population, an aliquot of each sample was pasteurized for 15 minutes at 75º C, following the procedure developed by Barbeau *et al.* (1997), and then plated as described above. Negative controls and blanks were analyzed as well. Heavy metal concentrations in the HVAC filter, floor, and high surface dust were determined via atomic absorption spectroscopy (Perkin Elmer AAnalyst 600). Dust samples were digested according to the microwave-assisted digestion method 3030K (APHA, 1998) and the liquid extract from each sample was analyzed for selected heavy metals (Pb, As, Cd) according to method 3111B (APHA, 1998). A nonparametric statistical method, the Wilcoxon Rank-Sum Test, which does not assume any specific distribution of the data, was applied to compare and identify dissimilarities among the different data groups. A significance level of 0.1 was assumed owing to the small sample size and the conservative nature of this statistical test.

3.3 MICROBIAL COMMUNITY INVESTIGATION

In the second phase of the investigation, the analysis was expanded to a culture-independent approach potentially capable of more fully characterizing the microbial communities present indoors. Additional information regarding the methodology employed can be found in Appendices B and D. This phase was conducted in a subset of four of the above residences and in an unoccupied 110 m^2 manufactured home (test house) where the fan of the HVAC system was operated continuously during the investigation. The test house was mainly unoccupied, which reduced localized particle and microbial emissions and represented a good site to conduct detailed measurements. High-efficiency (minimum efficiency reporting values, MERV > 11) polyester HVAC filters were installed in all of the sites at the beginning of this phase and, at filter

algorithm (Stamatakis *et al.*, 2005). Finally, the microbial communities present in the different samples were compared using the Weighted UniFrac algorithm (Lozupone *et al.*, 2006; Lozupone *et al.*, 2007). A significance level of 0.1 was assumed due to the reduced number of sites and the exploratory nature of the investigation. More details on these metrics and their limitations appear in Appendix E.

3.4 FATE ANALYSIS

The objective of this phase of the dissertation was to investigate the fate of indoor airborne particles and the likelihood that HVAC filters can be effective samplers for indoor particle-bound contaminants. A scaling analysis was performed to estimate the probability that 0.001-100 μm particles would be removed from indoor air in a typical residence by deposition, exfiltration, or filtration through the HVAC filter. The volume of the residence was selected considering a typical floor area of 163.3 m^2 (US Bureau of Census, 2005) and assuming a ceiling height of 2.4 m for a total volume (V_T) of 391.9 m^3. To estimate the removal probability for each mechanism, the size-dependent characteristic time was considered for each removal process. The characteristic time for deposition was the particle size-resolved deposition loss rate coefficients (β), for exfiltration, the air exchange rate (λ) was utilized, while for filtration, the recirculation rate (λ_r) multiplied by the size-dependent filter removal efficiency (η) was used. The air exchange rate is the ratio between the flow rate in and out of a building (Q) and the volume of the residences, while the air recirculation rate is the ratio between the airflow through the HVAC system (Q_r) and the volume of the residences.

For deposition, the β values summarized by Riley *et al.* (2002) were utilized in the model. For exfiltration, the 10^{th}, 50^{th} and 90^{th} percentile values of the λ distribution reported by Murray and Burmaster (1995) were utilized. These values, corresponding to

However, it has limitations including the fact that similar percentage compositions may not necessarily mean that similar species are present. Species in the same phylum could be either extremely similar or quite distant from a phylogenetic standpoint. The same could be true for the opposite example, where different compositions in terms of phyla/classes could illustrate communities extremely different or rather similar, depending on the distance in evolution of the species present in the communities. Table 4 presents the UniFrac values for the comparisons between the HVAC filter and high surface dust samples in the residential sites. From the p-values, it appears that, although some differences in composition are present (Figs. 4 and 5), both bacterial and fungal communities in the filter and high surface dust samples within each residence investigated are not statistically different. Thus, the UniFrac results suggest that in a given residence, the microbial community present in high surface dust is similar to that present in HVAC filter dust and high-efficiency filters may be suitable samplers for assessing the composition of indoor microbial communities. This similarity seems to contradict some of the compositional findings described above, possibly because of the nature of the Unifrac analysis, which relies on a phylogenetic distance comparison rather than a species by species comparison.

While the UniFrac comparisons indicate that the microbial communities in HVAC filter dust and high surface dust are similar in a given residence, Site 7 had the lowest p-values of all the residences for both the bacterial and fungal community comparisons, with a bacteria p-value of 0.10, the threshold of significance. One possible explanation is the location of the HVAC filter in this residence. The filter was located at the return grille in the hallway, away from some of the rooms where the high surface dust sample was collected (living room and two of the four bedrooms) potentially resulting in differences in the particles collected on the filter versus those being deposited on indoor

5. SUMMARY AND CONCLUSIONS

The main contribution of this dissertation to the indoor air field is the evaluation of HVAC filters as a sampling mechanism for indoor contamination. To my knowledge, the relationship between the contaminants observed on HVAC filters and those observed in other indoor locations has not been explored in sufficient detail before to assess their potential application as samplers for residences. Specifically, this dissertation revealed that HVAC filters could be used to assess culturable microbial concentrations in buildings with levels similar to those observed in other indoor sampling locations and to the values reported in the literature. HVAC filter dust seems to be a favorable environment for microorganisms for the specific conditions present during this study (mostly warm and humid). The concentrations observed in different indoor sampling locations and across filters with varying efficiencies suggest that microbial concentrations are not likely to be influenced by particle size. Metal concentrations observed in the field investigation of residences revealed that Pb is present in higher levels than Cd and As, and may be associated with the age of the buildings suggesting a possible correlation with leaded-based paint. Metal concentrations in HVAC filter dust are statistically lower than that observed in high surface and floor dust samples. Additionally, dust samples from low efficiency filters had greater metal concentrations than did high efficiency filters. The last two points indicate that small particles may have greater metal concentrations than larger particles as previously suggested by other studies.

