of airborne dust samples showing differences in amounts of bacteria (a) and fungi (b) collected onto settling plates over 2.5 months of sampling.

effect of the sex of the occupants on bacterial community composition is unlikely to be driven by differences in the number of room occupants or differences in room location within the dormitory.

The qPCR-based estimates of bacterial abundances in the collected passive samples were not significantly correlated with any of the measured factors, except for the sex of the room occupants. In particular, we found that bacteria were, in general, 67% more abundant in male versus female-occupied rooms (Kruskal-Wallis test, $p = 0.006$) (Figure 3.1). Although the reasons for these differences remain uncertain, our guess is that these differences are driven by differences in the rates at which skin-associated bacteria are resuspended into room air given the importance of skin as a source of bacteria in the collected samples (see below). Males may simply shed more skin bacteria into their surrounding environments than females, a hypothesis that has some support in the literature [19, 94], though additional research is warranted to determine if this is a valid explanation for the higher abundances of bacteria observed in male-occupied rooms. . Estimated skin surface area based on occupant height and weight was not a significant predictor of bacterial abundances, eliminating that parameter as a confounder for sex differences in bacterial shedding. Other factors, however, such as the use of skin lotion [43] or clothing type [21], may contribute to differences in bacterial dispersal.

Given the significant effect that the sex of occupants had on bacterial community composition in both the passive and filter samples, we set out to determine how accurately we could predict the sex of occupants from information on bacterial community composition alone. To do this, we used machine learning models to assess the probability of whether a sample originated from a female-inhabited or male-inhabited room by examining the relative abundance of bacterial genera. Given the smaller number of passive samples (38 rooms sampled), this analysis was only conducted on filter samples (91 rooms sampled). We found that we could predict, with 79% accuracy, the sex of the room occupants from the relative abundances of different bacterial genera. The bacterial genera that had the highest contribution in the model, i.e. those that were most differentially abundant between male and female-occupied rooms, are shown in descending order of model contribution in Figure 3.2. Note that the genus *Lactobacillus* had the largest contribution to the model and the

relative abundance of this genus was the most useful for discriminating between male and female-occupied rooms. Members of this genus can be found in a wide range of habitats and are often abundant in environments as varied as human skin [32], the gut [77], the vagina [103], or in dairy products [11].

Although the genus level assignments proved useful for identifying male versus female-occupied rooms, we looked at the data at a finer level of taxonomic resolution to identify which specific OTUs were differentially abundant between the male and female-occupied rooms. Indicator value analyses revealed specific OTUs that were indicators of female versus male-occupied rooms. Table 3.1 displays the top five indicator OTUs for each sampling and occupant type. While those individual OTUs identified as being differentially abundant between male and female-occupied rooms varied depending on whether we examined the results from the passive or filter samples, a number of these taxa overlapped between the two sample types. Specifically, *Lactobacillus iners* was consistently more abundant in female occupied rooms while *Dermabacter hominis*, *Facklamia*, and *Corynebacterium* were consistently more abundant in male-occupied rooms, regardless of the sample type (Figure 3.3). *Lactobacillus iners* is a relatively abundant member of the vaginal microbiome, being detected in 83.5% of subjects in a recent cross-sectional study of 396 healthy asymptomatic women and dominating 34.1% of the communities analyzed [103]. Although the source of *Dermabacter hominis* in these rooms remains difficult to determine, members of this species are commonly found in semen [118]. Three male-associated OTUs classified as *Corynebacterium* are common skin inhabitants [41] and members of this genus have previously been shown to be significantly more abundant on male than on female hand surfaces [32].

OTU number	Taxonomic identity	Indicator value	Occupant sex	Sample type
OTU 14	<i>Lactobacillus iners</i>	0.73	Female	Passive
OTU 317	<i>Dialister microaerophilus</i>	0.63	Female	Passive
OTU 179	<i>Prevotella</i> sp.	0.53	Female	Passive
OTU 353	<i>Sphingobacterium multivorum</i>	0.45	Female	Passive
OTU 6078	<i>Mycoplana</i> sp.	0.45	Female	Passive
OTU 11	<i>Lactobacillus crispatus</i>	0.83	Female	Filter

OTU 14	<i>Lactobacillus iners</i>	0.78	Female	Filter
OTU 5851	<i>Corynebacterium</i> sp.	0.70	Female	Filter
OTU 65	<i>Anaerococcus</i> sp.	0.67	Female	Filter
OTU 78	<i>Dialister</i> sp.	0.65	Female	Filter
OTU 234	<i>Dermabacter hominis</i>	0.91	Male	Passive
OTU 6181	<i>Facklamia</i> sp.	0.86	Male	Passive
OTU 8580	<i>Corynebacterium</i> sp.	0.78	Male	Passive
OTU 35	<i>Corynebacterium</i> sp.	0.75	Male	Passive
OTU 28	<i>Corynebacterium aurimucosum</i>	0.68	Male	Passive
OTU 170	<i>Corynebacterium</i> sp.	0.81	Male	Filter
OTU 8580	<i>Corynebacterium</i> sp.	0.77	Male	Filter
OTU 6181	<i>Facklamia</i> sp.	0.72	Male	Filter
OTU 234	<i>Dermabacter hominis</i>	0.70	Male	Filter
OTU 28	<i>Corynebacterium aurimucosum</i>	0.68	Male	Filter

Table 3.1: Top OTUs that most effectively distinguished between male and female-occupied rooms, as determined by the indicator value analyses. OTUs with high indicator values indicate that they were more common and more abundant in rooms occupied by either men or women. For details on the proportional abundances of these OTUs in male versus female-occupied rooms, see Figure 3.3.

To quantitatively compare the potential sources of bacteria in male versus female-occupied rooms, we compiled a list from the literature of bacterial taxa indicative of specific source habitats (Table 3.2). This list includes many of the taxa we identified as indicators of male and female environments. We found that male rooms had significantly higher proportions of skin-associated bacteria and female rooms had significantly higher proportions of vagina-associated bacteria (Kruskal-Wallis test, $p < 0.01$) (Figure 3.4). Male rooms yielded significantly higher proportions of stool-associated bacteria only in filter samples (Kruskal-Wallis test, $p < 0.01$).

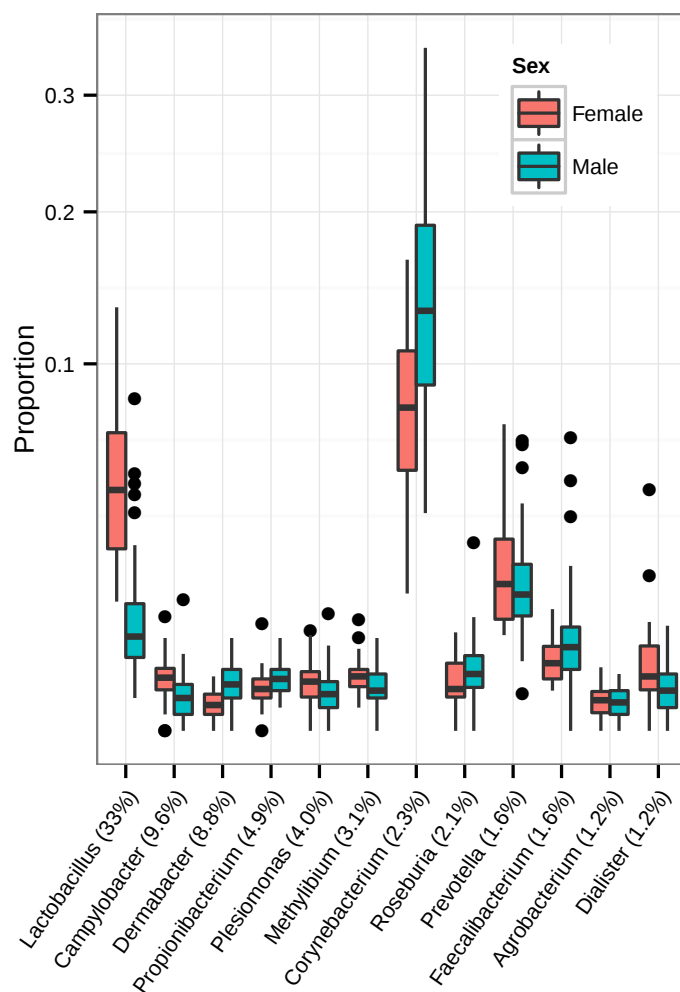


Figure 3.2: Proportional abundances of bacterial genera from the HVAC filter samples listed in descending order of their contribution to the boosted regression tree model (model contributions are listed in the x-axis labels).

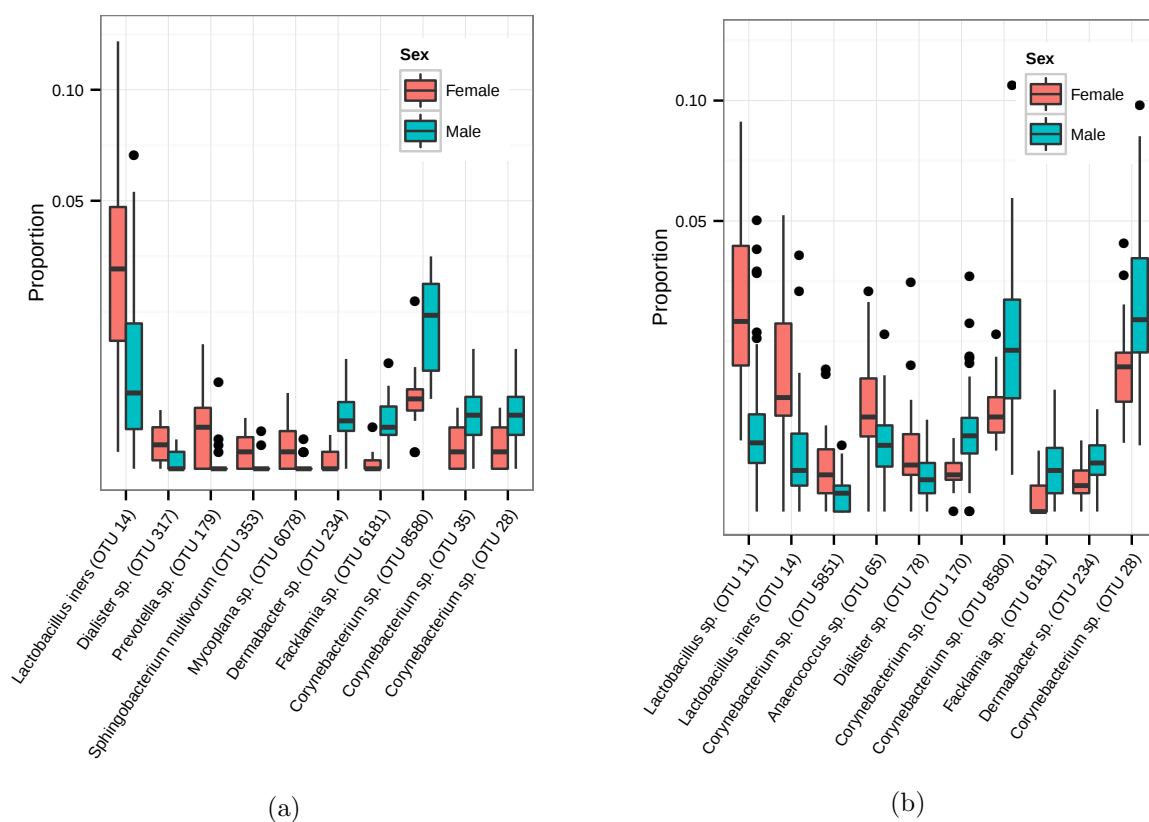


Figure 3.3: Proportional abundances of top OTUs identified using indicator value analysis (listed in Table 1) comparing female and male rooms from passive samplers (a) and HVAC filters (b).

Source environment	Indicator taxa	References
Plants	Chloroplast	[10]
Insects	<i>Wolbachia</i> , <i>Buchnera</i> , <i>Rickettsiella</i> , <i>Cardinium</i> , <i>Rickettsia</i> , <i>Rhabdochlamydia</i> , <i>Fritschea</i> , <i>Regiella</i> , <i>Hamiltonella</i> , <i>Blochmannia</i> , <i>Portiera</i> , <i>Tremblaya</i> , Endomicrobia, Entomoplasmatales, Bartonellaceae, <i>Blattabacterium</i> , <i>Sulcia</i> , <i>Thorsellia</i> , <i>Baumannia</i>	[10]
Skin	<i>Propionibacterium</i> , <i>Staphylococcus</i> , <i>Corynebacterium</i> , <i>Streptococcus</i> , <i>Rothia</i> , <i>Micrococcus</i> , <i>Anaerococcus</i> , <i>Brevibacterium</i>	[10]
Soil	Solibacteraceae, Chloracidobacteria, Koribacteraceae, Acidobacteria-iii1.15, Acidobacteriaceae, <i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Mesorhizobium</i> , <i>Rhodoplanes</i> , <i>Chitinophaga</i> , <i>Solirubrobacter</i> , <i>Opitutus</i>	[10]
Stool	<i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Lachnospira</i> , <i>Oscillospira</i> , <i>Roseburia</i> , <i>Coproccoccus</i> , <i>Ruminococcus</i> , <i>Parabacteroides</i> , <i>Phascolarctobacterium</i> , <i>Sutterella</i> , <i>Blautia</i>	[10]
Vagina	<i>Lactobacillus iners</i> , <i>L. crispatus</i> , <i>L. gasseri</i> , <i>L. jensenii</i> , <i>L. johnsonii</i> , <i>Prevotella bivia</i> , <i>Atopobium vaginae</i> , <i>Gardnerella vaginalis</i> , <i>Peptostreptococcus anaerobius</i>	[103, 73]

Table 3.2: Bacterial taxa, or chloroplasts, were used as indicators of the potential source environments to identify the relative importance of each source environment in each of the collected samples. Details on this approach and how these taxa were selected are provided in [10]. For the determination of those bacteria taxa indicative of the vaginal microbiome, we used data from [103, 73] to identify taxa abundant in vaginal samples, but uncommon in other source environment types.

There has recently been a call for more standardized sample collection protocols in order to improve meta-analysis of microbiota in the built environment [2]. Passive airborne dust collectors are frequently used as a way to monitor bioaerosol exposures [5] given their ease of deployment and low cost. Preliminary evidence suggests that HVAC filters may represent another, relatively low-cost, option for longer-term investigations of airborne microbial communities [95, 28]. With this dataset, we could assess if the two sampling strategies (passive versus HVAC filter samples) yielded similar assessments of room microbiomes given that we had 26 rooms that were sampled using both strategies. In general, the two sampling strategies yielded qualitatively similar results

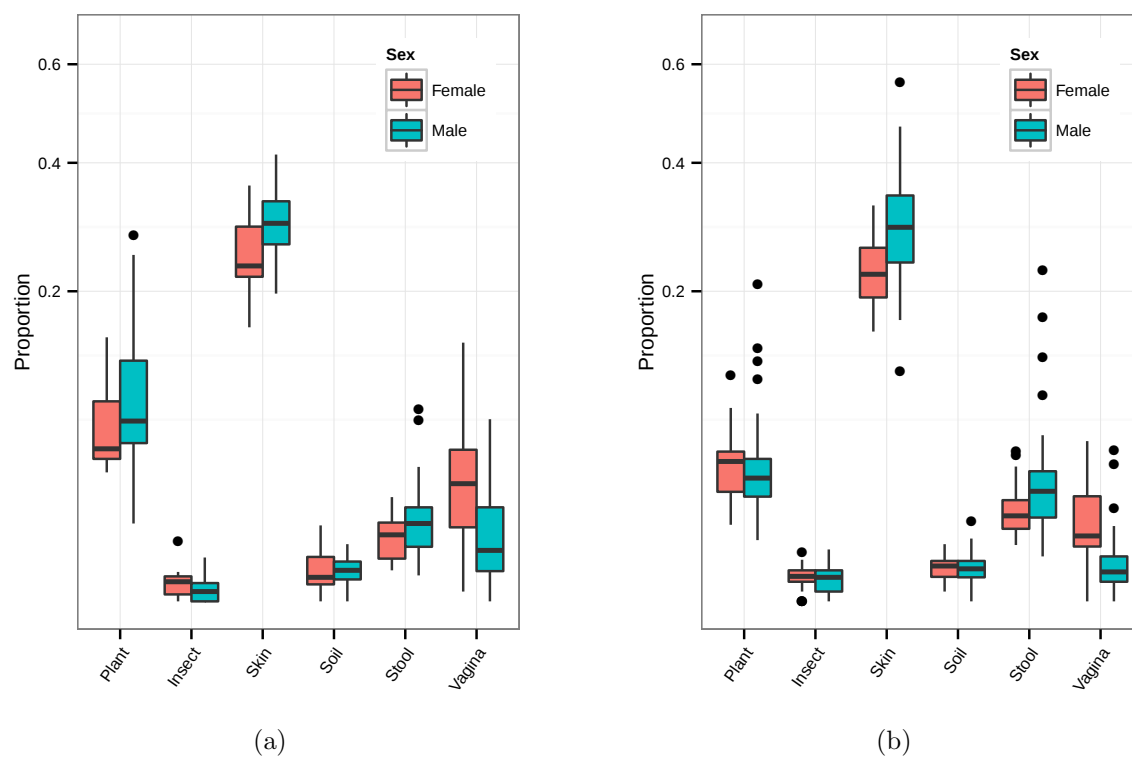


Figure 3.4: Differences in the relative abundances of bacterial taxa indicative of potential source environments (listed in Table 3.2) between female and male rooms. Results are based on analyses of either the passive samplers (a) or the HVAC filters (b).

as the community similarity patterns between the two sampling strategies were correlated (Mantel test; $r_M = 0.43$; $p < 0.01$). The most common bacterial and fungal OTUs were similar across the two sample types in the 26 overlapping rooms (Figure 3.5) and various OTUs found in the two sample types overlapped as bacterial indicators of the sex of occupants (see above), reinforcing the idea that the microbial communities sampled from HVAC filters are similar to longer-term indoor air samples [95]. Interestingly, a few of the more common OTUs had significantly different relative proportions between the two sample types. *Streptococcus*, *Lactobacillus*, and *Haemophilus* were relatively more abundant in passive samplers while *Micrococcus*, *Corynebacterium*, and *Staphylococcus* were relatively more abundant in HVAC filters (Kruskal-Wallis test, $p < 0.05$). This subtle pattern could be driven by differences in sampling time or by differences between the sampling strategies in the size distributions of the collected particles. It is also possible that sampling from HVAC filters may have introduced some mechanical microbial lysis, which has the potential to selectively change community composition, as discussed in [28]. Together these results highlight that both sampling strategies are useful and yield generally similar assessments of the indoor microbiome, but caution must be considered when quantitatively comparing results that were obtained using different sampling strategies, a point that has been made in a previous meta-analysis of indoor microbiomes [2].