The investigation of culturable microorganisms revealed similarity in the concentrations in different indoor sampling locations. However, although the concentrations are similar, the composition of the microbial communities could still be different, emphasizing the importance of fully characterizing the microbial communities.

of PM_{10} is much larger than $PM_{2.5}$ suggesting that even the low MERV filters can retain many of the larger particles from vacuuming activities.

7	1	Low	49 ± 0.5 (.20 ± 0.002)	1300 ± 91 (763 ± 54)	26.0 ± 0.30 (78.8 ± 33)	60.1 ± 2.9	87	
	2	Mid	81 ± 0.8 (.32 ± 0.003)	1140 ± 79 (669 ± 46)			92	4.1 ± 0.002
8	1	Mid	48 ± 0.52 (.19 ± 0.002)	1150 ± 80 (676 ± 47)2				
	2	Low	27 ± 0.3 (.11 ± 0.001)	1200 ± 84 (705 ± 49)	24.7 ± 1.3 (76.5 ± 34)	52.5 ± 3.9	88	
9	1	High	76 ± 0.82 (.30 ± 0.003)	2730 ± 190 (1610 ± 110)				
	2	Mid	81 ± 0.8 (.32 ± 0.003)	2790 ± 200 (1640 ± 120)	24.0 ± 1.6 (75.2 ± 35)	54.2 ± 5.3	82	6.5 ± 0.002

[1]only initial measurement
[2]only final measurement

Figure 1 presents the mean culturable microbial concentrations in the HVAC filter dust from the nine sites investigated, expressed as CFU/g dust. Since two filters were collected and analyzed from each site, 18 total samples are represented in Figure 1 and the mean value for each site is shown. For each site, the left bar indicates the culturable concentration of bacteria while the right bar represents the culturable fungal concentration. The height of each bar indicates the mean culturable concentration and originated from the counts of the microbes with the ability to form colonies on the specific agar plates described in the Methodology section. The bottom section of each bar represents the spore forming fraction of the population, which is the fraction of the viable microbial concentration able to survive the pasteurization treatment. Only the error bars for the total height of the columns are shown in the figure and the bars on the lower portions were of similar magnitude.

these microbial concentrations in context, these values are similar to those observed in soil for both bacteria and fungi (Lovell *et al.*, 1995; Toro *et al.*, 1997).

The culturable bacterial and fungal concentrations observed in the current study are slightly higher than the values reported in the literature for settled dust (Bouillard *et al.*, 2005; Chew *et al.*, 2003). This difference may be attributable to the HVAC airflows that deliver airborne microbes and nutrients to HVAC filters. Many studies have suggested that microbial contamination of HVAC filters occurs because filters collect sufficient organic material and nutrients to support microbial growth (Burge, 1987; Kemp *et al.*, 2001; Pejtersen, 1996). Kemp *et al.* (1995) also observed enhanced fungal growth when additional nutrients were delivered to HVAC filters. The culturable microbial concentrations encountered in this study suggest that HVAC filters in residential buildings in a humid environment like central Texas during the cooling season represent a hospitable environment for microbial proliferation.

The microbial concentrations measured in this study represent only the culturable fraction of the microbial population able to grow on the specific media utilized. Toivola *et al.* (2002) estimated that only 1% of the microbial population indoors is culturable and molecular based tools offer the promise of being able to detect a much greater fraction of the microbial community, not just the culturable fraction. However, the extraction of DNA directly from HVAC filter dust cake is particularly challenging and, as reported by Ramakrishnan *et al.* (2006), the use of standard commercial DNA extraction kits often generates inconsistent results. Nevertheless, the authors are currently investigating these techniques and their applicability to further characterize microbial populations on HVAC filters.

Table 4 summarizes the median microbial concentrations observed on filters with different MERV ratings. Median microbial concentrations on HVAC filters were

relatively consistent across filters with different removal efficiencies. The median concentrations were typically within one order of magnitude of each other and application of the Wilcoxon Rank-Sum Test to the data did not find any significant differences between filters with different MERV ratings. Despite this general similarity, high-efficiency filters had the lowest median microbial concentrations for bacteria, fungi and fungal spores. As reported by Waring and Siegel (2008), the particle mass that accumulates on HVAC filters strongly depends on their removal efficiency, and high-efficiency filters capture a greater mass of particles. Typical bacteria and fungi cell sizes vary from less than a micron to several microns, depending on the microbial species. Therefore, high-efficiency filters are more likely to retain an elevated number of microbial cells. A high-efficiency filter also captures more non-biological particles, potentially providing microorganisms with a greater amount of substrate and nutrients, and therefore promoting their growth. However, the presence of non-biological particles will also increase the mass captured on the filters and serve to diminish the measured microbial concentration because it is based on CFU per unit mass (both biotic and abiotic) of dust captured. This is one possible explanation for the decreased microbial concentrations observed on the dust captured in the high-efficiency filters.

Table 4: Median microbial concentrations in HVAC filter dust for filters with different efficiencies.

Filter MERV	Bacteria	Bacterial spores	Fungi	Fungal spores
	CFU/g			
Low	$6\times10^{6} \pm 6\times10^{5}$	$5\times10^{4} \pm 9\times10^{3}$	$4\times10^{5} \pm 7\times10^{4}$	$1\times10^{3} \pm 1\times10^{3}$
Mid	$9\times10^{5} \pm 2\times10^{5}$	$7\times10^{4} \pm 7\times10^{3}$	$6\times10^{5} \pm 1\times10^{5}$	$8\times10^{2} \pm 1\times10^{3}$
High	$3\times10^{5} \pm 6\times10^{4}$	$7\times10^{4} \pm 3\times10^{3}$	$1\times10^{5} \pm 9\times10^{4}$	$6\times10^{2} \pm 7\times10^{2}$

Figure 2 summarizes the mean HVAC filter dust concentrations of lead, cadmium and arsenic for each site. Pb had consistently the greatest concentration in all the samples

with values ranging from 5.4 to 28.6 µg/g dust. The median Pb concentration across all samples was 13.0 µg/g. HVAC filter dust concentrations for Cd and As were lower than Pb concentrations with values varying in the 0.5 - 6 and 0.8 - 7.3 µg/g ranges, respectively. The median concentrations of Cd and As across all the samples analyzed were 1.9 µg/g and 1.4 µg/g, respectively. The metal concentrations reported in the literature for indoor dust are similar to those reported here for HVAC filter dust and are typically in the µg/g range, with Pb and Zn concentrations that tend to be higher than the other metals and can reach the mg/g range (Al-Rajhi *et al.*, 1996; Lisiewicz *et al.*, 2000; Turner *et al.*, 2006).

Sites 5, 6 and 7 had higher Pb concentrations than the rest of the sample. None of the three sites had attached garages or is located adjacent to a major highway, suggesting that leaded gasoline is not the major source of indoor lead. Sites 5 and 6 were the oldest sites investigated and we hypothesize that the elevated Pb concentration was derived from leaded paint, still in use when the residences were built. Several researchers (Chattopadhyay *et al.*, 2003; Kim *et al.*, 1998; Tong, 1998) provide evidence for this hypothesis. There was uncertainty about the age of Site 7, the other site with an elevated Pb concentration although it was located in a neighborhood constructed in the 1970s and was likely to have contained leaded paint. Site 3, the newest residence investigated had the lowest Pb concentration again supporting the hypothesis that leaded paint is an important contributor to indoor lead levels. A correlation between the age of a property and Pb levels in settled dust has also been observed by other researchers (Adgate *et al.*, 1998; Kim *et al.*, 1998; Tong, 1998). However this correlation is not entirely consistent throughout our study; for instance, Site 4, which is also a new residence, had a higher Pb concentration than several older sites in the study so other factors may be important. At a given site, our data suggests that a correlation between the Pb, Cd and As metal

interest. Once the impact of these factors is better delineated, HVAC filters may become a useful, widely-available sampling tool that can be collected with minimal effort and analyzed for a broad spectrum of contaminants.

CONCLUSIONS

We measured microbial and metal concentrations in HVAC filter dust collected from nine sites. We detected culturable bacterial and fungal concentrations in the 10^5 - 10^7 and 10^4 - 10^6 CFU/g ranges, respectively. Spore concentrations represented a smaller fraction, typically two to three orders of magnitude lower than the total concentrations. The microbial concentrations in the filter dust were slightly higher than settled dust concentrations reported in the literature and are in the same range as those reported for soil. These results indicate that HVAC filters in humid environments such as central Texas represent a hospitable environment for microbial proliferation. Microbial concentrations on filters with different removal efficiencies were relatively similar, typically within one order of magnitude. Mean Pb concentrations in the HVAC filter dust were particularly elevated with mean values as high as 29 µg/g, while Cd and As concentrations were on the order of a few µg/g. A possible correlation between the age of the site and the Pb concentration was observed suggesting that leaded paint is a possible source of indoor Pb dust. Differences in heavy metal concentrations were observed between buildings and filters, suggesting that several factors including the influence of filter efficiency, system run time and indoor contaminant distribution need more exploration before filters can be used as sampling devices.

REFERENCES

Adgate, J. L., R. D. Willis, T.J. Buckey, J.C. Chow, J.G. Watson, G.G. Rhoads, and P.J. Lioy 1998. Chemical mass balance source apportionment of lead in house dust. Environmental Science & Technology, 32, 108-114.

Kemp, S.J., T.H. Kuehn, D.Y. Pui, D. Vesley, and A.J. Streifel. 1995. Growth of microorganisms on HVAC filters under controlled temperature and humidity conditions. ASHRAE Transactions, 101 (1), 305-316.

Kemp, P.C., H.G. Neumeister-Kemp, G. Lysek, and F. Murray. 2001. Survival and growth of micro-organisms on air filtration media during initial loading. Atmospheric Environment, 35, 4739-4749.

Kim, K.W., J.H. Myung, J.S. Ahn, H.T. Chon. 1998. Heavy metal contamination in dusts and stream sediments in the Taejon area, Korea. Journal of Geochemical Exploration, 64, 409-419.