This work not only confirms the importance of human occupants in shaping the bacterial communities found in indoor air, it also shows that even the sex of the occupants can alter those communities. *Lactobacillus* is the genus that most contributed to the differences between female-inhabited and male-inhabited environments. More generally, taxa often identified as members of the vaginal microbiota were more common in female-occupied rooms while taxa associated with human skin or the male urogenital microbiota were more common in male-occupied rooms. The results presented here have potential relevance to forensics as it shows that we can predict the sex of the occupants with fairly high accuracy. Just as other studies have shown that skin-associated bacterial communities are highly personalized and could be used for forensic identification of items touched by an individual [33], these results show that dust samples can also be identifying. In cases

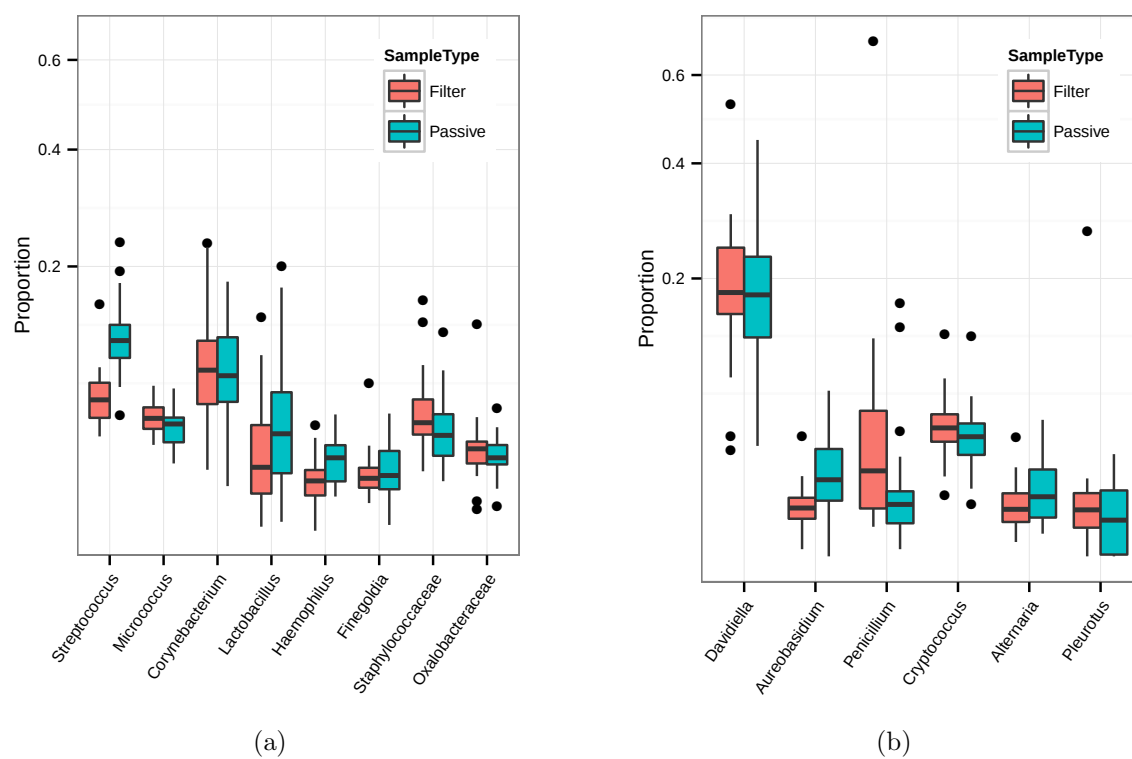


Figure 3.5: Proportional abundances of the most common bacterial (a) and fungal (b) taxa between the two sample collection strategies.

where human DNA cannot be obtained, the bacterial cells dispersed in the indoor environment may represent a novel forensic approach for identifying the sex of the occupants of a given room, but additional work is required to determine the potential applications of such an approach.

3.1.6 Acknowledgements

We gratefully acknowledge financial support for this project from Unilever Industries. We also want to thank the dormitory residents for participating in this study and Zoey Craun for her enormous help with recruitment and sample/data collection. Andrew Baugher, Prateek Shrestha, Mohamed Eltarkawe, Sarah Fosco, and Amanda Makowiecki helped with sampler deployment and collection.

3.2 HVAC system characterization measurements and documentation of occupant health symptoms

3.2.1 Abstract

With increasing interest in quantifying indoor exposures, the question remains how we can use building mechanical systems to control and reduce these exposures. Recent studies have increased our knowledge of indoor microbial exposures, but gaps still exist in quantifying health effects associated with HVAC or building parameters, specifically studies of epidemiologic design (see Chapter 4). One of the biggest challenges of conducting a well-designed health effect or epidemiologic study of the indoor environment is accurately and thoroughly characterizing the mechanical building characteristics that may be affecting occupant health. This work demonstrates various measurement methods for characterizing air change rates as well as a method for measuring pressure differences between zones to qualitatively assess airflow direction between zones. The standardized methods described here can inform future studies looking to measure ventilation or pressure differentials. We also describe the health data that was collected, but due to low statistical power from various limitations, were unable to see any associations in our analyses.

⁰ *This work is unpublished.*

3.2.2 Introduction

Our study took place in a dormitory on the CU-Boulder campus. All rooms housed either one or two individuals. The HVAC system was made up of two separate systems: a central air handler that supplied 100% outdoor air at a constant air volume (CAV) at 70°F to each room as well as a fan coil unit (FCU) in each room that provided heating and cooling by recirculating air from within the room through a heat exchanger. All windows were operable.

3.2.3 Measurement Methods

3.2.3.1 Ventilation Measurements

Several methods exist for measuring airflow rates in HVAC systems. These methods are often grouped into two categories: direct and indirect. Direct measurements use instruments that measure the airflow directly, such as an anemometer. Indirect techniques measure other parameters that are dependent on airflow, such as tracer gas decay or energy balances. The choice of which method to use depends on many factors such as the design of the HVAC system and how accessible it is, the objective of the measurements, the equipment available, etc.

Ventilation measurements were simplified due to 100% outdoor air delivery and a CAV delivery. We used one direct measurement to characterize the outdoor air delivery and two indirect measurement methods to estimate the air change rate (outdoor air ventilation plus infiltration from either outdoors or adjacent zones) in several rooms.

Direct Outdoor Air Delivery Measurement. Direct measurements at the outdoor air supply register were made with an airflow hood (TSI ALNOR Balometer Capture Hood) two times on different days to ensure airflows were constant across varying outdoor air conditions. Although airflows were constant, not all rooms matched the design airflow rate of 30 cfm of outdoor air delivery (regardless of room size).

Overnight Equilibrium Analysis. One of the indirect measurement methods used steady state overnight CO₂ concentrations to estimate the outdoor air supply to the room. This method has

been demonstrated previously in field [114] and laboratory studies [111]. This method assumes that CO₂ is being generated at a constant metabolic rate while occupants are sleeping, having the ability to reach a steady state/equilibrium concentration overnight. This approach is a special case of the constant-injection technique described in ASTM E741 [7]. We continuously monitored CO₂ concentrations for one week in a subset of rooms. We then found the average steady state CO₂ concentration in each room during the hours of 3:00am to 6:00am. An example of one week of CO₂ monitoring in a dorm room is shown in Figure 3.6. The flat portion of the peaks were used to estimate the overnight steady state concentration. A mass balance of CO₂, assuming air entering the space is at background/outdoor levels, allows us to estimate the outdoor air supply as follows [99]:

$$Q_o = \frac{10^6 \times G}{(C_{in,eq} - C_{out})} \quad (3.1)$$

where Q_o is the outdoor airflow rate into the space (cfm), G is the carbon dioxide generation rate in the space (cfm), $C_{in,eq}$ is the equilibrium carbon dioxide concentration in the space overnight (ppm), and C_{out} is the outdoor carbon dioxide concentration (ppm). Equation 3.1 can be used for any indoor steady state concentration but is not recommended when $C_{in,eq}$ is not much higher than outdoor level (less than 3-4 times the uncertainty of your instrument) because uncertainties in CO₂ generation rates and measurement can contribute to high uncertainties in outdoor air supply estimates.

Carbon dioxide generation rates were calculated based on the height, weight, and metabolic activity of the students sleeping in the space. First, the DuBois surface area was calculated for each occupant:

$$A_D = 0.660H^{0.725}W^{0.425} \quad (3.2)$$

where H is the body height in feet and W is the body weight in pounds. The CO₂ generation rate (or the rate of oxygen consumption) is then calculated with the following equation [99]:

$$G = \frac{0.000543A_DM}{(0.23RQ + 0.77)} \quad (3.3)$$

where RQ is the respiratory quotient and M is the level of physical activity, or the metabolic

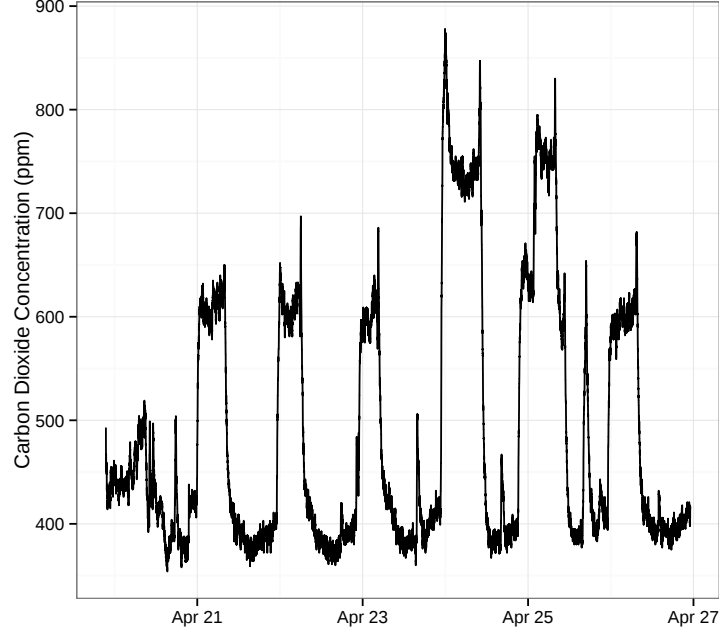


Figure 3.6: An example of one week of continuous CO_2 monitoring in a dormitory room. The flat peaks overnight were used for estimating outdoor air ventilation with an equilibrium analysis.

rate per unit of surface area. The value of RQ depends on diet, the level of physical activity, and physical condition of the person but we assumed $RQ=0.83$, the level for an average size adult doing sedentary activities. We assumed $M=0.7$ for a sleeping person [111].

The advantage to using an indirect measure of ventilation is that it accounts for both mechanical ventilation and infiltration as diluting the air in the room with air from both outside the building as well as from the hallway or adjacent rooms. In this study, the comparison between direct and indirect measurements of outdoor air dilution may help distinguish rooms that often open their windows versus rooms that do not. A list comparing results from the multiple ventilation measurement methods is shown in Table 3.3.

Tracer Gas Decay with Injected CO_2 . The second indirect method we used to measure air change rates was a tracer gas decay analysis of injected CO_2 . We followed procedures described in [99] and [7]. In summary, we introduced CO_2 into the room to the point of reaching a concentration of 5000 ppm. Mixing fans were used to ensure uniform distribution of the tracer gas. We then

allowed the concentration of CO₂ to decay, measuring the concentration continuously. We estimated the mean air change rate with the following equation [7]:

$$ACH = \frac{[\ln C(t_2) - \ln C(t_1)]}{(t_2 - t_1)} \quad (3.4)$$

where $C(t_2)$ is the mean CO₂ concentration at time t_2 (the end of the decay), $C(t_1)$ is the mean CO₂ concentration at time t_1 (the beginning of the decay), and $t_2 - t_1$ is the elapsed time of the decay. Given the volume of the room, the outdoor (or dilution) air delivery rate can be estimated. Results from tracer gas decay tests are included in Table 3.3. Note that all ventilation measurements reported can be standardized to be ventilation rate per person, per floor area, or per volume of the room for ease of comparison across studies.

	Direct Measure- ment	Indirect Measurements	
Room	Balometer (cfm)	Equilibrium Analysis (cfm)	Tracer Decay (ACH/cfm)
E123	25	23.3	0.54 ACH/13.7 cfm
E125	25	25.1	
E131	35	45.6	
E133	25	38.3	1.96 ACH/30.9 cfm
E141	37	58	
E142	60	118	
E143	34	41.6	1.17 ACH/30.8 cfm

Table 3.3: Results from multiple measurement methods of outdoor air dilution in dorm rooms.

3.2.3.2 Pressure Differences Between Zones

Considering that every room had a constant volume of air delivery, we assumed the pressure difference between each room and the hallway would remain constant at all times. Upon further investigation, we discovered that the operation of the FCU would generate a positive pressure in each room. Figure 3.7 shows 30 minutes of monitoring pressure differences between many rooms and the hallway on one floor. The hallway was treated as the reference pressure. There is a clear indication that rooms with the pressure differential stepped up have the FCU running and rooms

near neutral pressure do not.

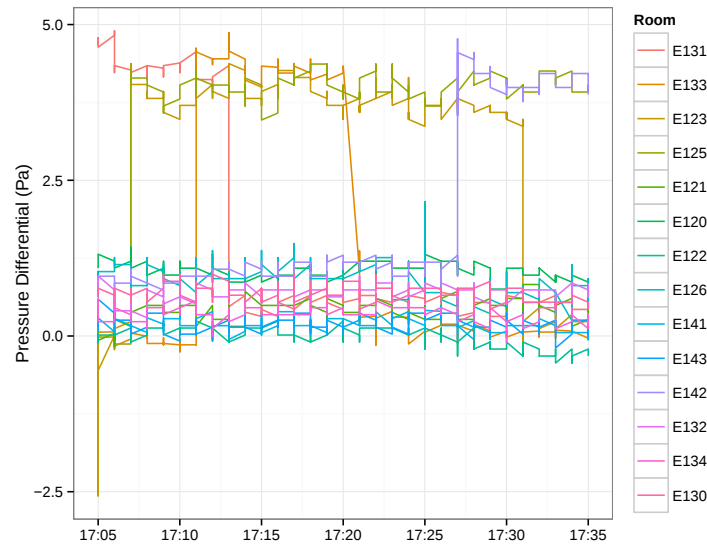


Figure 3.7: Monitoring of pressure differences over 30 minutes between rooms and the hallway (reference pressure). Rooms with stepped-up positive pressure have the fan coil operating and rooms near neutral pressure do not.

The building automation system (BAS) was able to provide logged data of FCU usage and window switch (“On” corresponding to a closed window), logging one sample per hour of whether the FCU was turned on and if the window was open during that hour. Using this data, we were able to calculate a proportion of fan coil usage and window opening frequency, which we used in a regression to test whether there was any association with illness incidence. We assumed that a higher proportion of FCU usage would decrease the risk of transmission since a positive pressure would not allow air to flow from the hallway into the room and a higher proportion of window switch being “On” would increase risk of illness incidence due to lower outdoor air dilution rates. Figure 3.8 shows examples of trended FCU fan status data for two rooms and Figure 3.9 shows examples of trended window switch data. Note that FCU usage and window switch are not dependent on each other.

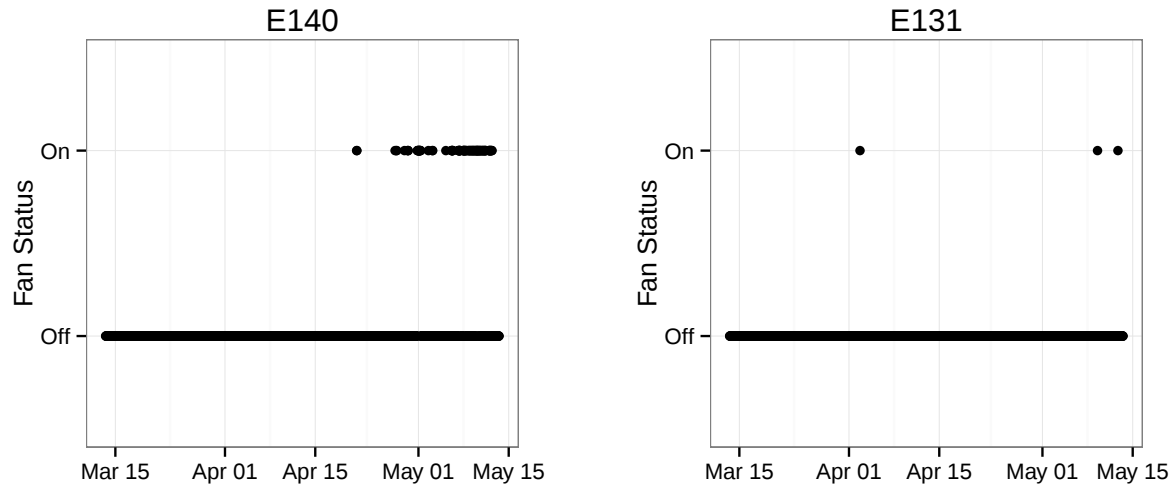


Figure 3.8: Trended FCU fan status data for two rooms.

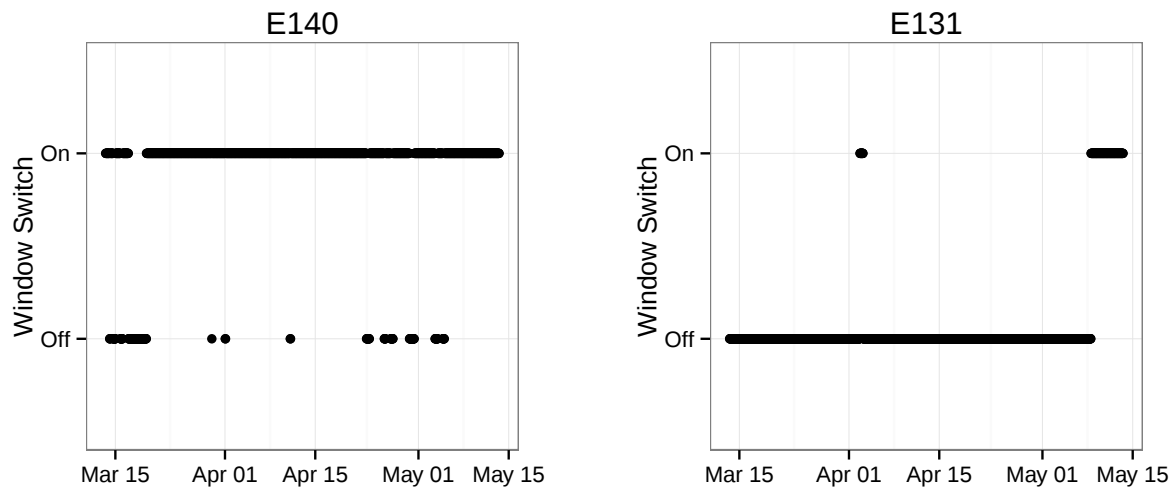


Figure 3.9: Trended window switch data for two rooms.

3.2.3.3 Health Data

We were able to recruit 48 student participants that lived in the dormitory of study. Participants were given an initial questionnaire at the beginning of the semester to gather basic information and habits. From then on, students were messaged every 2 weeks to inquire if they had been ill. Positive responses received a short questionnaire inquiring about symptoms, length of illness,

whether their roommate was ill, etc. The initial questionnaire was as follows:

- (1) How old are you?
- (2) Sex?
- (3) Height and weight?
- (4) Do you have or currently treat dandruff?
- (5) How many roommates do you have?
- (6) In general, how often do you or your roommate clean your room?
 - (a) Every day
 - (b) Once a week
 - (c) Once every 2 weeks
 - (d) Once a month
 - (e) Less than once a month
- (7) Have you ever been told by a doctor that you have any of the following?
 - (a) Asthma?
 - (b) Allergy to dust?
 - (c) Allergy to molds?
 - (d) Seasonal allergies?
- (8) On average, how many hours per day (out of 24) do you spend in your room, to the nearest hour?
- (9) On average, how many hours per day (out of 24) do you spend in your dorm building, to the nearest hour?
- (10) On average, how many hours per day (out of 24) do you open your window, to the nearest hour?
- (11) What is your tobacco smoking status?
 - (a) Never smoked
 - (b) Former smoker
 - (c) Current smoker
- (12) Are you currently sick?
- (13) Is your roommate sick?

The bi-weekly check-in message was as follows:

- (1) Since the previous check-in, on average, how many hours per day (out of 24) did you spend in your room, to the nearest hour?
- (2) Since the previous check-in, on average, how many hours per day (out of 24) did you spend in your dorm building, to the nearest hour?
- (3) Since the previous check-in, on average, how many hours per day (out of 24) did you have your window open, to the nearest hour?
- (4) Since the last questionnaire, how many hours of sleep a night are you averaging?
- (5) Which of the following symptoms have you experienced?
 - (a) Wheezing
 - (b) Sore or dry throat
 - (c) Unusual fatigue, tiredness, or drowsiness
 - (d) Chest tightness
 - (e) Stuffy or runny nose
 - (f) Sinus congestion
 - (g) Cough
 - (h) Sneezing
 - (i) Nausea or upset stomach
 - (j) Fever
- (6) Have you gone to see a healthcare professional for your symptoms?
- (7) How long did your symptoms last?
- (8) Since the last questionnaire, how many hours of sleep a night are you averaging?
- (9) Is your roommate sick?

3.2.4 Statistical Analyses

Data collected were analyzed to assess relationships between illness incidence and any of the following parameters: direct measurement of outdoor air supply with balometer, average overnight steady state CO₂ concentration, fan status proportion, window switch proportion, number of room occupants, self-reported hours in room and dorm, self-reported cleaning frequency, and self-reported hours of window opening. Data analyses was performed in R (<https://www.r-project.org/>). Negative binomial and Poisson regression models were used but no significant covariates were found.

3.2.5 Conclusions

Unfortunately our health study had multiple limitations that may have contributed to the lack of associations seen, the most important ones being the short duration of the study and the limited power. The insufficient statistical power resulted primarily due to difficulty recruiting participants, as well as data loss from lack of survey participation and difficulty retrieving trended data from the BAS. It is difficult to avoid limitations of this kind [80], but future health-related observational studies would improve with wider variance in covariates (such as ventilation rates or some exposure measure), longer study duration, larger sample size and participation/response rates, and a more objective outcome measure of illness than self-reported, perhaps employer or physician-provided illness or absence data.

Chapter 4

Role of Mechanical Ventilation in the Airborne Transmission of Infectious Agents in Buildings

Julia C. Luongo¹, Kevin P. Fennelly², Julia A. Keen³, Zhiqiang (John) Zhai⁴, Byron W. Jones⁵, Shelly L. Miller^{1,*}

¹Department of Mechanical Engineering, University of Colorado, Boulder, CO, USA

²College of Medicine, and Emerging Pathogens Institute, Division of Infectious Diseases and Global Medicine, University of Florida, Gainesville, FL, USA

³Department of Architectural Engineering and Construction Science, Kansas State University, Manhattan, KS, USA

⁴Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, CO, USA

⁵Department of Mechanical and Nuclear Engineering, Kansas State University, Manhattan, KS, USA

* *Corresponding author: [shelly.miller\[at\]colorado.edu](mailto:shelly.miller[at]colorado.edu)*

⁰ This chapter is modified from a publication in the following journal [71]:
Luongo, J. C., Fennelly, K. P., Keen, J. A., Zhai, Z. J., Jones, B. W., & Miller, S. L. (2015). Role of mechanical ventilation in the airborne transmission of infectious agents in buildings. *Indoor Air*. doi:10.1111/ina.12267

4.1 Abstract

Infectious disease outbreaks and epidemics such as those due to SARS, influenza, measles, tuberculosis, and Middle East Respiratory Syndrome-coronavirus have raised concern about the airborne transmission of pathogens in indoor environments. Significant gaps in knowledge still exist regarding the role of mechanical ventilation in airborne pathogen transmission (APT). This review, prepared by a multidisciplinary group of researchers, focuses on summarizing the strengths and limitations of epidemiologic studies that specifically addressed the association of at least one heating, ventilating and/or air-conditioning (HVAC) system-related parameter with airborne disease transmission in buildings. The purpose of this literature review was to assess the quality and quantity of available data and to identify research needs. This review suggests that there is a need for well designed observational and intervention studies in buildings with better HVAC system characterization and measurements of both airborne exposures and disease outcomes. Studies should also be designed so that they may be used in future quantitative meta-analyses.

4.1.1 Practical Implications

Studies to date show an association between increased infectious illness and decreased ventilation rate, however, there are insufficient data to quantify how mechanical ventilation may affect the airborne transmission of infectious agents. Our review reveals a strong need for more epidemiologic studies and meta-analyses. Specifically, we call for well designed prospective observational or intervention studies in buildings to establish causal relationships between airborne exposures and outcomes and between HVAC system factors and exposures. Future studies will benefit greatly from improved experimental design, standardized measurement methods, and better collaboration between epidemiologists and HVAC engineers.

4.2 Introduction

Infectious diseases cause millions of deaths annually around the world. Morbidity from infectious diseases keeps children and adults home sick, away from work and school, and costs billions of dollars each year in lost productivity, treatments and hospitalization [86, 97]. The annual cost of respiratory infections in the US was approximately \$64 billion (productivity losses plus health care costs) and changing building-related factors would save 20-30% of the cost, or \$6-\$19 billion [36]. The estimated mean total productivity losses from absenteeism and presenteeism (lost productivity while at work) was \$181 per employee per year for respiratory infections [65]. Efforts to prevent transmission of infectious diseases are expected to help further social and economic development around the world.

Infectious diseases are caused by a variety of pathogens. Person-to-person transmission of pathogens occurs via direct contact, indirect contact via fomites, impact of projectile large droplets (droplet transmission), and aerosolized fine particles (airborne transmission). The World Health Organization (WHO) and Center for Disease Control (CDC) define droplets as being <5 microns and airborne pathogen transmission (APT) to occur from desiccated droplets (droplet nuclei) <5 microns in size but there is still discussion about the size criterion of a droplet [40, 93]. A fomite is an object or a material that can carry pathogens, transferring them from one person to another, for example a doorknob. Infections are a result of the interaction between susceptibility factors in the exposed host, the concentration and virulence of the pathogen in the environment, and the extent and nature of the exposure.

Although individuals may change their behaviors to reduce or eliminate exposure to pathogens, e.g., by washing hands more frequently or using hand sanitizers, avoiding airborne infections can be more difficult. The relative importance of the modes of transmission for many diseases, including influenza, remains unclear. It is likely that influenza and other respiratory pathogens are transmitted by multiple modes of transmission, i.e., contact of hands or body and both large droplets and fine aerosols. Features of the infectious agent, host, or the environment may affect mode of

transmission [110].

A better understanding of how building characteristics affect different modes of transmission can help develop optimal intervention strategies. While the majority of studies reviewed here characterize the HVAC system using measurements of ventilation rates or CO_2 , other building factors that may be of importance include recirculation rates, temperature and humidity, occupant density, proximity and movement patterns, presence of control technologies like filtration and ultraviolet (UV) germicidal lamps, and static pressure differentials between zones driving transport.

In many studies reviewed here, terminology introduced confusion. For example, some studies report air change rates that may be interpreted as either outdoor air change rate or supply air change rate. There is similar confusion with the term ventilation as to whether it refers to outdoor or supply air. It is advised to refer to these terms with the descriptor of either “outdoor” (OA) or “supply” (SA) for clarity. An excellent review of nomenclature and best practices in field measurements of ventilation rates can be found in Persily [98].

For this review, we focused on diseases transmitted by either aerosolized fine particles and/or impact of projectile droplets containing pathogens. The impact of projectiles is important when a susceptible person is in close proximity to the infectious person. Measuring airborne pathogens documents that an airborne exposure may have occurred. Assessing disease outcomes documents that transmission occurred (possibly through numerous routes). In this paper we refer to APT as a measure of exposure. However, in most of the epidemiological studies we reviewed, they document disease outcome, which implies pathogen transmission occurred. We did not encounter any study that reported a measurement of both of these parameters. We acknowledge that documentation of airborne exposure and disease outcome does not quantify the contribution of specific modes of transmission, but given the difficulty of measuring these contributions, data on airborne exposure, outcomes, and how HVAC system characteristics affect exposure can provide meaningful insights about risk of airborne transmission, associations with outcomes, and potential control strategies.

Several literature reviews have been published on the topic of ventilation rates and health [69, 78, 107]. The most recent was Sundell et al. [115], which summarized published articles

through the end of 2005 investigating associations of ventilation rates in buildings with human health responses. Our review is more specifically focused on the transmission of infectious aerosol in high occupancy density settings and the role of mechanical ventilation systems.

This review was prepared by a multidisciplinary group of researchers to better understand how epidemiologic studies of airborne diseases can inform future studies of mechanical ventilation and APT. We focused our review on epidemiologic studies investigating the association of at least one HVAC-related parameter with an infectious disease-related outcome in buildings (almost all studies reported ventilation rates or CO₂). This review emphasized studies after 2000 with a brief summary of studies prior to 2000. The majority of papers prior to 2000 used categorical characterizations of building factors while almost all studies after 2000 made measurements. This review introduces engineers and building scientists to topics in epidemiology as they relate to APT indoors, summarizes available data, and discusses research needs. Emphasis is placed on reviewing experimental designs to inform best practices in future collaborative studies among building engineers and epidemiologists.

4.3 Methods

Literature databases such as Science Direct, Web of Knowledge, MEDLINE/PubMed, Engineering Village, and Google Scholar were searched for any studies pertaining to the topic of APT where HVAC system attributes were measured. Searches were made using various combinations of the following keywords: Ventilation, HVAC, disease transmission, infectious disease, APT, building, infectious aerosol, epidemiology, illness incidence, and measurement. Citation statistics were only conducted on studies related to APT, infectious disease outcomes, and HVAC systems (modelling studies are not included). The number of publications related to this topic increased from six in 2003 to 25 in 2013, and totalled 172, which demonstrates that there is a growing interest and increasing number of publications on this topic in recent years. All of these papers were initially reviewed and papers that specifically used an epidemiologic study design and that described or measured some HVAC parameter within the context of the hypothesized associations were included. A total of

13 papers that had measurements or descriptors of the HVAC system(s) and an infectious disease health outcome were reviewed in detail and are discussed here. Summaries of the building-related epidemiologic studies described in this review are listed in Table 4.1.

4.4 Epidemiologic Studies

Epidemiology is “the study of the occurrence and distribution of health-related states or events in specified populations, including the study of the determinants influencing such states, and the application of this knowledge to control the health problems [101].” Epidemiology is a cornerstone of public health, informing evidenced-based practice by identifying risk factors associated with disease. Epidemiologic studies generally report observations or evaluate the effects of interventions. A graphic of various types of epidemiologic study designs is shown in Figure 4.1.

Observational studies evaluate changes in outcomes over a range of study factors and can take many forms. There is no artificial manipulation in observational studies, and they are carried out in natural settings. Observational studies can be either prospective or retrospective and generally take three forms: cohort, cross-sectional, or case-control. Most studies of mechanical ventilation and APT are of the prospective cohort design. They start with information about the study factor such as building type and then gather information prospectively about disease incidence as the study progresses. A cross-sectional study is a descriptive survey or prevalence study in a defined population and involves random sampling. They aim to gather data on the entire population under study. Some HVAC-related APT studies have also used this study design. Case-control studies are retrospective, comparing cases of disease with one or more groups of non-cases with respect to a current or previous study factor level. This is a good study design for a disease outbreak. Only one study we reviewed used the case-control design.

Intervention studies involve the prospective introduction of a factor over a range of values or against a placebo or sham negative control, and changes in the health outcome are then evaluated. Two of the studies we reviewed used an intervention study design.

Generally, observational studies are less expensive and logistically easier to conduct than

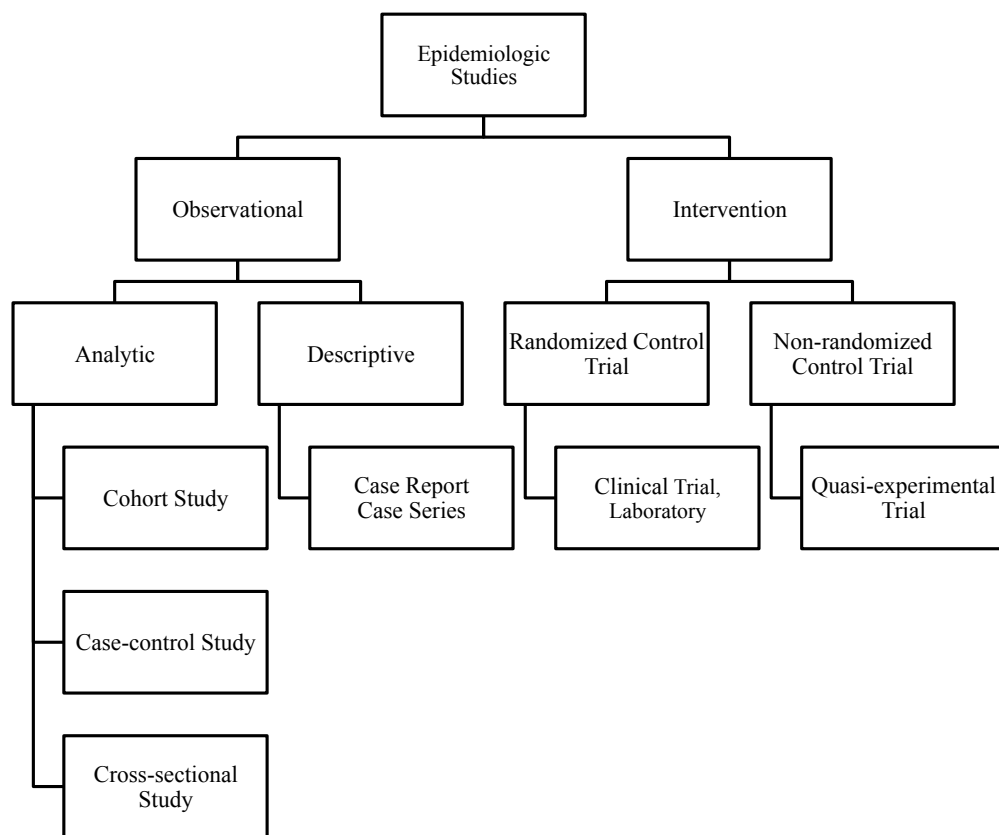


Figure 4.1: Types of epidemiologic studies (adapted from [63]).

intervention studies, but intervention studies can usually provide more robust data if well designed.

The success of epidemiologic studies is dependent on there being sufficient variability in the study factors and the health outcomes of interest to detect an association. Note that an association does not prove causality. Minimal conditions necessary to provide adequate evidence of a causal relationship between variable and outcome are known as *Hill's criteria for causation* [46]. Some HVAC factors are more amenable to observational studies than others, depending on whether or not there is sufficient variability in the HVAC variables of interest and if there are sufficient data. Other HVAC factors may be better suited to intervention studies. The health outcome of interest necessarily would inform what HVAC factor would be best to measure.

We have noted in our review whether the epidemiological studies are population-based or based on disease outbreaks. In *population-based* studies a large number of environments or occupants may be investigated looking for an association between a specific health endpoint and an HVAC system factor. In *disease outbreak* studies, a disease outbreak has occurred in a specific environment and investigators want to know if an HVAC system factor played a role.

It should be evident that the airborne transmission of infectious disease occurs within a complex matrix of diseases, mechanisms, and paths, some of which the HVAC system can impact and many of which it cannot. In attempting to assess the impact of HVAC systems on APT, there are large challenges in identifying and quantifying those factors that can impact APT and in measuring an impact given all of the other transmission pathways that occur simultaneously. A limited number of epidemiologic studies that have attempted to specifically address the role of one or more HVAC-related factors on APT are identified in this review. In the following discussion, the strengths and weaknesses of each study are addressed. Given the complexities of APT and the wide variety of HVAC systems, it should be understood that no one study will answer the general question as to the role of HVAC systems in APT. At best, a given study will be able to address one or very few HVAC factors and one or very few diseases. This review is not intended to criticize the studies that have been conducted but rather is intended to evaluate them in a way that makes it possible to see what was learned from them about how to assess the impact of mechanical

ventilation on APT in future studies. It should be understood that it is one thing to identify desired features and methodologies; however, resource constraints are almost universally a limiting factor in epidemiologic studies.

4.5 Building Studies before 2000

Studies prior to 2000 used descriptive approaches to assessing ventilation rates (Table 4.1). An exception was the investigation of a respiratory infection outbreak in an overcrowded Houston jail by Hoge et al. [47]. The case-control and cohort studies began after two inmates died on the same day of pneumococcal sepsis. The first 25 case patients hospitalized were enrolled in a case-control study. For each case, three inmates living in the same cellblock were randomly selected as controls. The jail had a constant volume, recirculated-air HVAC system operating at maximum capacity; 20% of the total volume of air delivered was outdoor air and 80% was recirculated. Each cellblock had its own recirculation system. Outdoor air ventilation was evaluated by measuring CO₂ levels and airflow rates to the living areas of the jail. Carbon dioxide levels were measured using detector tubes (this method usually results in high potential errors and is not recommended [107]). Airflow was measured in the center of duct using a thermal anemometer. There is no mention of a traverse of the duct to show that the velocity at the center was a valid estimate of average velocity (recommended). Volume of outside air delivered per person was calculated but there is no mention if it came from CO₂ levels or from an assumed percentage of outside air from total supply air. The respiratory infection incidence rate was highest among inmates in cellblocks with the highest CO₂ levels and the lowest volume of outside air delivered by the HVAC system. This study showed the impact of occupancy and OA ventilation rate per person on disease outcome and investigators concluded that over-crowding and inadequate ventilation contributed to the outbreak. It was an advance forward compared to other studies during this period because it used quantitative measures collected close to the time of investigation.

Among military barracks, two studies report that tighter buildings with HVAC systems with recirculation showed a higher rate of upper respiratory symptoms or infection [14]. Richards et al.

(1993) report on a cross-sectional study conducted with questionnaires. This study assessed the health impact of exposure to sand storms and crowded barracks in Saudi Arabia. The accommodations in which the soldiers slept were categorized as tent, warehouse, non-air conditioned (AC) and AC buildings. There was a trend of increasing risk for complaints of a sore throat and cough by troops who were less exposed to the outdoor environment, with troops sleeping in AC buildings having the highest risk of developing these symptoms. It is difficult to draw conclusions about what factor related to AC buildings contributed to higher risk of symptoms without a more quantitative comparison of the differences between building categorizations.

Brundage [14] was a prospective cohort study on a population of newly trained soldiers in the army. This study's strengths were that it was conducted over four years, had a very large sample size, the population was diverse geographically and demographically, was randomly assigned to building type, and it captured variability in illness years, meaning the time frame encompassed two epidemics as well as years of low illness rates. Incidence rates of febrile acute respiratory disease were significantly higher among trainees in modern barracks that had lower design outdoor air change rates due to energy conservation measures. An important strength of this study was the standardized diagnosis and treatment of disease across locations and conditions of the study. Quantitative measurements comparing HVAC characteristics of modern, more energy efficient buildings versus older, less energy efficient buildings as well as better descriptions of the HVAC systems themselves would have enhanced our understanding of the differences in building type ventilation performance.

Two studies by Drinka et al. [23, 22] were designed to determine whether a newly constructed nursing home had a lower incidence of influenza compared to the older building designs. The 1996 study was a surveillance study with retrospective analysis during the 1993/94 influenza season. A limitation of this study was that data was only collected over the course of one year. They reported that the newer building with a 100% outdoor air HVAC system and filtered room supply had fewer influenza cases. The other buildings recirculated between 30-70% of the air and did not have filtration. Buildings were descriptively characterized, and no measurements of any HVAC

parameters were conducted. Filter efficiency was not reported; they were described as 8-micron fiberglass filters. Drinka et al. [22] negated the 1996 study and reports on five subsequent years of sampling after the 1996 study, finding that their initial report was based on a statistical outlier of high incidence rates during the initial one year of sampling. The updated 2004 study found no clear associations and encouraged the use of larger datasets. These series of studies illustrate that periods of epidemic illness need to be considered in prospective studies of respiratory infections.

4.6 Building Studies from 2000 to Present

Since 2000, studies of ventilation and APT have almost all incorporated some measurements to characterize the building(s) HVAC system. Two categories of studies were reviewed, population-based studies and outbreak studies. Many studies sought to determine the relationship between ventilation rates or CO₂ levels and illness absence in environments such as hospitals, classrooms, and office buildings. Reviewing these studies shows an evolution in HVAC system understanding and the realization that there is a need to collect quantitative data. HVAC systems do not always perform as designed and ventilation rates (both outdoor and/or supply) can change with occupancy, season, etc.

A cross-sectional observational study conducted across seventeen Canadian hospitals studied the association of tuberculin conversion among over 1200 healthcare workers (HCW) with ventilation of patient care areas [82]. This study performed an assessment of the HVAC system by measuring air change rates using a tracer gas method with pure CO₂ and noting the direction of airflow at all doors, windows, and vents with smoke tubes. Note that this measurement accounted for both mechanical ventilation and any infiltration. The percentage of rooms on each nursing unit that met the current ventilation standard was also calculated for each hospital, with results varying from 19% to 87% of rooms meeting the standard for a given hospital. Researchers concluded that in Canadian hospitals that admitted at least six patients with TB annually, tuberculin conversion among HCW was strongly associated with inadequate ventilation in general patient rooms defined as <2.0 air changes per hour (ACH). It was also associated with type and duration of work. Of

note is that researchers accounted for community exposure as well, which would be an important confounder. Ventilation measurements and inspection were performed in 339 patient care areas. One of the largest limitations of the study is that ventilation was assessed based on tracer gas measurements of air change rate during the course of one day. Although the type of HVAC system is not mentioned, it is likely that air change rates varied significantly throughout the year, especially considering that the hospitals were located in areas with extreme outdoor conditions. For this reason, a single day of measurements may not represent the HVAC system operation over the course of three years, the time period in which tuberculin conversions were documented. Additionally, ACH can be an unclear measure if the composition of the dilution air is unknown (outside air, air from an adjacent space, recirculated air from other spaces, etc.). It can refer to the outdoor air change rate if dilution air comes solely from outdoors, but in many cases the composition of the dilution air is unknown.