Lisiewicz, M., R. Heimburger, and J. Golimowski. 2000. Granulometry and the content of toxic and potentially toxic elements in vacuum-cleaner collected, indoor dusts of the city of Warsaw. Science of the Total Environment, 263, 69-78.

Lovell, R.D., Jarvis, S.C., Bardgett, R.D. 1995. Soil microbial biomass and activity in long-term grassland: effects of management changes. Soil Biol. Biochemistry, 27, 969-975.

Momani, K. A., Q. M. Jaradat, A. Q. Jbarah, I. F. Momani, A.A. Omari. 2002. Water soluble species and heavy metal contamination of the petroleum refinery area, Jordan. J. Environ. Monit. 4, 990–996.

Moritz, M., H. Peters, B. Nipko, and H. Ruden. 2001. Capability of air filters to retain airborne bacteria and molds in heating, ventilating and air-conditioning (HVAC) systems. Int. J. Hyg. Environ. Health, 203, 401-409.

Pejtersen, J. 1996. Sensory pollution and microbial contamination of ventilation filters. Indoor Air, 239–248.

Ramakrishnan, M.A., S. Anantharaman, S.W. Kim, N.J. Stankey, T.H. Kuehn, P.C. Raynor, and S.M. Goyal. 2007. Detection of airborne bacteria in HVAC filters by polymerase chain reaction. Abstracts from the American Association of Aerosol Research Annual Meeting, Minneapolis, MN, 2006.

Schleibinger, H., and H. Ruden. 1999. Air filters from HVAC systems as possible source of volatile organic compounds (VOC) – laboratory and fields assays. Atmospheric Environment, 33, 4571-4577.

Simmons, R.B., S.A. Crow. 1995. Fungal colonization of air filters for use in heating, ventilating and air conditioning (HVAC) systems. J. of Industrial Microbiology, 14, 41-45.

Tong, S.T. 1998. Indoor and outdoor household dust contamination in Cincinnati, Ohio, USA. Environmental Geochemistry and Health, 20, 123-133.

Toivola, M., S. Alm, T. Reponen, S. Kolari, and A. Nevalainen. 2002. Personal exposures and microenvironmental concentrations of particles and bioaerosols. Journal of Environmental Monitoring, 166-174.

Toro, M., Azcon, R., Barea, J. 1997. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (32P) and nutrient cycling. Applied and Environmental Microbiology, 63, 4408-4412.

Tringe, S., Zhang, T., Liu, X., Yu, Y., Lee, W., Yap, J., Yao, F., Suan, S., Ing, S., Haynes, M., F., Rohwer,Wei, C.,Tan, P., Bristow, J., Rubin, E., Ruan, Y., 2008. The airborne metagenome in an indoor urban environment. PLoS ONE 3(4): e1862.

Turner, A., and L. Simmonds. 2006. Elemental concentrations and metal bioaccessibility in UK household dust. Science of the Total Environment, 371, 74-81.

Verhoeff, A.P., and H.A. Burge. 1997. Health risk assessment of fungi in home environments. Annals of Allergy Asthma & Immunology, 78, 544-554.

Waring, M. S., and J. A. Siegel. 2008. Particle loading rates for HVAC filters, heat exchangers, and ducts. Indoor Air in press.

Yoon, Y. H., and P. Brimblecombe. 2000. Contribution of Dust at Floor Level to Particle Deposit within the Sainsbury Centre for Visual Arts. Studies in Conservation, 45, 127-137.

Appendix B

A potential alternative to the use of settled dust and air samples for microbial evaluation is the use of HVAC filters for indoor environment investigations. Collecting samples of HVAC dust may improve our understanding of indoor occupant exposure by providing an integrated measure of pollutant concentrations associated with indoor particles. Greater than 70% of the residential buildings in the United States have a central, forced HVAC system (US Bureau of Census, 2005), almost all with a built-in filtration system. These filters essentially serve as passive, long-term samplers that can be collected with minimal effort and analyzed for a broad range of indoor contaminants. Recently, Stanley *et al.* (2008) utilized filters in two large public buildings as bioaerosol sampling devices to determine the culturable bacteria concentrations and to identify selected culturable species present in air. While most of the molecular-based studies described above focused solely on settled dust, Tringe *et al.* (2008) investigated the microbial communities present on the dust that collected on two HVAC filters in two large shopping centers in Singapore. They reported that the two air samples (HVAC filters) have more in common to each other than with environmental samples (outdoor soil and water) collected in the proximity and originate from indoor niche. They also found more similarity between filter samples and indoor floor dust compared to outdoor ground-level dust. The purpose of the current study is to explore the microbial concentrations and communities on filters and compare them to indoor settled dust and air communities as a first step towards using HVAC filter dust as an integrated measure of microbial levels in residences. This paper focuses on bacterial and fungal culturable concentrations and communities present in HVAC filter dust and other indoor sampling locations in occupied residences and in an unoccupied full-scale test house. The investigation was divided into two phases: 1) Investigation of culturable microbial concentrations in settled and HVAC filter dust in eight occupied residences in Austin,

approximately four weeks apart from each other. In the same five buildings where three floor and high surface dust samples were collected, air samples were also collected from a height of 1 m to 1.5 m above floor level. An impinger (SKC Biosampler, Eighty Four, PA) was connected to a vacuum pump operating at a constant volumetric flow rate of 12.5 L min^{-1} for a period of 1 hour. The microorganisms were captured in a phosphate buffer solution (PBS) consisting of 8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4. All the samples were stored in a 4º C environmental chamber maintained at approximately 70% RH until the analyses were performed.

Sample Analysis

The enumeration of culturable microorganisms (both bacteria and fungi) present in the bioaerosol samples, settled dust, and HVAC dust samples were completed using the standard spread plate method 9215C (APHA, 1998). For the settled dust and the HVAC dust samples, the microorganisms present in the dust were transferred into PBS by sonication and vortexing for 10 minutes each. An aliquot of PBS was plated on R_2A agar containing 0.04 % cycloheximide and incubated at 30 °C for bacterial enumeration or on Sabouraud dextrose agar (SDA) plates containing 0.01% chloramphenicol and incubated at room temperature (approximately 23 °C) for fungal quantification. To estimate the spore-forming fraction of the population, an aliquot of each sample was pasteurized for 15 minutes at 75º C and then plated as described above. The Wilcoxon Rank-Sum Test, which does not assume any specific distribution of the data, was applied to compare and identify dissimilarities between the different data groups. A significance level of 0.1 was assumed owing to the small sample size and the conservative nature of this statistical test.

Sample Analysis

Approximately 50 mg of high surface dust sample was immersed into 50 ml of PBS, sonicated and vortexed for 10 minutes each to transfer the microorganisms to the liquid phase. Subsequently, the liquid solution was filtered first through a Whatman #41 (Whatman Inc., Piscataway, NJ) to remove large particles and then through a 0.2 µm GTTP Membrane Filter (Millipore, Billerica, MA) to separate the microbes from the particles, and the filters were stored at -80 °C until analysis. To sample the dust from each HVAC filter, nine 2.54 cm square pieces of filter material distributed in each quadrant were cut from the filter. Subsequently, they were all immersed into 50 ml of PBS, sonicated, vortexed and filtered as described above for the surface dust samples. For the bioaerosol samples, the PBS containing the microorganisms was directly filtered through a 0.2 µm filter.

The DNA from the microorganisms captured on the 0.2 µm filters was extracted using the Power Soil DNA (MoBio Laboratories, Carlsbad, CA) kit per manufacturer's specifications except for the following modifications. 100 µl of lysozyme (3 mg/ml) and 300 µl of a phenol-chloroform-isoamyalcohol (24:24:1) solution were added at the initial step in addition to the normal reagents. Also, the MP FastPrep-24 (QBiogene) was used instead of vortexing step. DNA samples were then PCR amplified using bacterial specific primers 8F (5'-AGAGTTTGATCCTTGGCTCAG-3') and 1492R (5'-GCYTACCTTGTTACGACTT-3') or fungal specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). These primers have been used successfully in several other microbial community studies (O'Brien *et al.*, 2005) and are useful for delineating and comparing the fungal community present in the samples collected from different indoor locations. Each 50 µl PCR reaction contained 1X PCR buffer, 1.6 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM

Lozupone *et al.*, 2007). A significance level of 0.1 was assumed due to the limited number of sites and the exploratory nature of the investigation.

RESULTS AND DISCUSSION

Phase 1: Culturable microorganisms

Fig. 1 shows the mean bacterial and fungal culturable concentrations for each of the sampling locations investigated in the residences. Multiple samples at the same site are given equal weighting, so there are 21 samples each of floor and high surface dust, 16 HVAC filter dust samples and five air samples shown in the figure.