Milton et al. [85] analyzed sick leave data for 3,720 employees among 40 buildings with 115 independently ventilated work areas at Polaroid Corporation corporate offices. Corporate records for building characteristics and indoor environmental quality complaints were used. Myatt et al. [90, 91] studied buildings in the same Polaroid Corporation offices. Outdoor air ventilation rates were estimated from continuous CO₂ measurements for a subset of work areas using ASHRAE Standard 62-1989. A rating of moderate (around 25 cfm/person, 12 L/s-person) or “high” (around 50 cfm/person, 24 L/s-person) was given to each floor by an expert industrial hygienist. There was a consistent association of increased sick leave with lower levels of outdoor air supply. Some limitations of this study include a broad categorization of OA ventilation rates, sampling in what appears to be a single location to characterize a floor, calculations from measurements that may have a high degree of uncertainty (not reported), and not all work areas were sampled. A strength, however, is that the engineer taking measurements and assigning OA ventilation ratings had prior knowledge of the system performance and any modifications that had been made to existing HVAC systems.

Myatt et al. [90] conducted an intervention study in two Polaroid Corporation office buildings

using short-term sick leave and CO₂ measurements as a surrogate for outdoor air supply rates. It was an intervention because they blindly varied the outdoor air supply dampers. They calculated the CO₂ concentration differential by subtracting the nightly background from the daily average levels. There was no association between CO₂ differential at the values studied with sick leave, albeit differential concentrations were relatively low, ranging from 37250 ppm. They hypothesized that the buildings had an excess of OA ventilation capacity relative to the volume of office space and number of workers. Researchers estimated that <450 ppm above background, it was unlikely that rhinovirus would be transmitted from one office worker to another via the airborne route and for that reason no association between CO₂ and sick leave was found. Some of the methods developed in this study were used in the Myatt et al. (2004) study. Conducting a preliminary study of methods prior to implementing a larger study is a good approach, as this will ensure enough power to see an association, improve data collection methods, and identify any possible confounding. Power is the probability that the null hypothesis is rejected if a specific alternative hypothesis is true. It is a measure of Type II error and is influenced by other factors such as significance level (Type I error) and sample size.

Myatt et al. [91] conducted a prospective intervention study. It is one of the strongest study designs to date, because it used a direct airborne exposure measurement; air filters were collected for rhinovirus detection and analyzed using polymerase chain reaction (PCR) techniques. While the direct exposure measurement of an airborne pathogen is an improvement from an indirect exposure measurement such as CO₂, it is the outcome of infection in conjunction with the presence of airborne pathogens that best informs the risk assessment of aerosol transmission. Ultimately, clinical confirmation of infection would be ideal for outcome measurements, but even outcome measurements with relatively high uncertainty such as absence data would be better than no measurement. Three Polaroid Corporation office buildings were assessed over 20 months. Occupancy was recorded every week. Outdoor air supply dampers were artificially and blindly adjusted every three months to reduce or increase the outdoor air supply and thereby extend the range of CO₂. This was a unique approach to experimenting with the outdoor airflow rates. Carbon dioxide differential above

the background was used according to the methods of Myatt et al. (2002). A significant positive relationship was found between the frequency of virus detection in air samples and average CO₂ concentrations greater than 100 ppm above background. In addition, one sample from a nasal lavage contained single-stranded conformational polymorphism assay (SSCP) band patterns that were an exact match to a building air sample collected during the same week, indicating that the same virus was found on both samples. This suggests that occupants in buildings with low outdoor air supply may have an increased risk of exposure to infectious droplet nuclei emanating from a fellow building occupant. The methods used in Myatt et al. (2004) provide a good starting point for protocols to measure exposure using air sampling in which RNA is extracted from air filters and amplified using PCR.

The series of studies by Milton and Myatt included strong epidemiological and building measurements. Over the course of four years, in which these investigators were focusing on this research, methods evolved to make the study more significant and have more power. In the Milton study, they first used CO₂ to categorize high or low OA ventilation rates based on a few CO₂ measurements and primarily the judgment of an industrial hygienist assigning ventilation rates. In the work of Myatt, they instead took CO₂ measurements above background more frequently and intervened by adjusting and locking outdoor air intake dampers into place over specified time intervals to increase the range of CO₂ concentrations. In the health assessment they moved away from outcome measurements of sick leave to an objective exposure measure of airborne rhinovirus.

Wong et al. [119] conducted a retrospective cohort study of a SARS outbreak at a Hong Kong hospital. They did an inspection of the hospital HVAC system after the outbreak, measuring the airflow rates, velocities, temperatures, and relative humidity (RH) at all supply diffusers and exhaust grilles in the ward. A subsequent study simulated the bioaerosol dispersion in the hospital ward using computational fluid dynamics and provided a more detailed description of the HVAC system [68]. The air change rate was found to be 7.8 ACH for the entire ward; but it was not reported whether this was the outdoor or supply air change rate, and methods for determining ACH were not described. Based on the measurement methods and presence of recirculation, we

believe the reported ACH is likely the supply air change rate. It was found that the supply and exhaust airflow rates were imbalanced, with some supply diffusers and exhaust grilles not functioning properly. The index patients cubicle was found to have the highest supply flow rate, while the adjacent exhaust grille had the lowest flow rate among all four functional exhaust grilles in the ward. It is possible that this imbalance in the supply and exhaust airflow rates promoted the dispersion of infectious aerosol from the index patients cubicle to other hospital areas. Since this study was for only one building, the measurement approach could be more comprehensive in nature. An issue was that they could not collect measurements at the time of outbreak, so it was done four months later, in July. The outbreak was in March, so a subsequent comparison of seasonal differences in ventilation performance would have been important since spring and summer weather in Hong Kong can be quite different and thus the outdoor air supply may vary significantly depending on design. The design of the system supplying air to the ward was not reported (i.e. variable versus constant air volume), so it is possible that supply airflow also varied based on factors such as heat loads.

Haselbach and colleagues [44] reported a re-analysis of data collected from a cross-sectional study of six different military barracks and over 5000 cases of acute respiratory infections (ARI). Data were collected in Feb-May 2004. Researchers documented the rate of ARIs, average room occupancy, and average HVAC contact population (sum of occupants from multiple zones that share return air that is mixed within the same HVAC system and redistributed as supply air). Average contact population was a unique variable in this study as it placed more emphasis on airborne transmission via the HVAC system than direct and indirect contact transmission. Distance between bunks, CO₂ levels, and RH were also measured. It is not clear from the study description how they used these measurements in the data analysis. They categorized the barracks according to age, construction, and HVAC system configuration and then assessed disease prevalence in each category of barrack. Results showed that occupancy affected risk of ARI transmission and the resulting prevalence rate ratios suggest that there was a significant risk of airborne ARI transmission through HVAC systems. Higher rates of infection were found in the systems with both higher HVAC

contact populations and less access to operable windows. Depending on the HVAC configuration, average HVAC contact population may be a useful parameter to include in future epidemiologic studies. In military barracks, large numbers of military recruits live in close proximity, and they are usually young and healthy. Numerous studies have documented epidemic respiratory infections in military barracks, and the pathogens reported include *Streptococcus pneumonia*, adenoviruses, and influenza viruses [55].

A study in China showed that crowded college student dormitories (these were not mechanically ventilated) with low outdoor air ventilation rates were associated with higher rates of respiratory infections among college students [114]. In Phase I, they conducted a cross-sectional study. Researchers collected demographic information, the health status of 6500 students, incidence and duration of common colds in the previous 12 months, and building/room characteristics for 2117 dorm rooms at Tianjin University using questionnaires. Students in six-person dorm rooms were about two times as likely to have an incidence of common cold >six times per year and a duration >two weeks, compared to students in three-person rooms. Outdoor air ventilation rates varied significantly between all rooms regardless of occupancy due to ventilation solely being provided from open windows and doors. The association between OA ventilation rates and the incidence of common cold were adjusted for room occupancy.

In phase II of the Sun et al. study, air temperature, RH, and CO₂ concentrations were measured in 238 occupied dorm rooms for 24 hours. The advantage of continuously monitoring overnight is that the occupancy and metabolic generation rate of CO₂ are fairly constant for a long period of time (78 hours) since the students were sleeping. Measurements were made during the summer and winter seasons. Dorm occupants reported opening status of doors and windows during measurements. Questionnaires on cold incidence and duration were only administered in Phase I, one year prior to the ventilation assessment in Phase II. Results of Phase II were that 90% of the dorm rooms had an outdoor airflow rate less than the Chinese standard of 8.3 L/s-person (17.6 cfm/person) during the heating season. A mean OA ventilation rate of 5 L/s-person (10.6 cfm/person) in dorm buildings was associated with only 5% of self-reported common cold six times,

compared to 35% at 1 L/s-person (2.1 cfm/person).

This study calculated outdoor airflow rates into the dorm rooms from an analysis of the build-up period of CO₂ produced from sleeping occupants. This method of air change rate calculation from CO₂ build-up from sleeping occupants was tested and validated in laboratory experiments and the uncertainty was found to be less than 10% [111]. A drawback of this study design was that the ventilation assessment was done after the cross-sectional data were collected on common cold incidence the year before. Cold incidence varies from season to season. A prospective study design would have collected cold information during the same year as the ventilation assessment. A repeat of the ventilation assessment a few times during the cold season to explore variability would have been useful. Since the dorms were only ventilated by opening windows and doors the difference in rates from one year to the next would be related to differences in the weather (assuming no work on the buildings had been done) and the frequency and habits of window opening. Additionally susceptibility of occupants to infection varies year to year as well as the infectiousness of the virus that would be accounted for in a multi-year study.

The school study by Shendell et al. [109] investigated the association between average daily attendance (ADA) as the health outcome and ventilation rates as estimated from CO₂ data. Carbon dioxide was measured in short-term, 5-min averages on a single school day, at variable times of the day. All but 2 of 434 classrooms had individual HVAC systems. A 1000-ppm increase in CO₂ was significantly associated with a 0.5-0.9% decrease in annual ADA, corresponding to a relative 10-20% increase in student absence. The authors stated: “In general, random errors in an independent variable, in this case the errors from using short-term CO₂ as a measure of long-term average ventilation rate, will tend to obscure and weaken associations with the dependent variable (in this case, attendance or absence).” This conclusion supports our recommendation that more accurate data are desirable and the total variability in the study factor must be estimated.

Mendell et al. [81] collected classroom-level illness absence and demographic data from participating school districts in 28 schools in three different climate zones within California. They measured real-time CO₂ concentrations in each participating classroom for two years. This study

found a significant relationship between classroom OA ventilation rates and illness absence for all combined schools: for every additional 1 L/s-person of ventilation (2.1 cfm/person), illness absence was reduced by 1.6% (within the studys ventilation rate range of 2-20 L/s-person, 4.2-42.4 cfm/person). Mendell et al. installed an internet-connected indoor environmental quality sensor in each classroom. Sensors transmitted CO₂, temperature, and RH data as five-minute averaged values. Outdoor air ventilation rate per person was calculated with a mass-balance model that used the indoor CO₂ equilibrium concentration minus the outdoor value. The analysis assumed well-mixed conditions, and that steady state was reached for CO₂. The steady-state approximation is particularly questionable as simple transient mass balances indicate that steady state is unlikely to be reached during the time period in which the students were continuously in the classroom. While the lack of true steady-state conditions may limit the accuracy of the OA ventilation rate calculations, the CO₂ levels measured provide a good indication of actual instantaneous exposure to exhaled air. Additionally, 37% of classrooms studied were ventilated naturally from window opening, not mechanically. These classrooms were not likely to have a fixed daily outdoor air ventilation rate, and ventilation rate estimates from CO₂ concentrations may have introduced more error than mechanically ventilated spaces that have far less transient and dynamic airflows.

This study encountered many limitations with the sensors used. High failure rates were experienced due to problems from software communicating with school data networks along with implausible values for indoor and outdoor CO₂. Due to implausible and erratic outdoor concentrations, all outdoor CO₂ levels were estimated to be 400 ppm, which might have introduced some error in estimation of ventilation rates. Implausible indoor concentrations were excluded from analyses. The specified measurement uncertainty for the sensors used was also quite high: 100 ppm, which allows for the sensor to be reasonably priced, but does not provide as good data quality as laboratory grade sensors which typically have much better accuracy (1-50 ppm). Nevertheless, Mendell et al. is the only study that we are aware of that succeeded in collecting measurements in every indoor environment in which the health outcome was measured for the entire length of the study. A few important conclusions were drawn from this and a previous study conducted by this research

team [35]. Single location sensors (e.g. those used for demand control ventilation) frequently have large errors (as demonstrated in Mendell et al. [81]). Also, multi-location measurement systems with higher-grade sensors may be necessary for acceptably accurate measurements.

Reference	Study Design	Epidemiological Measure	Building-Related Factor	Associations
Brundage, 1988	Observational: Retrospective Cohort	Incidence rates, relative risk	Categorical: Modern, “tight” construction (~3 ACH) vs. old “leaky” construction	Rates of febrile acute respiratory disease were significantly higher among trainees in modern barracks
Richards et al., 1993	Observational: Cross-sectional Questionnaire Study	Prevalence odds ratio	Categorical: Type of sleeping accommodation (Tents, Warehouses, Non-air-conditioned buildings, air-conditioned buildings)	Upper respiratory illnesses were frequent, related both to housing and exposure to the extreme outside environment
Hoge et al., 1994	Observational: Case-control and Cohort	Incidence rate, relative risk, odds ratio	Measured: Ventilation rate from duct velocity using thermal anemometer	Incidence rates highest among inmates in cells with highest CO ₂ concentrations and lowest rate of outside air delivery
Drinka et al., 1996	Observational: Prospective surveillance with retrospective analysis	Incidence rates	Categorical: Amount of public space, percent outside air delivery, filter type	Inconclusive after a study update (Drinka et al., 2004), encourage larger datasets and longer data collection to eliminate statistical outliers
Menzies et al., 2000	Observational: Cross-sectional Survey	Hazard ratio	Measured: Air changes per hour from CO ₂ tracer gas method	Tuberculin conversion among healthcare workers was strongly associated with inadequate ventilation in general patient rooms and with type and duration of work, but not with ventilation of respiratory isolation rooms
Milton et al., 2000	Observational: Prospective Cohort	Relative risk	Categorical: high vs. low ventilation	Increased sick leave with lower levels of outdoor air supply and IEQ complaints

Myatt et al., 2002	Intervention: Quasi-experimental Trial	Absence rate	Measured: Indoor CO ₂ concentrations above background	No association of relatively low indoor CO ₂ concentrations above background and sick leave
Myatt et al., 2004	Observational: Prospective Cohort Study	Risk of exposure via probability of detecting airborne rhinovirus	Measured: Amount exhaled breath from indoor CO ₂ concentrations above background	Association found between the probability of detecting airborne rhinovirus and a weekly average indoor CO ₂ concentration greater than 100ppm above background
Wong et al., 2004	Observational: Retrospective Cohort	Incidence rate	Measured: Air changes per hour, Flow rates through supply and exhaust diffusers	Proximity to index patient was associated with transmission; risk of contracting SARS was sevenfold greater among students who entered index patients cubicle
Shendell et al., 2004	Observational: Prospective Cohort	Average daily attendance	Measured: Indoor CO ₂ concentration above background	A 1000-ppm increase in CO ₂ above background was associated with 0.5-0.9% decrease in annual average daily attendance, corresponding to a relative 10-20% increase in student absence
Haselbach et al., 2009	Observational: Prospective Cohort	Incidence rate ratio	Categorical: HVAC Contact Population	Higher incidence rates were associated with additional opportunities for airborne transmission via the HVAC system design (i.e. higher HVAC contact populations)
Sun et al., 2011	Observational: Measurements + questionnaire	Self-reported incidence rate	Measured: Ventilation rate from Overnight CO ₂	Crowded dorms with low outdoor air ventilation rates are associated with more respiratory infections among college students
Mendell et al., 2013	Observational: Prospective Cohort	Incidence rate ratio	Measured: Ventilation rate from real-time CO ₂	Increasing classroom ventilation rates from CA average of 4 L/s-person to the State standard of 7.1 L/s-person would decrease illness absence by 3.4%

Table 4.1: Summary of Study Designs, Reported Measure, and Findings in Building-Related Epidemiologic studies

4.7 Discussion

Ventilation rate was a key factor reported in many of the studies we reviewed. There were three primary methods used for measuring outdoor air ventilation rates: (1) mass balance with occupant-generated CO_2 ; (2) tracer gas decay (often with CO_2); and (3) direct measurement of systems airflows. Myatt et al. [91], Sun et al. [114], Shendell et al. [109], and Mendell et al. [81] used indoor-outdoor CO_2 differences to estimate the contribution of outdoor air. Menzies et al. [82] used a CO_2 -injected tracer decay to measure air change rates assuming well-mixed conditions. Tracer gas decay only provides an estimate of the outdoor air ventilation rate at the time it was performed. Many researchers have cautioned against the use of CO_2 to estimate ventilation rates (e.g. Seppänen et al. [107]). CO_2 methods do not always just measure the outdoor air component of ventilation. If the zone of measurement is not the entire building, then the zone may have dilution from other areas of the building, such as infiltration through connecting doorways. In this case the calculated ventilation rate is a dilution rate not an outdoor airflow rate. Measuring ventilation rates is a complex topic in its own right and cannot be fully explored here; several references are available [7, 8, 98].

To determine outdoor air ventilation flows using a mass balance with occupant-generated CO_2 , there must be sufficient CO_2 generation within the space to raise the concentration well above background levels so the difference can be measured accurately. Most occupancy patterns are such that steady-state concentrations are not achieved, which adversely impacts the accuracy of the ventilation rate calculation unless complex corrections are made. When CO_2 concentrations vary the mass balance can be analyzed to give ventilation rate as a function of time. In some spaces, CO_2 concentrations may vary considerably from one location to another and measurements at a given location may not accurately reflect the space in question. The slow, transient response of

CO₂ levels resulting from occupancy changes creates serious challenges in estimating the ventilation rate at a point in time using this method.

Direct measurements of supply ventilation flows may be made using a balometer at supply diffusers for a given space or by inserting anemometers or pitot tubes into air ducts to measure air velocities. If only the airflow into a space is measured, then the measurement is of total air supply. If the air supply to a space is a mixture of outdoor and recirculated air, additional system measurements are required to determine the outside air component of the ventilation. Anemometers or pitot tubes could be permanently installed in the appropriate ducts to measure ventilation rates continuously throughout an epidemiologic study but we encountered no such application in our review. Typically, it is a one-time set of measurements. Depending upon the application, it can be a relative straightforward set of measurements, e.g. a few balometer measurements at the supply diffusers for the spaces included in the study. It can also be quite complex involving identifying and accessing many ducts at different locations, installing anemometers in these ducts, and recording and interpreting these measurements.

Regardless of the method used to determine ventilation rates, several important factors should be kept in mind. Most buildings do not have constant ventilation rates (outdoor and/or supply). Depending upon the HVAC system design, the ventilation rates may vary throughout the day or seasonally. They may vary with heating or cooling load and the outdoor air component may be varied with outdoor air temperature. A measurement at a single point in time, no matter how accurate, is unlikely to adequately characterize ventilation rates for a space or building for the duration of an epidemiologic study. Most HVAC systems do not use 100% outside air and anytime there is return air supplied to a space, quantifying the outside air ventilation rate becomes considerably more complex. At a minimum, there is the question of whether it is outside air or total supply air that is being measured. There is also the question of whether or not infectious pathogens are present in the return air and whether or not this air is effectively filtered or treated with UV germicidal lamps. These questions become even more complex if return air from multiple zones is recirculated in the supply.