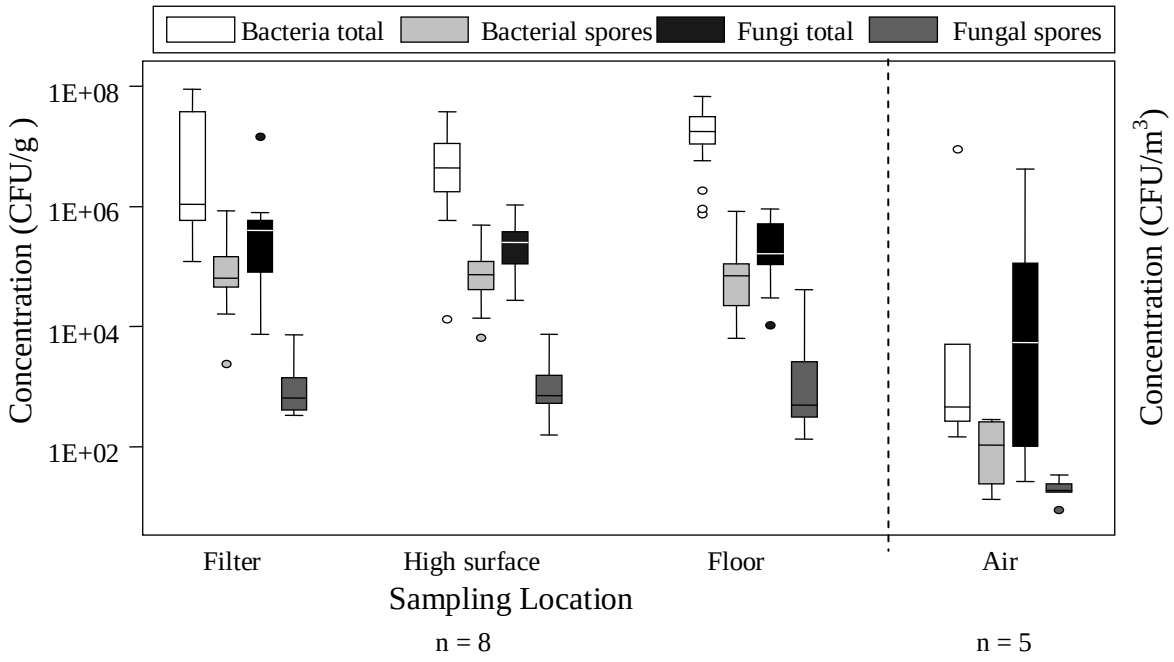

Fig. 1. Culturable microbial concentrations by sampling location. Air samples have dimensions of CFU/m^3 and all others have dimensions of CFU/g, with n= number of residences. The lowest end of the box represents the 25th percentile, the top represents the 75th percentile, and the horizontal bar inside the box indicates the median of the distributions. Single points outside the box are the outliers.

The culturable concentrations for both fungi and bacteria in Fig. 1 are generally consistent with the published literature. Indoor concentrations for bacteria and fungi vary

considerably with reported values ranging from 10^2 to 10^4 CFU m^{-3} for indoor air and from 10^5 to 10^7 CFU g^{-1} for settled dust (Andersson *et al.*, 1999; Bouillard *et al.*, 2005; Dales, 1997; Koch *et al.*, 2000; Ren *et al.*, 1999; Ross *et al.*, 2000). For all of the sampling locations, viable bacterial concentrations are higher than fungal concentrations and the estimated spore concentrations are approximately two orders of magnitude lower than total concentrations. Total bacteria concentrations range from 10^4 to 10^7 CFU/g, with a greater median concentration found on the floor, followed by high surfaces, and HVAC filter samples with median concentrations of 1.9×10^7, 4.4×10^6 and 1.1×10^6 CFU/g, respectively. This would suggest that larger particles or clusters of bacterial cells that are more likely to settle may have greater bacterial concentrations than small particles that remain suspended in air and are captured on the filter. Another possible explanation could be that the survival/growth conditions and nutrient availability on surfaces may be more favorable than on the filters. Fungal concentrations in the dust samples ranged from 10^3 to 10^7 CFU g^{-1} with reasonably consistent distributions across the dust sampling locations. It is important to note that airborne microorganisms may be attached to particles, and the size of the particles to which they are attached may have the greatest influence on their fate in an indoor environment (Hairston *et al.*, 1997).

A greater variation in fungal spore concentrations was observed in the floor dust samples, possibly due to the different types of flooring (i.e., carpet and hardwood floor) present in the different residences. However, there was a small variation in the bacterial concentrations suggesting that other factors beside floor surface characteristics may be important. In the air samples, there the median fungal concentration was greater than the median bacterial concentration and the concentration of culturable fungi varied considerably.

Several studies showed that indoor air fungal concentrations have elevated temporal and spatial variability (Hyvärinen *et al.*, 2001; Koch *et al.*, 2000) and thus the short sampling time may have affected the results. Stanley *et al.* (2008) calculated low indoor air culturable concentrations for selected bacterial species, often below 4 CFU/m^3, based on HVAC filter concentrations. The results in the current study may diverge from those due to differences in quantification techniques and the fact that HVAC systems in the current study supplied 100% recirculated indoor air and operated intermittently when the thermostat called for conditioning.

Fig. 2 summarizes the mean culturable concentrations of bacteria and fungi in the floor dust, high surface dust and HVAC dust at each of the eight sites investigated. The concentration of bacteria was fairly consistent within one order of magnitude across most sites except for Sites 1, 2 and 3. At Site 3, HVAC filter and high surface dust concentrations were quite similar but the floor dust samples had much greater concentrations and may have been influenced by tracked-in particles from outside. The difference between the HVAC filter and the high surface dust samples at Sites 1 and 2 may be due to the reduced efficiency of the filters collected from these sites, specifically one low- and one mid-efficiency filter for Site 1 and two low-efficiency filters for Site 2. The reduced efficiency of these filters makes them less ideal sampling devices and increases the probability of observing differing levels on the filters than on the floor or high surface. The difference in microbial concentrations on the filters and those found in surface and floor dust at these three sites may also be attributable to the cycling of the HVAC system (Noris *et al.*, 2009), suggesting that HVAC filters in residential buildings where the HVAC system is operated sporadically may be less representative of indoor contaminant levels. Nevertheless, despite some site-specific differences, the Wilcoxon

study. For all the residential sites investigated, Proteobacteria were present in greater proportion in the filter dust samples than in the high surface samples, with mean values of 65% and 39% respectively. Tringe *et al.* (2008) utilized a DNA-based technique similar to the current study and also observed an elevated proportion of Proteobacteria on HVAC filters in two commercial buildings. These results contrast to those reported by Stanley *et al.* (2008) who observed that the gram positive *Bacillus* (of the Firmicutes phylum) was the most commonly identified group in a culture-based study of HVAC filter bacterial communities. Thus, the prevalence of gram-positive bacteria in the Stanley *et al.* (2008) study may be due to a bias of culturing techniques that favor gram-positive bacteria. The results from culture-independent studies described herein and by others suggest that Proteobacteria represent a significant fraction of the indoor air bacterial community and that this phylum may better tolerate the environmental conditions encountered in air (Brodie *et al.*, 2006; Fierer *et al.*, 2008) and on HVAC filters. One explanation could be that they possess a greater fraction of key genes involved with resistance to desiccation and oxidative damage, as suggested by Tringe *et al.*, (2008). While Proteobacteria dominated the filter dust samples, an opposite trend was observed for Actinobacteria, with the mean percentage in the high surface samples more than four times higher than that found on the filters, 26% versus 6%.

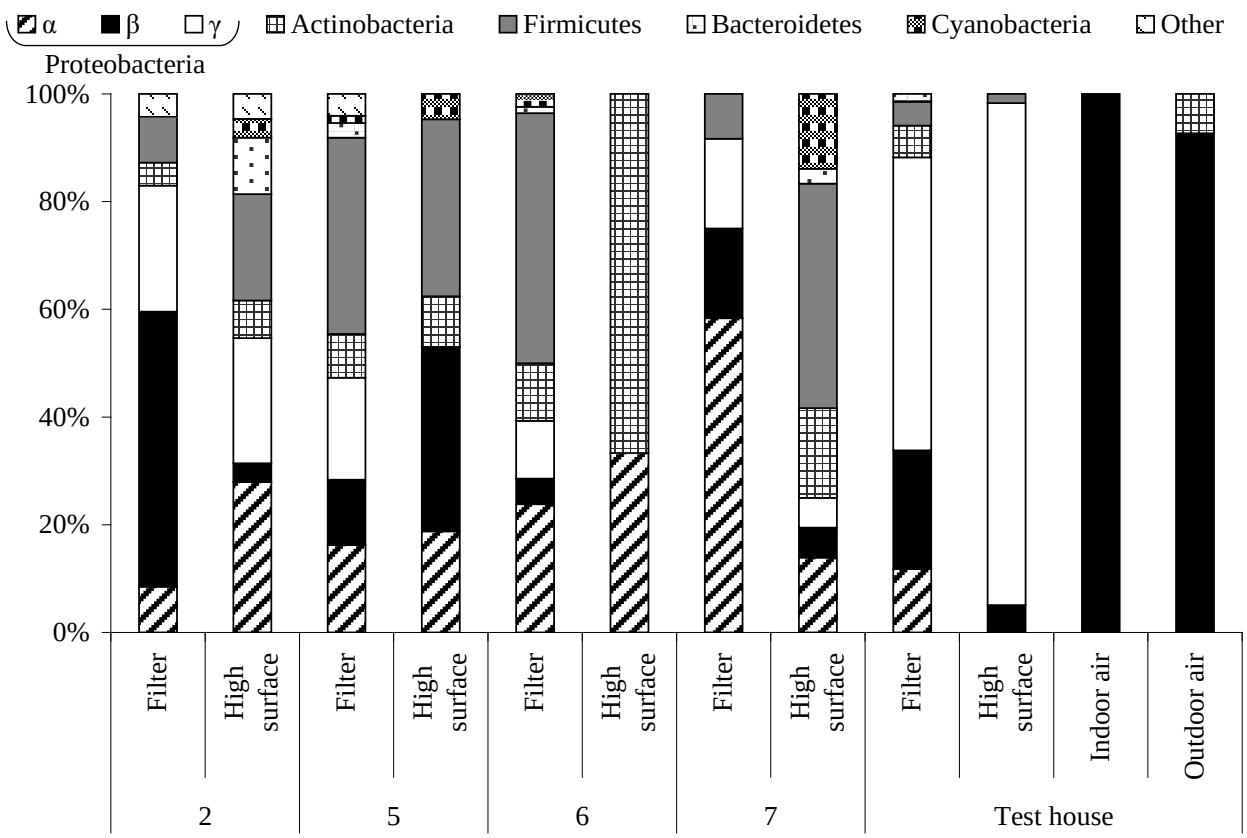

Figure 3. Bacterial composition at the phylum level for the sequence libraries obtained from all the samples analyzed.

Comparison of the clone libraries generated from the dust samples in occupied residences to those in the unoccupied test house indicates that a much greater proportion of gram-positive bacteria, mainly Firmicutes and Actinobacteria, were present in the residences versus in the test house, with mean values of 41% and 6% respectively. This increased proportion of gram-positive bacteria in occupied buildings supports the speculation that many gram-positive bacteria found indoors may be attributable to human sources (Horak *et al.*, 1996; Pakarinen *et al.*, 2008; Rintala *et al.*, 2008; Täubel *et al.*, 2009). Rintala *et al.* (2008) examined the bacterial communities in surface dust in two buildings across seasons. They observed higher variation in microbial composition between buildings than between seasons suggesting the development of site-specific

most common phylum was Basidiomycota. However, they also observed an increase in Dothideomycetes, and therefore in Ascomycota, during the summer months which represent a more similar climate to that encountered in central Texas in summer and fall. In the *Sordariomycetes* class, the genus *Fusarium* spp. was the most commonly detected which is consistent with results of other studies (O'Brien *et al.*, 2005; Pitkäranta *et al.*, 2008) which also used a molecular-based approach. Some culture-based studies reported elevated concentrations of the genera *Penicillium* and *Aspergillus* spp. in indoor and outdoor communities (Koch *et al.*, 2000; Ren *et al.*, 1999). However, in the current study, we observed a limited proportion of the class corresponding to these genera, *Eurotiomycetes*. This discrepancy could be due to a specific bias of the culturing methods that favor these species.