The studies reviewed here were all conducted in mechanically ventilated buildings, where ventilation rates are typically a good estimate of an HVAC systems contribution to dilution of potential pathogens. Mechanically ventilated buildings are often slightly pressurized to reduce infiltration, although infiltration may range from a small fraction of the outdoor air delivered to a space to 12-19% [53] or up to 23-61% [42] of total outdoor ventilation air flow. We did not identify an epidemiologic study that measured infiltration as a study parameter, but we urge that this parameter not be neglected in future studies as its range of potential contribution to outdoor ventilation may confound results if not quantified. Given the complexity of HVAC system configurations and myriad of factors with the potential to influence APT, we cannot feasibly discuss all possible HVAC configurations and their potential to affect APT.

For the reasons discussed above, generally applicable recommendations as to methods that should be used to determine ventilation rates cannot be made. We do recommend that studies need to collect information to establish ventilation parameters on both per person and per unit area (or unit volume) basis as a means of more easily comparing data across studies. It is recommended that epidemiologic studies addressing the role of HVAC systems in APT incorporate a knowledgeable HVAC engineer who can study and fully understand the air supply systems for every building in the study and then devise a measurement strategy that will effectively quantify the ventilation rates (accounting for infiltration) for the spaces and buildings for the duration of the study. Inadequate characterization of ventilation has been the Achilles heel of many of the epidemiologic studies conducted to date and adequately quantifying ventilation rates has been a difficult challenge even when the team included members with detailed HVAC system and related measurements knowledge. One possible explanation for the lack of detail in reporting HVAC system characteristics is that many indoor air, health-related studies are conducted by epidemiologists, physicians, or individuals from other fields with limited knowledge of HVAC system design and operation. Another reason is that every HVAC system is different. Better collaboration between epidemiologists and HVAC engineers may lead to more powerful interdisciplinary studies.

The distinction between mechanical and natural ventilation is important, especially when

advocating for standardized measurement methods and reporting for use in future meta-analyses and epidemiologic studies. Mechanically ventilated spaces (without operable windows) are far more controlled in their airflows and control strategies, making it easier to measure, report, and compare across buildings and studies. Naturally ventilated spaces are often heavily influenced by temperature gradients, wind, number and location of open windows, and other factors that may be more difficult to quantify or replicate. Naturally ventilated and mechanically ventilated spaces are not well suited for making comparisons.

Very few studies discussed filtration in the buildings under study. Two studies that did address filtration are Drinka et al. [23] and Haselbach et al. [44]. It is important in future studies to report building filtration for HVAC systems that operate in recirculation mode. Filtration is potentially an important mechanism for removal of airborne pathogens if filters have a rating intended to capture the particle size ranges of the pathogen(s) of concern. The presence of filters within the system is important but ensuring the filters are maintained (replaced on a regular basis in a manner that does not allow reintroduction of particulate to the air stream) and working properly (i.e. no blow-by) are also items to consider.

In the context of population-based studies, it is usually not logistically possible to conduct in-depth measurements of filter removal efficiencies for a large number of buildings. If the study is designed for only a few buildings, these measurements may be possible. In the case of a disease outbreak study, detailed filter efficiency and integrity measurements should be considered. If the population-based epidemiologic study is being performed under the assumption that filters were installed and maintained correctly and it has a large enough dataset that any outliers of poorly installed or maintained filters will not influence significantly the association, recording the filter ratings and age for all filters being used in the study space should suffice. Filters are different than primary factors such as ventilation rate because the efficiency only indirectly impacts exposure; they only impact the air that passes through them and they only impact the outcome if the air that passes through them affects exposure.

To use filter rating as a factor across many environments and varying HVAC designs, one

option could be to normalize it in some way, for example, by the volume of recirculation air given the volume of the space, the equivalent to the recirculation air change rate (assuming pathogen source is indoors). By normalizing the filter rating, the effect of filter rating on exposure can be isolated across many different buildings. This is one possible alternative to an intervention study that changes filter performance to reflect the actual impact on exposure.

Many studies report that occupant density or crowding is commonly positively associated with disease outcomes (Hoge et al. [47], for example). Studies need to collect sufficient information to establish occupant density (people per unit area) and total occupancy of a space. Similarly, a separate but related variable is the total number of people in an air handler zone that share recirculated air. The occupancy effect is confounded by the fact that diseases are also transmitted in close contact environments by fomites and droplets. How to differentiate these transmission modes remains an important research question.

Studies need sufficient power to detect associations between environmental conditions or ventilation factors and illness incidence or pathogen transmission. Myatt et al. (2004) was the only study that we reviewed that directly measured airborne pathogen exposure using filter collection and PCR analyses. Myatt et al. [91] was unfortunately underpowered for exposure measurements but not by much (181 filters analyzed, 58 tested positive for rhinovirus). Mendell et al. 2013 was also underpowered in two of the three school districts that were studied. They used classroom level outcome data, not individual data, which may have underpowered the study. Another reason for under-powering was loss of CO₂ monitoring data due to equipment failure. It is important in epidemiologic studies to limit data loss since it is so costly to gather the data in the first place, and loss of data can severely limit the use of disease incidence or prevalence data.

Although there are currently a reasonable number of studies of HVAC system characteristics and APT, a meta-analysis is not yet a viable option [113]. Meta-analysis is a statistical method for combining results from different studies, to identify patterns among the study results and to aggregate information to achieve a higher statistical power for the associations, such as between ventilation rate and disease. A quantitative meta-analysis is intended to combine estimates from

multiple studies of the same exposure and health outcome, considering the individual studies as simply repetitions of the same study with randomly varying findings around a true value of the association. The design of a study included in a meta-analysis is important; a meta-analysis of badly designed studies will result in biased estimates, with the direction of the bias depending on the flaws in the study. For example, an improved study design would use representative sampling, such as random, systematic, stratified, and cluster sampling, or a combination of these. Note that a meta-analysis will usually include studies of differing quality, and can usefully see if the better designed studies are more consistent when the weaker studies are excluded. A research finding is less likely to be true when the studies conducted are smaller, when effect sizes are smaller, and where there is greater flexibility in designs, and outcomes [51]. Additionally, HVAC system characteristics and measurement methods should be reported in sufficient detail for proper interpretation of results and use in future meta-analyses. Clarity of reported data and measurement methods is essential to the success of future meta-analyses.

4.8 Conclusions

Some progress has been made in understanding the role of mechanical ventilation and APT in the past 20 years. Much more remains to be done. The current available studies in buildings are mostly observational analytic or etiologic epidemiologic studies; although observational, they were designed to explore causal connections. While an advantage of such studies is that they reflect real-world conditions, they afford the least control over the multiple variables that may be involved. Observational studies cannot positively establish causality. These are good studies to explore hypotheses, but intervention studies are needed to confirm a causal relationship. In many cases, however, observational epidemiology is considered sufficient to establish likely causality because many epidemiological questions cannot be answered by conducting experimental studies. The weight of the data implies that HVAC system factors in buildings have a role in APT; however, more studies need to be completed, with the eventual goal of a meta-analysis to integrate results. We specifically recommend that individual studies should be designed in a way aimed at future

meta-analyses of key relationships so that all study methods are focused on these relationships of interest and also include parallel measurements to other such studies so they can be combined.

Of course as more studies are completed, and meta-analyses are conducted, the weight of evidence may be enough to establish a causal link between building factors and risk of transmission; for example, lower outdoor air ventilation rates result in higher incidence of influenza. Confirmation of these findings by prospective, preferably experimental studies using detailed HVAC-parameter, exposure, and outcome measurements is needed. The study of buildings lends itself well to experimental studies, however such studies are difficult and expensive, especially with randomization implemented and blind interventions. Interventions could be made on various HVAC-related parameters, including outdoor air rates, supply air rates, humidity, or control technologies such as UV germicidal lamps, or filter type and rating. We need better-powered evidence from larger studies and meta-analyses. There is also a need for more studies with strong measurement methods of building characteristics, exposures, and outcomes, which can be greatly facilitated by increased collaboration between epidemiologists and engineers. Large-scale evidence should be targeted for research questions where the pre-study probability is considerably high, so that a significant research finding would be considered definitive [51]. The pre-study probability in this case does seem high, given the studies completed to date, so that a well-designed well-funded study should result in clear evidence. Ioannidis states the large-scale evidence is also particularly indicated when it tests major concepts. The concept in question is a broad one what is the causal association between mechanical ventilation and APT, so in fact it is a worthwhile endeavor to pursue.

Establishing causality, however, is just the first step. It is generally believed and accepted that HVAC system factors can and do impact APT so establishing causality is mostly a matter of confirming the obvious. Quantitative relationships between HVAC factors and APT are needed for effective design and operation of HVAC systems. For example, how much outdoor air ventilation (at a given recirculation filter rating) is needed for a classroom with a given occupancy to avoid elevated disease outcomes? Thus, additional epidemiologic studies as well as meta-analyses will establish the causality that is needed to focus on providing definitive quantitative relationships.

4.9 Acknowledgements

We thank Mark Mendell, William Fisk, and Dick Menzies for their courtesy review of this manuscript and helpful comments. This research was funded in part by ASHRAE.

4.10 Supplementary Information

Recommended measurement protocols of parameters related to mechanically ventilated buildings for use in epidemiologic study designs can be found in Appendix A.

Chapter 5

Summary and Concluding Remarks

The work presented here has reviewed a compilation of studies that have increased our knowledge of how building characteristics affect indoor air microbiology and energy efficiency, two salient qualities of a healthy building. Important limitations of building and health studies were also outlined in hopes of improving future indoor environment research endeavors.

First, we took an in-depth look at UVGI coil cleaning technology in the first controlled laboratory experiment of its kind (Chapter 2). We found that the efficacy of UVGI cleaning depended heavily on the mode of operation (i.e. whether fin surfaces were wet). We observed a 3–7% increase in airside heat transfer effectiveness for the UV-irradiated coil under condensing conditions but found no significant effect on heat transfer when coil surfaces were dry. We did not observe any effect on pressure drop but we hypothesize that the small-scale and mild latent loads in our experimental setup may have contributed to smaller effects than would be observed in large commercial systems. It is clear, however, that energy and monetary savings from UVGI coil cleaning resulted primarily from improved heat transfer, not decreased pressure drop. Upon seeing that decreased biofouling resulted in improved heat transfer effectiveness, we investigated this technology further by quantifying the reduction of microbial loading on heat exchanger fin surfaces as well as the fate of this microbial contamination. We found that prior to UV-irradiation, the downstream surfaces of cooling coils had the highest microbial loading during condensing conditions, suggesting that placement of the UV lamps downstream would be the most effective location. Conversely, under dry surface conditions, surface concentrations were higher upstream for both the

UV-treated and control coils. UVGI reduced surface microbial loading by 55% on average during condensing conditions and inhibited bacterial attachment causing clusters of bacterial matter to become airborne downstream of the cooling coil. Additionally, it was found that desiccation also inhibited surface microbial loading and yielded cluster detachment but to a lesser degree than UVGI treatment. This work suggests that UVGI is most effective at reducing biofouling in humid climates with high latent loads, so installation of this technology should be carefully considered depending on the climatic region. It also suggests that filters downstream of cooling coils in humid climates should be carefully monitored for proper installation and possible heavy loading after UV installation or coil desiccation, particularly after periods of high latent heat transfer loads that corresponds to large amounts of condensation on coil surfaces. We recommend replacing final filters one or two months after initial UVGI installation due to likely microbial fouling, especially if an increase in pressure drop across final filters is observed after the first few months. With better understanding of this technology, it can be properly deployed to improve the energy efficiency and quality of our indoor environments.

Next, we delved deeper into understanding what building characteristics affect the microbiology of indoor air (Chapter 3). We found that occupants had the greatest effect shaping bacterial communities in indoor air, specifically that the sex of occupants could be predicted based solely on bacterial community composition, showing the potential relevance to forensics. *Lactobacillus* is the genus that most contributed to the differences between female-inhabited and male-inhabited environments. More generally, taxa often identified as members of the vaginal microbiota were more common in female-occupied rooms while taxa associated with human skin or the male urogenital microbiota were more common in male-occupied rooms. We found no significant predictors of fungal communities indoors, but we believe the lack of variance in our HVAC parameters (such as ventilation rate) made it difficult to find associations. The same limitation existed in health study of dormitory students, combined with low statistical power from low participation and response rates. We recommend that future health-related observational studies incorporate environments with wider variance in covariates (such as ventilation rates or some exposure measure), longer

study duration, larger sample size and participation/response rates, and a more objective outcome measure of illness than self-reported, perhaps employer or physician-provided illness or absence data.

Finally, we conducted a comprehensive literature review surveying the current state of knowledge of epidemiologic studies of HVAC system or mechanical ventilation parameters and health-related endpoints (Chapter 4). Studies to date showed an association between increased infectious illness and decreased ventilation rate, however, there are insufficient data to quantify how mechanical ventilation may affect the airborne transmission of infectious agents. Our review revealed a strong need for more epidemiologic studies and meta-analyses. Specifically, there is a need for well-designed prospective observational or intervention studies in buildings to establish causal relationships between airborne exposures and outcomes as well as between HVAC system factors and indoor exposures. Future studies will benefit greatly from improved experimental design, standardized measurement methods, and better collaboration between epidemiologists and HVAC engineers.

This dissertation provides a glimpse into the complex and interdisciplinary nature of indoor air and building science, which is still a young and ripe field of research. A recent editorial piece acknowledges that indoor air scientists often research “in narrow trenches, interacting primarily with those they have interacted with for years, content to dig more deeply into that of which they already have significant knowledge, and unaware of the connections that their work may have to those who dig in other trenches [17].” This work makes connections across building energy systems, HVAC science, and microbiology to demonstrate the nuances of how building characteristics or design decisions can affect indoor exposures. It is my sincere hope that research in this field continues to make connections and advancements that get us closer to quantitative evidence and, ultimately, defining what makes a healthy building.

Bibliography

- [1] ACHR News. UVC Lights Keep Hospital Cool , Efficient. The HVACR Contractor's Weekly News Magazine, pages 1295–1296, 2007.
- [2] Rachel I. Adams, Ashley C. Bateman, Holly M. Bik, and James F. Meadow. Microbiota of the indoor environment: a meta-analysis. Microbiome, 3(1):49, 2015.
- [3] Rachel I. Adams, Marzia Miletto, Steven E. Lindow, John W. Taylor, and Thomas D. Bruns. Airborne bacterial communities in residences: Similarities and differences with fungi. PLoS ONE, 9(3), 2014.
- [4] Rachel I Adams, Marzia Miletto, John W Taylor, and Thomas D Bruns. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. The ISME journal, 7(7):1262–73, 2013.
- [5] Rachel I. Adams, Yilin Tian, John W. Taylor, Thomas D. Bruns, Anne Hyvärinen, and Martin Täubel. Passive dust collectors for assessing airborne microbial material. Microbiome, 3(1):46, 2015.
- [6] Rudolf I Amann, Wolfgang Ludwig, and Karl-heinz Schleifer. Phylogenetic Identification and In Situ Detection of Individual Microbial Cells without Cultivation. Microbiological Reviews, 59(1):143–169, 1995.
- [7] ASTM. ASTM Standard E741-11, Standard Test Method for Determining Air Change in a Single Zone by Means of a Tracer Gas Dilution. Technical report, American Society for Testing and Materials, Philadelphia, 2011.
- [8] ASTM. ASTM Standard D6245-12, Standard Guide for Using Indoor Carbon Dioxide Concentrations to Evaluate Indoor Air Quality and Ventilation. Technical report, American Society for Testing and Materials, Philadelphia, 2012.
- [9] Albert Barberán, Robert R Dunn, Brian J Reich, Krishna Pacifici, Eric B Laber, Holly L Menninger, James M Morton, Jessica B Henley, Jonathan W Leff, Shelly L Miller, and Noah Fierer. The ecology of microscopic life in household dust. Proceedings of the Royal Society B, 282(1814), 2015.
- [10] Albert Barberán, Joshua Ladau, Jonathan W. Leff, Katherine S. Pollard, Holly L. Menninger, Robert R. Dunn, and Noah Fierer. Continental-scale distributions of dust-associated bacteria and fungi. Proceedings of the National Academy of Sciences, 112(18):5756–5761, 2015.

- [11] M Bernardeau and JP Vernoux. Safety assessment of dairy microorganisms: the *Lactobacillus* genus. International journal of food microbiology, 126(3):278–285, 2008.
- [12] J P Biggerstaff, M Le Puil, B L Weidow, J Prater, K Glass, M Radosevich, and D C White. New methodology for viability testing in environmental samples. Molecular and cellular probes, 20(2):141–6, 2006.
- [13] E. Bloom, L. F. Grimsley, C. Pehrson, J. Lewis, and L. Larsson. Molds and mycotoxins in dust from water-damaged homes in New Orleans after hurricane Katrina. Indoor Air, 19(2):153–158, 2009.
- [14] John F. Brundage. Building-associated risk of febrile acute respiratory diseases in Army trainees. JAMA: The Journal of the American Medical Association, 259(14):2108, 1988.
- [15] Chang Y Chen and Ginette Séguin-Swartz. A rapid method for assessing the viability of fungal spores. Canadian journal of plant pathology, 24(2):230–232, 2002.
- [16] J Danny Cooley, Wing C Wong, Cynthia A Jumper, and David C Straus. Correlation between the prevalence of certain fungi and sick building syndrome. Occupational and Environmental Medicine, 55(9):579–584, 1998.
- [17] Richard L Corsi. Connect or stagnate: the future of indoor air sciences. Indoor air, 25(3):231–234, 2015.
- [18] K. C. Dannemiller, J. F. Gent, B. P. Leaderer, and J. Peccia. Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children. Indoor Air, 2015.
- [19] RR Davies and WC Noble. Dispersal of bacteria on desquamated skin. The Lancet, 1962.
- [20] DOE. Buildings Energy Data Book. Technical report, U.S. Department of Energy, 2011.
- [21] Caroline M Doig. The effect of clothing on the dissemination of bacteria in operating theatres. British Journal of Surgery, 59(11):878–881, 1972.
- [22] Paul J. Drinka, Peggy Krause, Lori Nest, and Doug Tyndall. Report of an outbreak: Nursing home architecture and influenza-A attack rates: Update. Journal of the American Geriatrics Society, 52(5):847–848, 2004.
- [23] Paul J. Drinka, Peggy Krause, M Schilling, BA Miller, P Shult, and S Gravenstein. Report of an outbreak: Nursing home architecture and influenza-A attack rates. Journal of the American Geriatrics Society, 44(8):910–913, 1996.
- [24] Marc Dufrêne and Pierre Legendre. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs, 67(3):345–366, 1997.
- [25] Robert R. Dunn, Noah Fierer, Jessica B. Henley, Jonathan W. Leff, and Holly L. Menninger. Home Life: Factors Structuring the Bacterial Diversity Found within and between Homes. PLoS ONE, 8(5):e64133, 2013.
- [26] EIA. Commercial Buildings Energy Consumption Survey (CBECS) Data. Technical report, Energy Information Administration, 2003.

- [27] J. Elith, J. R. Leathwick, and T. Hastie. A working guide to boosted regression trees. The Journal of Animal Ecology, 77(4):802–13, 2008.
- [28] Joanne B. Emerson, Patricia B. Keady, Tess E. Brewer, Nicholas Clements, Emily E. Morgan, Jonathan Awerbuch, Shelly L. Miller, and Noah Fierer. Impacts of Flood Damage on Airborne Bacteria and Fungi in Homes after the 2013 Colorado Front Range Flood. Environmental Science & Technology, 49(5):2675–2684, 2015.
- [29] EPA. The Inside Story: A Guide to Indoor Air Quality, 2015.
- [30] M. P. Fabian, S. L. Miller, T. Reponen, and M. T. Hernandez. Ambient bioaerosol indices for indoor air quality assessments of flood reclamation. Journal of Aerosol Science, 36:763–783, 2005.
- [31] Anne Fausto-Sterling. The Five Sexes, Revisited. The Sciences, 40(4):18–23, 2000.
- [32] N Fierer and M Hamady. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proceedings of the National Academy of Sciences, 105(46):17994–17999, 2008.
- [33] N. Fierer, C. L. Lauber, N. Zhou, D. McDonald, E. K. Costello, and R. Knight. Forensic identification using skin bacterial communities. Proceedings of the National Academy of Sciences, 107(14):6477–6481, 2010.
- [34] Joseph Firrantello, Jason DeGraw, and William Bahnfleth. Modeled Air Quality and Energy Use Impacts of UVGI for Cooling Coil Biofouling Mitigation. In ASHRAE IAQ 2013 Proceedings: Environmental Health in Low Energy Buildings, pages 207–214, 2013.
- [35] William J Fisk, Mark J Mendell, Molly Davies, Ekaterina Eliseeva, David Faulkner, Tienzen Hong, and Douglas P Sullivan. Demand Controlled Ventilation and Classroom Ventilation. Technical report, Lawrence Berkeley National Laboratory, Berkeley, CA, 2012.
- [36] William J. Fisk and Arthur H. Rosenfeld. Estimates of improved productivity and health from better indoor environments. Indoor Air, 7:158–172, 1997.
- [37] Gilberto E. Flores, Scott T. Bates, Dan Knights, Christian L. Lauber, Jesse Stombaugh, Rob Knight, and Noah Fierer. Microbial biogeography of public restroom surfaces. PLoS ONE, 6(11):e28132, 2011.
- [38] Kei E. Fujimura, Christine C. Johnson, Dennis R. Ownby, Michael J. Cox, Eoin L. Brodie, Suzanne L. Havstad, Edward M. Zoratti, Kimberley J. Woodcroft, Kevin R. Bobbitt, Ganesa Wegienka, Homer A. Boushey, and Susan V. Lynch. Man’s best friend? The effect of pet ownership on house dust microbial communities. The Journal of allergy and clinical immunology, 126(2):410–412, 2010.
- [39] GDB. Green Dashboard - Energy - LEED Buildings, 2014.
- [40] Jan Gralton, Euan Tovey, Mary-Louise McLaws, and William D Rawlinson. The role of particle size in aerosolised pathogen transmission: a review. The Journal of infection, 62(1):1–13, 2011.

- [41] Elizabeth A Grice and Julia A Segre. The skin microbiome. Nature reviews. Microbiology, 9(4):244–253, 2011.
- [42] R.A. Grot and A.K. Persily. Measured air infiltration and ventilation rates in eight large office buildings. In H.R. Trechsel and P.L. Lagus, editors, Measured Air Leakage of Buildings, ASTM STP 904, pages 151–183. American Society of Testing and Materials, Philadelphia, 1986.
- [43] GS Hall, CA Mackintosh, and PN Hoffman. The dispersal of bacteria and skin scales from the body after showering and after application of a skin lotion. Journal of hygiene, 97(02):289–298, 1986.
- [44] Liv Haselbach, James Hussey, Charles E Feigley, and James R Hebert. Airborne transmission via HVAC of acute respiratory infections in military facilities? Review of a basic training cohort study. Journal of Green Building, 4(1):114–120, 2009.
- [45] David L Hawksworth. Davidiella, a New Generic Name for the Teleomorph of Cladosporium S. Str. Mycological Research, 107(11):1250, 2003.
- [46] Austin Bradford Hill. The Enviroment and Disease: Association or Causation. Proceedings of the Royal Society of Medicine, 58(5):295–300, 1965.
- [47] Charles W. Hoge, Mary R. Reichler, Edward A. Dominguez, John C. Bremer, Timothy D. Mastro, Katherine A. Hendricks, Daniel M. Musher, John A. Elliott, Richard R. Facklam, and Robert F. Breiman. An epidemic of pneumococcal disease in an overcrowded, inadequately ventilated jail. The New England Journal of Medicine, 331(10):643–648, 1994.
- [48] P Hugenholtz, M A Cunningham, J K Hendrlkz, and J A Fuerst. Desiccation resistance of bacteria isolated from an air-handling system biofilm determined using a simple quantitative membrane filter method. Letters in Applied Microbiology, 21(1):41–46, 1995.
- [49] P Hugenholtz and J A Fuerst. Heterotrophic bacteria in an air-handling system. Applied and environmental microbiology, 58(12):3914–20, 1992.
- [50] ICC. International Conservation Code. Technical report, International Code COuncil, 2009.
- [51] John P a Ioannidis. Why most published research findings are false. PLoS Medicine, 2(8):e124, 2005.
- [52] John E Janssen. The History of Ventilation and Temperature Control. ASHRAE Journal, 41:47–52, 1999.
- [53] Daranee Jareemit and Jelena Srebric. A Characterization of time-dependent air infiltration rates in retail stores using calibrated multi-zone model. Science and Technology for the Built Environment, 21(4):420–428, 2015.
- [54] Peggy L Jenkins, Thomas J Phillips, Elliot J Mulberg, and Steve P Hui. Activity patterns of Californians: Use of and proximity to indoor pollutant sources. Atmospheric Environment, 26A(12):2141–2148, 1992.
- [55] Vivek Kak. Infections in confined spaces: cruise ships, military barracks, and college dormitories. Infectious disease clinics of North America, 21(3):773–84, ix–x, 2007.

- [56] Firouz Keikavousi. UVC : Florida Hospital Puts HVAC Maintenance Under a New Light. Engineered Systems, pages 60–66, 2004.
- [57] Scott T Kelley, Ulrike Theisen, Largus T Angenent, Allison St. Amand, and Norman R Pace. Molecular Analysis of Shower Curtain Biofilm Microbes. Applied and Environmental Microbiology, 70(7):4187–4192, 2004.
- [58] Steven W Kembel, Evan Jones, Jeff Kline, Dale Northcutt, Jason Stenson, Ann M Womack, Brendan Jm Bohannan, G Z Brown, and Jessica L Green. Architectural design influences the diversity and structure of the built environment microbiome. The ISME Journal, 6(8):1469–1479, 2012.
- [59] Steven W. Kembel, James F. Meadow, Timothy K. O’Connor, Gwynne Mhuireach, Dale Northcutt, Jeff Kline, Maxwell Moriyama, G. Z. Brown, Brendan J M Bohannan, and Jessica L. Green. Architectural design drives the biogeography of indoor bacterial communities. PLoS ONE, 9(1):1–10, 2014.
- [60] Jana Kesavan, Deborah Schepers, Jerold Bottiger, and Jason Edmonds. UV-C Decontamination of Aerosolized and Surface-Bound Single Spores and Bioclusters. Aerosol Science and Technology, 48(4):450–457, 2014.
- [61] Eric M. Kettleson, Atin Adhikari, Stephen Vesper, Kanistha Coombs, Reshmi Indugula, and Tiina Reponen. Key determinants of the fungal and bacterial microbiomes in homes. Environmental Research, 138:130–135, 2015.
- [62] A.K. Klauss, R.H. Tull, L.M. Roots, and J.R. Pfafflin. History of Changing Concepts of Ventilation Requirements. ASHRAE Journal, 12(6), 1970.
- [63] DG Kleinbaum, LL Kupper, and H Morgenstern. Epidemiologic research: principles and quantitative methods. John Wiley & Sons, 1982.
- [64] Elmira Kujundzic, Mark Hernandez, and Shelly L. Miller. Ultraviolet germicidal irradiation inactivation of airborne fungal spores and bacteria in upper-room air and HVAC in-duct configurations. Journal of Environmental Engineering and Science, 6(1):1–9, 2007.
- [65] Charles E Lamb, Paul H Ratner, Clarion E Johnson, Ambarish J Ambegaonkar, Ashish V Joshi, David Day, Najah Sampson, and Benjamin Eng. Economic impact of workplace productivity losses due to allergic rhinitis compared with select medical conditions in the United States from an employer perspective. Current medical research and opinion, 22(6):1203–1210, 2006.
- [66] Christian L Lauber, Kelly S Ramirez, Zach Aanderud, Jay Lennon, and Noah Fierer. Temporal variability in soil microbial communities across land-use types. The ISME journal, 7(8):1641–1650, 2013.
- [67] Estelle Levetin, Richard Shaughnessy, Christine A Rogers, and Robert Scheir. Effectiveness of Germicidal UV Radiation for Reducing Fungal Contamination within Air-Handling Units. Applied and Environmental Microbiology, 67(8):3712–3715, 2001.
- [68] Y Li, X Huang, I T S Yu, T W Wong, and H Qian. Role of air distribution in SARS transmission during the largest nosocomial outbreak in Hong Kong. Indoor air, 15(2):83–95, 2005.

- [69] Y Li, G.M. Leung, J.W. Tang, X. Yang, C.Y.H Chao, J.Z. Lin, J.W. Lu, P.V. Nielsen, J. Niu, H. Qian, A.C. Sleight, H.-J.J. Su, J. Sundell, T.W. Wong, and P.L. Yuen. Role of ventilation in airborne transmission of infectious agents in the built environment - a multidisciplinary systematic review. Indoor air, 17(1):2–18, 2007.
- [70] Julia C Luongo, Albert Barbern, Robin Hacker-Cary, Emily E. Morgan, Shelly L Miller, and Noah Fierer. Microbial analyses of airborne dust collected from dormitory rooms predict the sex of occupants. Indoor Air, pages n/a–n/a, 2016.
- [71] Julia C Luongo, Kevin P Fennelly, Julia A Keen, Zhiqiang John Zhai, Byron W Jones, and Shelly L Miller. Role of mechanical ventilation in the airborne transmission of infectious agents in buildings. Indoor air, 2015.
- [72] Julia C Luongo and Shelly L Miller. Ultraviolet Germicidal Irradiation on HVAC Cooling Coils Part 2: Decreased Surface Microbial Fouling and Resuspension of Cell Clusters. Manuscript submitted for publication, 2015.
- [73] Bing Ma, Larry J Forney, and Jacques Ravel. The vaginal microbiome: rethinking health and diseases. Annual Review of Microbiology, 66:371–389, 2012.
- [74] Pierre Alain Maron, David P H Lejon, Esmeralda Carvalho, Karine Bizet, Philippe Lemanceau, Lionel Ranjard, and Christophe Mougél. Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library. Atmospheric Environment, 39:3687–3695, 2005.
- [75] James F. Meadow, A. E. Altrichter, S. W. Kembel, J. Kline, G. Mhuireach, M. Moriyama, D. Northcutt, T. K. O’Connor, A. M. Womack, G. Z. Brown, J. L. Green, and B. J M Bohannan. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. Indoor Air, 24(1):41–48, 2014.
- [76] James F. Meadow, Adam E. Altrichter, Ashley C. Bateman, Jason Stenson, GZ Brown, Jessica L. Green, and Brendan J.M. Bohannan. Humans differ in their personal microbial cloud. PeerJ, 3(1):e1258, 2015.
- [77] James F. Meadow, Adam E. Altrichter, Steven W. Kembel, Maxwell Moriyama, Timothy K. O’Connor, Ann M. Womack, G. Z. Brown, Jessica L. Green, and Brendan J.M. Bohannan. Bacterial communities on classroom surfaces vary with human contact. Microbiome, 2(1):7, 2014.
- [78] M J Mendell. Non-specific symptoms in office workers - a review and summary of the epidemiologic literature. Indoor Air, 3:227–236, 1993.
- [79] M J Mendell, Q Lei-Gomez, A G Mirer, O Seppänen, and G Brunner. Risk factors in heating, ventilating, and air-conditioning systems for occupant symptoms in US office buildings: the US EPA BASE study. Indoor air, 18(4):301–16, 2008.
- [80] Mark J Mendell, Ekaterina A Eliseeva, Michael Spears, Wanyu R Chan, Sebastian Cohn, Douglas P Sullivan, and William J Fisk. A longitudinal study of ventilation rates in california office buildings and self-reported occupant outcomes including respiratory illness absence. Building and Environment, 92:292–304, 2015.

- [81] M.J. Mendell, E.A. Eliseeva, M.M. Davies, M. Spears, A. Lobscheid, W.J. Fisk, and M.G. Apte. Association of classroom ventilation with reduced illness absence: A prospective study in California elementary schools. Indoor air, 23:515–528, 2013.
- [82] Dick Menzies, Anna Fanning, Lilian Yuan, and Mark FitzGerald. Hospital ventilation and risk for tuberculous infection in canadian health care workers. American Society of Internal Medicine, 133(10):779–789, 2000.
- [83] Dick Menzies, Julia Popa, James A Hanley, Thomas Rand, and Donald K Milton. Effect of ultraviolet germicidal lights installed in office ventilation systems on workers’ health and wellbeing: double-blind multiple crossover trial. Lancet, 362(9398):1785–91, 2003.
- [84] Shelly L Miller, Jacqueline Linnes, and Julia C. Luongo. Ultraviolet germicidal irradiation: future directions for air disinfection and building applications. Photochemistry and photobiology, 89(4):777–81, 2013.
- [85] D K Milton, P M Glencross, and M D Walters. Risk of sick leave associated with outdoor air supply rate, humidification, and occupant complaints. Indoor air, 10(4):212–21, 2000.
- [86] Noelle-Angelique M Molinari, Ismael R Ortega-Sanchez, Mark L Messonnier, William W Thompson, Pascale M Wortley, Eric Weintraub, and Carolyn B Bridges. The annual impact of seasonal influenza in the US: Measuring disease burden and costs. Vaccine, 25(27):5086–96, 2007.
- [87] Ross D Montgomery and Robert Baker. Study Verifies Coil Cleaning Saves Energy. ASHRAE Journal, 48(11):34–38, 2006.
- [88] P.R. Morey. Microorganisms in buildings and HVAC systems: A summary of 21 environmental studies. In Proceedings of the ASHRAE Conference on Indoor Air Quality, American Society of Heating, Refrigeration, and Air-Conditioning Engineers, pages 10–24, Atlanta, GA, 1988.
- [89] E. S. Moyer, W. E. Miller, M. A. Commodore, C. C. Coffey, J. L. Hayes, S. A. Fotta, and G. Sims. Aerosol and Biological Sampling of a Ventilation Fan-bank Modified with Ultraviolet Germicidal Irradiation and Improved Filter Holders. Indoor and Built Environment, 19(2):230–238, 2010.
- [90] TA Myatt, J Staudenmayer, K Adams, M Walters, M Wand, S Rudnick, and DK Milton. An intervention study of outdoor air supply rates and sick leave among office workers. In Proceedings: Indoor Air 2002, pages 778–783, 2002.
- [91] Theodore A. Myatt, Sebastian L. Johnston, Zhengfa Zuo, Matthew Wand, Tatiana Kebabdz, Stephen Rudnick, and Donald K. Milton. Detection of airborne rhinovirus and its relation to outdoor air supply in office environments. American journal of respiratory and critical care medicine, 169(11):1187–90, 2004.
- [92] NCEI. Climate Data Online, National Centers for Environmental Information, NOAA.
- [93] Mark Nicas, William W Nazaroff, and Alan Hubbard. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. Journal of occupational and environmental hygiene, 2(3):143–54, 2005.
- [94] WC Noble. Dispersal of skin microorganisms*. British Journal of Dermatology, 1975.

- [95] Federico Noris, Jeffrey A. Siegel, and Kerry A. Kinney. Evaluation of HVAC filters as a sampling mechanism for indoor microbial communities. Atmospheric Environment, 45(2):338–346, 2011.
- [96] NRDC. Ten large US cities join major new initiative to reduce carbon pollution from buildings — Kaid Benfield’s Blog — Switchboard, from NRDC, 2014.
- [97] Ismael R Ortega-Sanchez, Noelle-Angelique M Molinari, Gerry Fairbrother, Peter G Szilagyi, Kathryn M Edwards, Marie R Griffin, Amy Cassedy, Katherine A Poehling, Carolyn Bridges, and Mary Allen Staat. Indirect, out-of-pocket and medical costs from influenza-related illness in young children. Vaccine, 30(28):4175–81, 2012.
- [98] Andrew Persily. Field measurement of ventilation rates. Indoor Air, 2015.
- [99] Andrew K. Persily. Evaluating Building IAQ and Ventilation with Indoor Carbon Dioxide. ASHRAE Transactions, 103(2), 1997.
- [100] M. Pitkäranta, T. Meklin, A. Hyvärinen, L. Paulin, P. Auvinen, A. Nevalainen, and H. Rintala. Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture. Applied and Environmental Microbiology, 74(1):233–244, 2008.
- [101] M. Porta, editor. A Dictionary of Epidemiology. Oxford University Press, 5th edition, 2014.
- [102] Aaron J. Prussin, Ellen B. Garcia, and Linsey C. Marr. Total Concentrations of Virus and Bacteria in Indoor and Outdoor Air. Environmental Science & Technology Letters, 2(4):84–88, 2015.
- [103] Jacques Ravel, Pawel Gajer, Zaid Abdo, G Maria Schneider, Sara S K Koenig, Stacey L McCulle, Shara Karlebach, Reshma Gorle, Jennifer Russell, Carol O Tacket, Rebecca M Brotman, Catherine C Davis, Kevin Ault, Ligia Peralta, and Larry J Forney. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America, 108 Suppl:4680–4687, 2011.
- [104] Nicholas G Reed. The history of ultraviolet germicidal irradiation for air disinfection. Public Health Reports, 125:15–27, 2010.
- [105] R M Ryan, G E Wilding, R J Wynn, R C Welliver, B A Holm, and C L Leach. Effect of enhanced ultraviolet germicidal irradiation in the heating ventilation and air conditioning system on ventilator-associated pneumonia in a neonatal intensive care unit. Journal of perinatology : official journal of the California Perinatal Association, 31(9):607–14, 2011.
- [106] Michael G Schmidt, Hubert H Attaway, Silva Terzieva, Anna Marshall, Lisa L Steed, Deborah Salzberg, Hameed A Hamoodi, Jamil A Khan, Charles E Feigley, and Harold T Michels. Characterization and control of the microbial community affiliated with copper or aluminum heat exchangers of HVAC systems. Current microbiology, 65(2):141–9, 2012.
- [107] O.A. Seppanen, W.J. Fisk, and M.J. Mendell. Association of Ventilation Rates and CO2 Concentrations with Health and Other Responses in Commercial and Institutional Buildings. Indoor Air, 9:226–252, 1999.

- [108] Brian G Shelton, Kimberly H Kirkland, W Dana Flanders, and George K Morris. Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States Profiles of Airborne Fungi in Buildings and Outdoor Environments in the United States. Applied and Environmental Microbiology, 68(4):1743–1753, 2002.
- [109] D G Shendell, R Prill, W J Fisk, M G Apte, D Blake, and D Faulkner. Associations between classroom CO₂ concentrations and student attendance in Washington and Idaho. Indoor air, 14(5):333–41, 2004.
- [110] Ian H. Spicknall, James S. Koopman, Mark Nicas, Josep M. Pujol, Sheng Li, and Joseph N S Eisenberg. Informing optimal environmental Influenza interventions: How the host, agent, and environment alter dominant routes of transmission. PLoS Computational Biology, 6(10), 2010.
- [111] P. Stavova, A.K. Melikov, J. Sundell, and K.G. Naydenov. A new approach for ventilation measurement in homes based on CO₂ produced by people - Laboratory study. In Proceedings of 17th Air-Conditioning and Ventilation Conference, Prague, 2006.
- [112] DC Straus. The possible role of fungal contamination in sick building syndrome. Frontiers in bioscience (Elite edition), 3:562–580, 2010.
- [113] Donna F Stroup, Jesse A Berlin, Sally C Morton, Ingram Olkin, G David Williamson, Drummond Rennie, David Moher, Betsy J Becker, Theresa Ann Sipe, and Stephen B Thacker. Meta-analysis of observational studies in epidemiology: A proposal for reporting. JAMA: The Journal of the American Medical Association, 283(15):2008–2012, 2000.
- [114] Yuexia Sun, Zhigang Wang, Yufeng Zhang, and Jan Sundell. In China, students in crowded dormitories with a low ventilation rate have more common colds: Evidence for airborne transmission. PloS one, 6(11):e27140, 2011.
- [115] J. Sundell, H. Levin, W.W. Nazaroff, W.S. Cain, W.J. Fisk, D.T. Grimsrud, F. Gynzelberg, Y. Li, A.K. Persily, A.C. Pickering, J.M. Samet, J.D. Spengler, S.T. Taylor, and C.J. Weschler. Ventilation rates and health: multidisciplinary review of the scientific literature. Indoor Air, 21(3):191–204, 2011.
- [116] Martin Täubel, Helena Rintala, Miia Pitkäranta, Lars Paulin, Sirpa Laitinen, Juha Pekkanen, Anne Hyvärinen, and Aino Nevalainen. The occupant as a source of house dust bacteria. The Journal of Allergy and Clinical Immunology, 124(4):834–40, 2009.
- [117] K. Bart Teeuw. Airborne Gram-negative Bacteria and Endotoxin in Sick Building Syndrome. Archives of Internal Medicine, 154(20):2339, 1994.
- [118] Silver Türk, Sandra Mazzoli, Jelena Stšepetova, Julia Kuznetsova, and Reet Mändar. Coryneform bacteria in human semen: inter-assay variability in species composition detection and biofilm production ability. Microbial ecology in health and disease, 25:1–6, 2014.
- [119] Tze-wai Wong, Chin-kei Lee, Wilson Tam, Joseph Tak-fai Lau, Tak-sun Yu, Siu-fai Lui, Paul K S Chan, Yuguo Li, Joseph S Bresee, Joseph J Y Sung, and Umesh D Parashar. Cluster of SARS among medical students exposed to single patient, Hong Kong. Emerging infectious diseases, 10(2):269–76, 2004.

- [120] Peng Xu, Jordan Peccia, Patricia Fabian, John W. Martyny, Kevin P. Fennelly, Mark Hernandez, and Shelly L. Miller. Efficacy of ultraviolet germicidal irradiation of upper-room air in inactivating airborne bacterial spores and mycobacteria in full-scale studies. Atmospheric Environment, 37(3):405–419, 2003.
- [121] Zheng Xue and Youngwoo Seo. Impact of Chlorine Disinfection on Redistribution of Cell Clusters from Biofilms. Environmental Science & Technology, 47:1365–1372, 2013.
- [122] Wan Yang, Subbiah Elankumaran, and Linsey C Marr. Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care centre and on aeroplanes. Journal of the Royal Society Interface, 8(61):1176–84, 2011.
- [123] Shibu Yooseph, Cynthia Andrews-Pfannkoch, Aaron Tenney, Jeff McQuaid, Shannon Williamson, Mathangi Thiagarajan, Daniel Bami, Lisa Zeigler-Allen, Jeff Hoffman, Johannes B. Goll, Douglas Fadrosh, John Glass, Mark D. Adams, Robert Friedman, and J. Craig Venter. A metagenomic framework for the study of airborne microbial communities. PLoS ONE, 8(12):e81862, 2013.

Appendix A

Recommended Measurement Protocols for Epidemiologic Studies of Mechanically Ventilated Buildings

A.1 Introduction

The purpose of this section of the report is to provide recommended protocols to survey and document the characteristics of a building or space that influence air movement and airborne pathogen transmission (APT). These protocols are to be used by researchers who are designing and implementing epidemiological studies of APT in buildings.

Protocols need to be selected by the research team to suit the building or environment under study. Not all protocols will be applicable to all settings. The appropriate protocol to use to make a measurement (or series of measurements) depends on the type of study for which it is being used and the objective of the study. Some of these recommended protocols have not been reported in the peer-reviewed literature on HVAC systems and APT. For those parameters that have been used and reported in the literature, we have provided a few representative citations that have measured this parameter. These citations are reviewed in more detail in the previous section of this report (Literature Review). Also the protocols are written to be informative, providing detail on the most important aspects of the measurement. They are not, however, exhaustive regarding the step-by-step process of the measurement. Additional references are provided in which more detail can be obtained on the measurement procedure. Here we recommend protocols we think are important. These are general protocols for use with future APT studies; while measurement method recommendations are split up for some possible HVAC configurations, not all possible

HVAC configurations are accounted for. Protocols appropriate to the specific building/HVAC type and study type will need to be carefully selected by researchers, engineers, etc. carrying out a study of APT and some building-related parameter. The researchers designing the study need to have knowledge of HVAC system operation and should use proper judgment when interpreting and applying these protocols in the field.

The measurement of an HVAC factor in a building can be considered a **proxy for exposure**. Since it is still almost impossible to directly measure exposure to an airborne pathogen that transmits a disease, epidemiologic studies use proxies. An example of one study that was able to directly measure exposure to an airborne pathogen was the measurement of *Mycobacterium tuberculosis* from coughing patients by Fennelly et al. (2004). This measurement can be considered a direct measurement of an airborne pathogen, however it was not measured in the actual room air, but in sputum-induced coughs. Another study, Myatt et al. (2004), directly detected rhinovirus both from air samples and nasal mucus samples in an office environment by polymerase chain reaction (PCR) technology. An example of an exposure proxy in a building is the amount of outdoor air a building brings into its occupied environments. This variable is appropriate to use since the potential for an airborne pathogen exposure decreases as the indoor air is diluted with pathogen-free outside air. It is not a direct measurement, however, and so epidemiological methods are needed to infer associations between the factor and the disease.

All epidemiology studies are dependent on there being sufficient **variability** in the study factors of interest as well as sufficient variability in the health outcomes of interest. Some HVAC factors are more amenable to observational studies than others, depending on whether or not there is sufficient variability in the HVAC variables of interest and if there is sufficient data from recorded measurements. Other HVAC factors may be better studied in interventional studies. The health outcome of interest necessarily would inform what HVAC factor would be best to measure.

Obviously the appropriate HVAC factor(s) must be identified and measured correctly. There are a number of more specific considerations that must be addressed in the identification of which factors are important.

- (1) There should be some **biological plausibility** that the factor may have an impact on disease transmission and would be a good proxy for exposure.
- (2) The factor needs to be something that can be measured. In epidemiology generally, the data analysis requires categorical variables. For example, how many individuals contracted influenza-A while working in a building that had a low ventilation rate per person. However, many of the variables of interest in buildings are continuous variables (e.g. air-exchange rate) and they must be measured with sufficient accuracy to distinguish various levels within the range of the variable reflected in the EPI study (see 4 below). In some cases, a variable can be discrete, for example, displacement ventilation vs. mixing ventilation, and these can be assessed by surveys, engineering drawings, and interviews with facility managers. In the context of our proposal, the factors measured should be directly affected by HVAC design or operation. For example, influenza-A virus per cubic centimeter of air may well relate to influenza-A infection rates. If we could measure this virus concentration, it would be a direct measure of exposure. In the context of this project, we would want to know how the HVAC system determines the value of this variable, e.g. determine how the airflow per person impacts this variable. Does it increase or reduce exposure? Does it increase or decrease disease incidence?
- (3) As suggested above, there must be enough variation in the predictor variables of interest for them to have an impact on the outcome of interest. For example, air-exchange rate may have a substantial impact on airborne disease transmission. However, if all of the spaces under investigation have similar air-exchange rates, there is no way for that study to determine the influence of that factor on disease transmission no matter how well the air-exchange rate is measured. In Myatt et al. (2002), the researchers were worried that there would not be enough variation in outdoor air rates, so they varied the dampers in the HVAC system to obtain more variability. Thus, it is important in the present project to consider the potential range of a factor and whether or not that range is sufficient for

it to be included in an EPI study. It must also be possible to match the variation in a factor to the measurement of the outcome. For example, the air-exchange rate in a space may vary by a factor of two or three or more during a typical day in response to thermal load changes. However, such variation may not be in a useful form if the infection rate can only be quantified over a period of days or weeks and the measured infection rate cannot be associated with a specific rate in this case. In comparison, two buildings with different air-exchange rates could be readily matched to infection rates since the affected population for each ventilation rate is different.

- (4) There are many factors that influence whether or not a given person will acquire a given disease at a given time and HVAC factors for a given space may or may not have a dominant impact. As long as the non-HVAC factors occur randomly within the population studied, appropriate statistical analysis can sift out the HVAC effects. However, these random effects tend to blur the results and increase the sample sizes required to obtain reliable results. Addressing these external factors is not within the scope of the present study except to the extent that they may impact the number of spaces that need to be evaluated which in turn may impact the types of measurements that are feasible. **Random sampling** has not been applied in any of the large population-based EPI studies of building factors and APT (except in Hoge et al. (1994), which was during a disease outbreak of pneumonia). This is an important design characteristic that must be addressed in future studies.
- (5) **Confounding** can be a limitation of nearly all epidemiology studies. “Confounding occurs when all or part of the apparent association between the exposure and outcome is in fact accounted for by other variables that affect the outcome and are not themselves affected by exposure (Porta 2014).” Confounding can be divided into two broad categories, external and internal. In the case of the present study, external confounding occurs when a non-HVAC factor that influences infection rates is associated with an HVAC factor of interest. Consider a hypothetical example where two buildings were studied, one with a

high air-exchange rate and one with a low air-exchange rate. However, it turns out that the occupants of the low air-exchange rate are predominately low income and the occupants of the high air-exchange rate building are predominantly high income. Also, suppose that higher income people have much higher immunization rates for influenza. So if the epidemiology study shows that the occupants of the higher air-exchange rate buildings have lower influenza infection rates, it may not be possible to determine if it is a result of the high air-exchange rate or the high immunization rate since the two variables are related to each other (confounded). Designers of good epidemiology studies must spend considerable effort at addressing potential confounding and attempting to eliminate its effects. Such external confounding is outside the scope of the present project. However, there is also internal confounding. Internal confounding occurs when there is a relationship between two or more of the factors of interest. For example, air-exchange rate and ventilation flow per person are both HVAC factors that may impact disease transmission. In general, spaces with high occupancy have higher air-exchange rates and lower ventilation airflow per person than spaces with low occupancy due to design considerations required to meet the thermal loads and ventilation needs. Hoge et al. (1994) found that the ventilation rate per person was low by a factor of two due to severe overcrowding of the jail under study. An epidemiology study that was not designed to carefully separate out these two factors could easily confuse air-exchange rate impacts with ventilation per person effects. This latter class of confounding is well within the scope of the present project and means to identify, address, and possibly eliminate such confounding should be a part of the protocols developed.

The primary functions of the HVAC system are to provide appropriate thermal conditions, appropriate humidity conditions, and appropriate air contaminant control. The HVAC system impacts disease transmission by its impact on concentrations of pathogens in the air, how long these pathogens remain in a space, how long these pathogens remain viable, and the locations to where these pathogens are transported. Once a susceptible person is exposed, the probability of

contracting the disease depends on many other biological factors, such as age, previous disease history, etc. With this perspective, HVAC related variables that are appropriate to consider in an epidemiology can be identified.

In the following discussion, key potential variables are identified and special considerations for each one are addressed. Then, measurements are **recommended** for future epidemiological studies based on the value of the measurement as well as the necessary resources/cost. Recommended measurement protocols are presented at four different levels corresponding to the order in which HVAC-related parameters should be considered when designing a ventilation-and-APT research study. The most important parameters to consider in the context of an EPI study are found in the earlier levels, and more exhaustive measurements and methods are found in the later levels. All research studies should begin by gathering the information described in Level 0. **Level 0** covers the fundamental information necessary prior to engaging in any measurement techniques – information such as HVAC system design and configuration – to ensure proper measurement methods are used further along in the study. Depending upon the objectives, experimental design, and resources available to the study, methods may then be selected from the following **Levels 1-2**.

A.2 Recommended Measurements

Any epidemiological study will be financially limited, especially if it is population-based and involves many buildings or zones, and trade offs will have to be made as to the number of buildings/zones included and the extent of the measurements conducted. In the discussion above we reviewed some of the many measurements that are possible in buildings for parameters that are related to APT. Many of these measurements are difficult, require access to restricted areas, require modification of some components of a building or HVAC system, or require many resources either in the context of costly equipment or of time/expertise. It is beyond the scope of the present study to address this trade off. Thus, the recommended measurements are presented more or less in order of priority given the financial resources available and value of the measurement in the context of the EPI study.

A.2.1 Level 0 – Space Description

A.2.1.1 Description of Study Space

General study space/building information collected will assist with identifying trends in epidemiological studies, while also providing insight for a disease outbreak studies. Most information can be obtained from the building construction documents and property management. We recommend obtaining the following:

- Building location
- Year of construction and any following renovation
- Gross square footage
- Number of stories above and below grade
- Primary function(office, manufacturing, hospital, education, etc.)
- General hours of operation
- Are windows operable?
- Does the building have an elevator?
- Are there any common mechanical issues of concern?
- Are there any common indoor environmental quality complaints?

Obtain copies of architectural and HVAC construction documents, preferably as-built drawings if available, that are pertinent to the subject space.

A.2.1.2 Description of HVAC System and its Operation

The boundary of the study space is primarily established by the HVAC systems that serve it. This makes understanding the HVAC system(s) and operation an essential first step when

surveying a building. The study space may be defined as just one HVAC zone or it may be made up of multiple HVAC zones. For example, if the study space of interest shares a common return and air handler with another space, it may be necessary to include the second space within the scope of the study, as depicted in Figure A.1. HVAC systems transport, mix, and dilute airborne pathogens within a space, provide filtration, and control temperature and humidity.

A.2.1.3 Distribution

Obtain copies of all architectural and HVAC construction documents, preferably as-built drawings if available, that are pertinent to the subject space. Review the HVAC floor plans and identify the air handlers serving the space of interest. Identify the complete supply and return duct networks associated with each air handler, as well as design airflow rates. The extent of the ductwork and zones served will define the boundary of the study space. If multiple air handlers serve one contiguous space, each air handler should be considered. Perform a site survey to confirm location of air handlers, associated ductwork, and spaces served.

If HVAC drawings are not available, as-built sketches that document air handler locations, associated ductwork routing, and geometry of spaces served should be produced during site survey. Figure A.1 illustrates how mechanical systems can transport air and airborne pathogens between spaces.

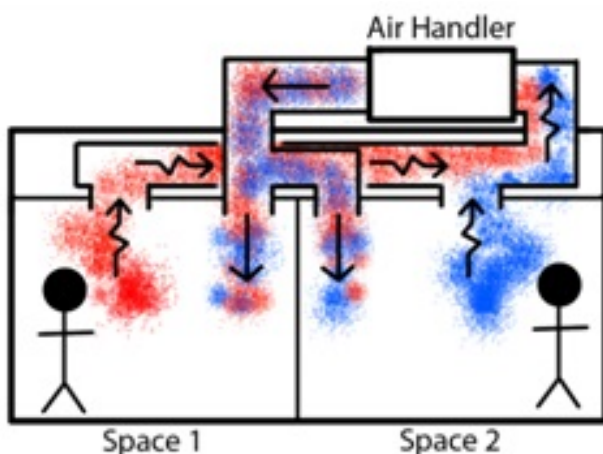


Figure A.1: HVAC transport and air mixing.

A.2.1.4 Air Handling Unit System Type

Review the available HVAC drawings and confirm the type of air handler unit(s) (AHU) that serves the subject space during the site survey. As discussed in the introduction, each AHU should be characterized by a few broad classifications: constant or variable air volume (CAV vs. VAV), existence of an economizer, and 100% outdoor air versus recirculation. Also, if the system does vary airflow rates or %OA, control sequences must be obtained to understand what set points control these airflow rates. For example, in the case of an economizer, outdoor airflow rates can be varied using many different control methods: fixed dry bulb, differential dry bulb, fixed enthalpy, electronic enthalpy, differential enthalpy, and dew-point, and dry-bulb temperatures.

Record the type, make, model, and serial number of the air handlers and terminal boxes serving the subject space so that product cut sheets can be obtained.

A.2.1.5 Filter Efficiency Rating

Record the observed Minimum Efficiency Rating Value (MERV) rating of filters identified in all AHUs and terminal boxes relevant to the study space. If there are multiple filters in series, record the order of filters relative to airflow. Interview property maintenance to understand the typical schedule of replacement.

A.2.1.6 Space Geometry and Characteristics

The geometry and characteristics of the envelope defines the subject space and influences infiltration and exfiltration with the outdoors and adjacent spaces. The locations of interior partitions and doors within the subject space influence circulation patterns.

As discussed above, the boundary of the subject space is primarily defined by the zoning of air handlers serving the space. This bounding “envelope” may be an entire building, a floor within a building, or a single room.

Identify each of the planes that separate the subject space from adjacent spaces, on all sides, making note of any substantial voids that might allow air to freely pass between spaces, such as

pipe penetrations. Use available floor plans, or create sketches to clearly define subject boundary conditions and location of interior partitions and doors within the space.

A.2.1.7 Occupant Density and Space Function

Understanding the functions of the space will provide insight for occupancy trends. Different space types have different occupancy characteristics. For example, an office conference room has a higher occupancy density potential than that of an open office floor plan. We are interested in understanding building usage and occupancy trends because people are the main vehicle of infectious agents, while also being susceptible to new infection. For example, Sun et al. (2011) tracked occupancy to test if there was an association between the incidence of common colds and crowding in college dorms. Hoge et al. (1994) documented occupancy that was twice as high as design, resulting in a lower ventilation rate per person than the ASHRAE standard.

Identify the function and floor area for each unique zone within the subject space. Count workstations, desks, bunks, etc., to estimate the maximum occupancy density in each space, if possible. Ask building management if building occupancy is tracked in any way. Use Table 6-1 in ASHRAE 62.1 - 2013 Ventilation for Acceptable Air Quality to estimate default occupancy densities for unknown spaces.

A.2.2 Level 1 – HVAC System Measurements

Many of the possible HVAC system measurements useful to epidemiologic study designs are difficult, tedious, or expensive, and may not have enough value for the cost in the context of an epidemiologic study. Level 1 provides a simplified and concise list of recommended HVAC system measurements that we believe are most valuable and cost effective for future epidemiological studies. Hierarchically, direct measurements are always recommended above indirect measurements when estimating ventilation flows. Additionally, outdoor air ventilation airflows are preferred over supply airflows, but both should be measured if possible. Our review of the current literature (Chapter 4) recommends that future epidemiologic studies include measurements of both exposure and out-

comes, but given that our protocols are organized hierarchically by importance and cost/difficulty, we do not include outcome measurements until Level 2.

A.2.2.1 Direct Measurements

As mentioned earlier in the discussion, direct measurements of outdoor airflow rates are very difficult for two main reasons: restricted access to outdoor air intakes or outdoor air ducts prior to mixing with recirculated air and low air velocities. The easiest and most suitable direct measurements for epidemiological studies can be made from within the space being studied. In the case of a DOAS or a 100% OA supply, a balometer can be used to directly measure outdoor airflow rates. In the more common scenario of a mixed OA and recirculated air supply, the balometer should still be used to measure supply airflow rates as this parameter is still useful by indicating the rate at which the room air is diluted with particle-free air, depending on the filtration efficiency. Using information gathered from Level 0 protocols, parameters of supply airflow rate per floor area and supply airflow rate per person should be calculated. In the case of 100% OA delivery to the space, outdoor airflow rate per floor area and outdoor airflow rate per person should be calculated.

A.2.2.2 Ventilation Flows Per Occupant and Per Area/Volume

Normalizing ventilation flows may aid with comparisons made across multiple studies in future meta-analyses of HVAC-related epidemiologic studies. If the study space in question has reasonably constant occupancy during data collection, we recommend reporting the outdoor and supply airflows on a “per occupant” basis. Additionally, outdoor and supply airflows should be reported “per floor area” and “per room volume”.

We would expect that pathogen generation be proportional, on the average, to the number of infectious persons present in that air space. Ignoring pathogen survival for the moment, the airborne pathogen concentration in the occupied zone, on average, will be determined by the amount of pathogen-free air provided to the space and the air exchange effectiveness. In systems with 100% outside air, or where all return air is effectively filtered, the flow of pathogen-free air is

simply the total supply airflow. As soon as return air gets involved, the definition and measurement of pathogen-free air gets much more complex. In a single-zone system without filtration, the outside airflow rate may be a reasonable measure of pathogen-free air. However, not all airborne pathogens are likely to survive the return air trip either due to becoming inactive or being deposited on system surfaces. Return air filtration also adds complexity as the filtration effectiveness is particle size dependent and pathogens are not necessarily distributed equally across particle sizes. In a single zone system with filtration, an approximation of the pathogen-free supply airflow rate would be as follows in equation A.1:

$$PFA = OA + RA \times E \quad (A.1)$$

Where PFA is the pathogen-free airflow rate, OA is the outdoor air rate, RA is the return airflow rate, and E is the system filtration efficiency¹ (%). Thus, determination of these three variables becomes important. When multiple zones are involved and connected via return air, the situation gets even more complex and it becomes very difficult, even conceptually, to separate out the effects of the adjoining spaces. If all of the spaces are similar in size and occupancy, then the above relationship could be applied, but on a multi-zone scale. If there is a lot of diversity between zones, it is almost necessary to do a case-by-case assessment. If there is reasonably effective filtration of return air, then total supply airflow to the space may be a reasonable approximation. If there is little or no filtration, then outdoor air may be a reasonable approximation.

A.2.2.3 Indirect Measurements

Indirect measurements can be made in the study space at fairly low costs and with little to no modifications necessary to the HVAC system and study space. Below are the indirect measurements that we recommend as alternatives if direct measurement is not a possibility. All measurements rely on the use of carbon dioxide sensors. We recommend using an instrument (or instruments)

¹ Use the efficiency for removal of particles of size 1 micron for bacteria, or 0.3 microns for virus.

with an accuracy of no more than ± 50 ppm (10% of the measured range or better would be best) between 0 and 5000 ppm.

A.2.2.4 Percent Outside Air

The percent outdoor air delivered to a space could be a parameter of interest affecting APT for use in future EPI studies, since outdoor air is usually assumed to be pathogen free and dilutes any airborne pathogens indoors. While outdoor airflow rate is the most common parameter measured among APT studies in the literature (Farant et al. 1991; Myatt et al. 2002; Myatt et al. 2004; Sundell et al. 2011), percent outdoor air may be another parameter of interest.

The percentage of outdoor air in the supply airstream can be found using CO₂ as a tracer gas based on mass balances of air and tracer at the air handler (Gothe et al. 1988; Olcerst 1994).

Based on a mass balance of air and CO₂ at the air handler, the percent outdoor air intake is given by equation A.2 (ASTM 2012; Persily 1997):

$$\%OA = 100 \times \frac{(C_r - C_s)}{(C_r - C_{out})} \quad (A.2)$$

Where %OA is the percent outdoor air intake, C_r is the CO₂ concentration in the recirculation airstream of the air handler, C_s is the CO₂ concentration in the supply airstream of the air handler, and C_{out} the CO₂ concentration in the outdoor air. When using this approach, C_r can be measured in the return duct, which is often more accessible than the recirculation duct. C_s should be measured at the air handler, as far downstream of where the outdoor air and return airstreams mix or in the supply duct supplying the space of interest being served by the AHU of interest. Care must be taken to ensure that C_s and C_r are representative of the average concentrations in those airstreams. Also, if a single sensor is being used, concentrations should be measured over the shortest time possible, within 10 minutes of each other is adequate (Persily, 1997). If multiple sensors are being used simultaneously, the longer the averaging period the better but caution should be used to ensure the instruments agree. If the system is equipped with an economizer, care must be taken to ensure

that the outside air damper position does not change over the duration of the measurements.

The precision in the percent outdoor air calculated with equation A.2 can be estimated using the following equation A.3 (ASTM 2012; Persily 1997):

$$\Delta\% = \%OA \sqrt{\frac{(\Delta C_r^2 + \Delta C_{out}^2)}{(C_r - C_{out})^2} + \frac{(\Delta C_r^2 + \Delta C_s^2)}{(C_r - C_s)^2}} \quad (\text{A.3})$$

where

$\Delta\%$ = precision of the percent outdoor air

ΔC_r = precision of the measured CO₂ concentration in the recirculation air

ΔC_s = precision of the measured CO₂ concentration in the supply air

ΔC_{out} = precision of the measured CO₂ concentration in the outdoor air

The equation above A.3 only accounts for the precision of the measured concentrations and neglects any bias due to calibration and operator error. The greatest factor affecting the precision of the %OA calculation is the difference between the indoor and outdoor concentrations of carbon dioxide, or C_r and C_{out} . In order to minimize the uncertainty of the estimated %OA, measurements should be made when the difference between indoor and outdoor carbon dioxide concentrations are at a maximum, or well into the occupied period of the day.

A.2.2.5 Air Exchange Rate

The average air-exchange rate (AER) can be measured using many different methods, including a mass-balance approach, tracer gas decay technique with an injected tracer gas (SF₆ for example; CO₂ can also be used or perfluorocarbons), or equilibrium analysis. These types of analyses are all based on the assumption of a well-mixed space. It may be difficult to gain permission to inject a tracer gas and equilibrium analysis relies on many underlying assumptions that must be accounted for to avoid large errors in ventilation estimates. For these reasons we recommend

continuously monitoring CO₂ and using a mass-balance approach to calculate AER.

Continuous Monitoring of Carbon Dioxide Concentrations

The indoor CO₂ sampling location should be selected to ensure a representative concentration value (not biased by the high concentration near CO₂ sources such as people or lower concentrations directly from ventilation air). Making measurements at multiple locations in a space and identifying one or more locations that yield a representative value should be used to select the indoor sampling location(s). A distance of 2 meters from any occupant is sometimes suggested as sufficient to avoid these effects (ASTM 2012). Sampling at return air grilles has also been suggested for representative concentrations (Fisk et al. 2012), but this should be verified in the study space as concentrations of CO₂ can be highly variable. A typical outdoor concentration range is between 350 and 600 ppm (or higher), and an indoor concentration range is equal to or higher than the outdoor concentration, and can often be much higher than 2000 ppm (Mendell et al. 2013).

Continuous monitoring of indoor carbon dioxide can be used to estimate outdoor airflow rates when steady-state conditions do not exist. This method involves applying a mass-balance model assuming well-mixed conditions to interpret CO₂ vs. time measurement data. All parameters in the mass-balance model can vary in time. Note that this approach can be used in an occupied space. This type of analysis has been used previously, for example by Lunden et al.(2003) to estimate the outdoor infiltration of PM2.5 into a residence with indoor sources.

Equation A.4 is the mass balance model for CO₂ in a space. This model describes the change in CO₂ concentration over time $\left(\frac{dC(t)}{dt}\right)$ in a space by tracking the amount of CO₂ generated $\left(\frac{E(t)}{V}\right)$, the amount of CO₂ in the air entering the space with outdoor air $\left(\frac{Q(t)}{V}C_{in}(t)\right)$, and the amount of CO₂ leaving the space $\left(-\frac{Q(t)}{V}C(t)\right)$. Use of the integral form of the mass-balance equation (A.5) allows for numerical integration of time series data from continuously monitored CO₂ in order to calculate ventilation rates $(Q(t))$ in the absence of steady state/equilibrium conditions.

$$\frac{dC(t)}{dt} = \frac{E(t)}{V} + \frac{Q(t)}{V}C_{in}(t) - \frac{Q(t)}{V}C(t) \quad (\text{A.4})$$

$$\int_{t_2}^{t_1} \frac{dC(t)}{dt} dt = \int_{t_2}^{t_1} \frac{E(t)}{V} dt + \int_{t_2}^{t_1} \frac{Q(t)}{V}C_{in}(t) dt - \int_{t_2}^{t_1} \frac{Q(t)}{V}C(t) dt \quad (\text{A.5})$$

The AER is estimated as $Q(t)/V$. Note that the model assumes that the air handler is only supplying air to that single space. In the case that the air handler recirculates air from other locations besides the space of interest (i.e. a multi-zone air handler whose recirculation air is made up of multiple return air streams), an extra term for the recirculation stream must be added to the model accounting for any CO₂ contributions from the return air of spaces other than the space of interest.

When equilibrium and steady-state conditions exist, a much simpler analysis using CO₂ measurements can be done (equation A.6):

$$\begin{aligned} QC &= E + QC_{in} \\ Q(C - C_{in}) &= E \\ Q &= E/(C - C_{in}) \end{aligned} \quad (\text{A.6})$$

This method was used by Mendell et al. (2013), Sun et al. (2011), Milton et al. (2000).

Further detail and discussion on these types of indirect measurement methods can be found in ASTM Standard E741 (2011), Persily (1994, 1997), or Mudarri (1996).

A.2.2.6 Environmental Parameters

A.2.2.7 Pressure Differences

In Level 0 of this procedure, visible compromises in the air barrier that encapsulates the subject space were documented. When a pressure differential is applied on both sides of these space boundaries containing voids, air movement occurs. This section focuses on defining those pressure differentials.

The following Equation A.7, commonly referred to as the Power Law Equation describes relationship between the pressure differential and openings.

$$Q = c(\Delta P)^n \quad (\text{A.7})$$

Q = airflow through opening, [CFM]

c = flow coefficient, [CFM/(in. of water)ⁿ]

n = pressure exponent, [dimensionless]

The values of c and n are determined by performing fan pressurization testing, otherwise known as a blower door test, where ΔP and the resulting Q is measured for multiple pressures. Blower door tests are more common in smaller residential buildings but they can technically be performed in any building as long as the blower has the capability of pressurizing the building to the desired pressure. This is a function of the leakage area, not the size of the building. A graph comparing Q and ΔP is generated and a curve fit is applied to the data to obtain values for c and n. The value of c accounts for the geometry and area of the total amount of openings in the space. (ASHRAE 2013)

Deploy differential pressure sensors with data logging capability between the subject space and adjacent spaces and record data for at least one week during each season. The differential sensor tubing should be routed where it will not be disturbed. It is also important for accurate data collection that the tubing be suspended in a location shielded from direct air movement

from diffusers and people walking, central in elevation. A nice “check” for differential pressure measurements is to compare the results with the design pressure of the space (found in Level 0) as well as with the differential between supply and exhaust airflow measurements made with a balometer.

A.2.2.8 Humidity and Temperature

Depending on the type of airborne pathogen, the infection rate and survivability may be dependent on the temperature and relative humidity of air. For example, a study by Noti et al. (2013) found that an indoor relative humidity $> 40\%$ will significantly reduce infectivity of influenza. Understanding the temperature and relative humidity in the subject space will provide insight for survivability and infection rate for infectious agents.

The temperature and relative humidity of an interior space will vary with HVAC operation, the opening and closing of windows, and infiltration. A temperature and relative humidity data logger should be deployed in the subject space for at least one week during each season, to understand the variations.

A.2.2.9 Carbon Dioxide Concentrations

Some of the very aspects that hinder the use of CO_2 for estimating outdoor airflow rates make it good for *exposure* assessment. It is transient and responds as exposure changes making it a good indicator of exposure at a given point in time whether or not conditions are steady state or transient. It automatically reflects the contribution of people who are present and the transient build up or decay of concentrations in the environment. Similarly, it automatically accounts for the differing contribution from people who are far away or close by. It automatically accounts for high and low levels of ventilation. Its spatial variation in space reflects the variation of exposure in a space.

In its most simple form, the difference between the CO_2 concentration in a space and the outdoor CO_2 concentration is an indicator of exposure to occupant-generated airborne pathogens

(Mendell et al. 2013; Myatt et al. 2002). However, there are several limitations to this simple approach:

- (1) It assumes there is an equal probability of all occupants generating infectious airborne pathogens. In most environments, this approximation is probably reasonable. There are a few situations where it clearly is not, such as in care of infectious patients.
- (2) It assumes there are no other sources of CO₂ other than occupant generation and outdoor air.
- (3) It does not account for air filtration, which removes pathogens but not CO₂. In such cases it will overestimate exposure. Corrections can be made for this effect but those corrections require knowledge about the various airflow rates to a space, the filtration efficiency for each of those airflow rates, and the CO₂ concentrations for those airflow rates.
- (4) It does not account for the decrease in survival with time of pathogens. Thus, in spaces with slow air-exchange or in buildings with long recirculation routes, a significant fraction of the pathogens originally present may no longer be infectious while CO₂ concentrations do not experience this decay. As a result, CO₂ concentration may overestimate exposure.
- (5) It does not account for self-generated CO₂. Since we cannot normally infect ourselves, our own CO₂ generation does not contribute to exposure. In a simple, perfectly mixed model, the CO₂ concentration can be corrected for self-generation as follows.

$$C'' = C_o + [(n - 1)/n](C - C_o) \quad (\text{A.8})$$

C'' is the corrected CO₂ concentration

C is the measured CO₂ concentration

C_o is the outdoor CO₂ concentration

n is the number of occupants in the space

Obviously, the correction is insignificant in spaces with more than a few people present. However, it can be applied in simple spaces where the well-mixed approximation applies at the measurement site, for example, a room with 100% outside air single air exhaust with the CO₂ concentration measured in that exhaust. Corrections can be applied in more complex situations such as with multiple zones and recirculation. However, as in Equation A.8 above, information about flows and CO₂ concentrations in those flows is required. Since most spaces of interest have values of “ n ” that are not small, this correction is normally not a factor but should be considered in small spaces (e.g. dormitory rooms).

While care must be used in applying indoor CO₂ concentrations for any environment, it is difficult to imagine a more promising, relatively low-cost indicator of airborne pathogen exposure for an epidemiological study. While continuous monitoring of CO₂ is already recommended in the ventilation measurements section, its value is further emphasized by the exposure assessment application as well.

A.2.2.10 Outcome Measurements: Absenteeism

If a study is able to incorporate measurements of both exposure and outcomes, associations can be drawn to estimate thresholds where certain reductions in exposure will also reduce illness incidence. This powerful association can inform the design of HVAC systems to ensure certain exposures are reduced below a certain level. However, outcome measurements can be very difficult to incorporate into a study design and require large sample sizes and long study duration. The simplest way to estimate illness incidence, albeit with high uncertainty, is to obtain absenteeism records from the study participants employer. Alternatively, illness incidence can also be recorded with self-reporting. Large sample sizes are needed due to often poor participation rates and uncertainty in reporting and long study duration is needed to distinguish seasonal illness trends.

A.2.3 Level 2 – Biological Exposure Measurements and Clinical Outcome Measurements

A.2.3.1 Speciation

To quantify the **abundances** of the known pathogens in a specific location in a building (supply duct or still room), air samples must be captured on sterile filters with a pore size relevant to the organism that is being sampled. It is recommended that as large an air volume be sampled as possible to ensure a high enough microbial load for amplification. Bowers et al. (2009) details a method that used 0.22 μm sterile, disposable, open-faced cellulose nitrate filters and collected aerosol on the filter using a vacuum pump with a flow rate of 7.5 L min⁻¹. They sampled a volume of between 2.5 and 5.4 m³. Unexposed negative control filters must be run alongside each collected sample to ensure that incidental contamination does not occur. Filters can be stored at -80°C until time for the DNA extraction assay.

A trained microbiologist, or experienced microbiology laboratory, should do this part of the protocol. DNA is extracted according to the method described previously (Bowers et al 2009). DNA concentrations are quantified using a PicoGreen dsDNA fluorometric assay, and the concentrations of all bacteria and/or of specific bacterial taxa are determined using established quantitative PCR assays. Briefly, all of the **quantitative PCR** assays are run in triplicate for each collected sample on a real-time PCR instrument, such as the Eppendorf Mastercycler ep realplex system, with data analyzed as described previously (Fierer et al. 2005). A DNA standard containing the targeted gene region must be generated for each assay. For “all bacteria” qPCR, this standard is the bacterial genomic DNA, e.g., from *E. coli*, and for qPCR for specific organisms, this is either genomic DNA from the organism of interest, or DNA from a plasmid standard. Plasmid standards are generated from PCR amplification of a gene of interest from DNA from the stock cultures, followed by cloning of the amplicons. Standard curves for each assay are generated using triplicate ten-fold dilutions of known amounts of standard DNA, and the genome or gene copy number (a proxy for abundance) is determined for each sample by comparison to the standard curve. PCR can be completed within

6 hours of sample collection to demonstrate near-real time assessment.

A.2.3.2 Real-Time Fluorescence Techniques

Fluorescence-based detection technologies were developed by military research communities, primarily to provide early warning against bio-warfare agents such as airborne pathogenic bacteria (Hairston et al. 1997; Pan et al. 1999; Pinnick et al. 1995; Sivaprakasam et al. 2004). Two instruments have been developed by leading US particle technology companies, and are widely commercially available: the ultraviolet aerodynamic condensation particle spectrometer (UV-APS Model 3314, TSI Inc.; Shoreview, MN) and the wideband integrated bioaerosol sensor (WIBS-4A, DMT Inc.; Boulder, CO). Several other companies have recently begun offering similar technologies within approximately the last year (e.g. Aerosol Fluorescence Sensor, Biral (Jung et al. 2012); BioScout, Environics Ltd. (Saari et al. 2014)).

Though providing relatively limited information about the biological identification of observed particles, these biosensors provide a first approximation screen of biological aerosol content at high resolution in particle size and time (seconds-minutes). These instruments are designed to measure total super-micron aerosol concentrations and to discriminate between airborne particles of biological and non-biological origin by detecting fluorescent emission. The UV-APS and WIBS each probe a mix of fluorophores present in biological systems. These **fluorophores** include molecular byproducts of cellular metabolism (e.g. NAD(P)H, riboflavin; excitation ca.360 nm) , including airborne microorganisms that may be more likely to be viable. The WIBS, additionally, probes fluorophores that are considered to be ubiquitous in all cells of biological origin (e.g. amino acids, proteins; excitation ca. 280 nm).

These instruments are semi-portable, loud for occupied indoor environments when in operation, and require a somewhat temperature-controlled environment in which to run. They are also currently designed to only measure from still air, however an inlet could be acquired to sample in moving air such as in an HVAC duct. Thus, placement and operation in a building is not straightforward. Typically they would need a dedicated office space in which to run, or a closet, with a

sample line run from the instrument to the location of interest. The sample line must be electrically conductive (e.g. metal or conductive rubber), grounded, and the horizontal distance between the instrument and location should be kept to an absolute minimum. Tight bends or stretches of tube longer than ~ 0.5 m can cause particles losses in lines that can perturb quantitative assessments and introduce size-dependent biases.

We recommend these types of instruments be deployed in a building such that they sample from the following locations depending on the study design: supply diffuser in the space of interest, or middle of the most occupied room or space of interest. The instrument inlet should be located away from occupants, at least 3 feet. These locations could help elucidate if a potential airborne pathogen was entering the space, or being generated within the space.

A.2.3.3 Clinical Outcome Measurement

Although incredibly costly, invasive, and difficult to implement, a clinical or clinical-like study design with careful control of building parameters and confirmation of illness incidence from a physician or medical expert would be the strongest outcome measurement possible. While this method is much less uncertain than absenteeism, one limitation of this type of outcome measurement is the inability to have larger sample sizes, limiting the statistical power of the study. It is possible, however, that this type of study setting would lead to more robust datasets for establishing associations between airborne exposures and outcomes.

A.3 Review – List of measurements useful to meta-analyses

Below we provide a concise list of HVAC-related measurements that we recommend standardizing across future HVAC-related epidemiologic studies in an effort to conduct more robust future meta-analyses.

- Record basic HVAC system description

- * Air Handling Unit System Type (i.e. CAV vs. VAV)

- * Detailed operating schedule/control sequences
 - For example, do airflows change seasonally? With changes in occupancy?
- Outdoor air ventilation rate
 - * L/min (cfm), L/min/person (cfm/person), L/min/m² floor area (cfm/ft² floor area),
L/min/m³ space volume (cfm/ft³ space volume)
- Supply air ventilation rate
 - * L/min (cfm), L/min/person (cfm/person), L/min/m² floor area (cfm/ft² floor area),
L/min/m³ space volume (cfm/ft³ space volume)
- Outdoor air, recirculation air, and/or supply air filter rating (MERV)
- Exposure variables
 - * i.e. detection or concentration of airborne rhinovirus, steady state CO₂ concentration,
air exchange rate
- Outcome variables
 - * i.e. Self-reported or employer-reported absenteeism, clinically-confirmed illness incidence
- Occupancy density and patterns
- Are windows operable?
- List any interventions affecting airborne pathogen transmission (i.e. UV)
- Is infiltration from outdoors or adjacent zones of concern?
 - * Measurement of pressure differentials, if possible (Pa or inH₂O)