(32%) and 6 (29%), the p-values are well above the threshold. However, the authors suggest caution in interpreting these results because of lack of direct monitoring of HVAC operation during the measurements described here.

Table 1 UniFrac significance value for the filter to high surface comparison in the four residential sites.

	UniFrac Significance (P-value)			
	Site number			
	2	5	6	7
Bacteria	0.41	0.35	0.48	0.10
Fungi	0.30	0.65	0.55	0.16

Table 2 shows the UniFrac significance values for the samples collected in the unoccupied full-scale test house. The p-values for the microbial community comparisons between the filter and high surface dust samples (second column) are lower than those determined for the residences (Table 1) suggesting that human-associated microorganisms dominate the communities in occupied residences, as reported by others (Rintala *et al.*, 2008; Täubel *et al.*, 2009). This could be due to the fact that in residences, occupants generate particles through their activities and introduce microorganisms that could deposit onto surfaces or be captured by the filter leading to a more homogeneous distribution of microorganisms in the indoor environment. In contrast, the fungal communities in the HVAC filter and high surface dust samples in the test house are statistically different, while bacteria are not, suggesting the fungi may be more prone than bacteria to develop communities adapted to the specific environment. The difference in the fungal communities observed in filter and high surface dust in the test house could be attributable to the increased proportion of Sordariomycetes in filter dust.

Table 2 UniFrac significance values for the samples collected in the test house

UniFrac Significance (P-value)

	Filter vs. high surface	Filter vs. indoor air	High surface vs. indoor air	Indoor vs. outdoor air
Bacteria	0.13	0.14	**0.01**	0.12
Fungi	**0.01**	0.24	**0.03**	0.19

The results from the month-long investigation in the unoccupied test house indicates that the filter and the composite indoor air sample (third column) were not statistically different supporting the findings of Tringe *et al.* (2008) that suggested that HVAC filter dust can be used as an integrated measure of airborne microbial communities, even though some specific differences in the community may occur. HVAC filters are in place for extended periods of time, therefore, during their usage, a great volume of air is filtered through them (Stanley *et al.*, 2008) and only the microorganisms that were, at some point in time, airborne have the opportunity to be captured on the filter. In this study, the airborne microbial communities derived from the analysis of a composite sample of 20 daily 1-hour samples represent a more integrated measurement. Short-term air samples are reported to have a great temporal variability (Brodie *et al.*, 2006; Fierer *et al.*, 2008) and by compositing daily collections we tried to overcome this limitation so as to be able to compare the indoor air samples to the dust that collected on the surfaces and on the filter over the same period.

The values reported in the fourth column of Table 2 indicate that both bacterial and fungal communities in high surface dust and indoor air were statistically different in the unoccupied test house. Therefore, high surface dust samples are not representative of airborne microbial communities possibly because surface dust samples may be influenced by the microorganisms attached to the particles that are more likely to deposit on surfaces (i.e., larger particles) rather than stay in air. Single bacterial and fungal cells range from 0.5 μm to 50 μm, with fungal spores generally larger than bacterial spores (Li and Li, 1996; Terzieva *et al.*, 1996). The size of these biological particles influences their fate

and the probability of being detected in the different sampling locations, since larger particles are likely to settle while smaller particles may stay longer in air and have more opportunities to be captured on HVAC filters. In the residences, the presence of occupants and the microorganisms associated with them tend to homogenize the communities. In the test house, these phenomena are more evident due to the limited occupancy. The significantly different bacterial community between these high surface and indoor in the test house seems to be largely attributable to the different composition of Proteobacteria. Air samples were dominated by β-Proteobacteria with 100% and 93% of the clone libraries for, respectively, indoor and outdoor air samples. In contrast, test house dust samples seem to be dominated by γ-Proteobacteria which constituted 54% of the bacterial clones encountered on the filter dust and 93% of those found on high surface dust sample (Fig. 3). For fungi the difference between high surface and air may reside in the composition of the *Dothideomycetes* class. The high surface sample is constituted by 53% of the subclass *Pleosporomycetidae* and 24% of the subclass *Dothideomycetidae*, while for indoor air the proportion for these two subclasses is inverted, with the former accounting for 21% and the latter 57% of the sequences (Fig. 4). Finally, we observed similar microbial communities in indoor and outdoor air (last column of Table 2). Most importantly for this investigation, the findings reported in Table 2 confirm that high-efficiency HVAC filters located in HVAC systems operating a great fraction of time can be used as a surrogate for long-term air samples that could be use as an alternative to extensive periodic air sample collections with not statistically different information. Given the results from the four occupied sites, we would anticipate that these results would also hold for occupied environments.

CONCLUSIONS

This study evaluated the use of HVAC filters as long-term air samplers for indoor biological contamination. Microbial concentrations and communities on HVAC filter dust samples were not statistically different from high surface dust samples in residences. However, differences in the community compositions may exist between samples collected in different indoor locations and between occupied and unoccupied buildings. Proteobacteria were present in greater proportion on HVAC filter dust samples than in high surface dust samples and in the unoccupied test house than in residences suggesting the outdoor air origin of this phylum. Gram-positive bacteria were present in greater proportion in occupied residences than in the unoccupied test house, confirming the potential association of this group with occupants. HVAC filter microbial communities were not statistically different from a composite indoor air sample in a mostly unoccupied test house. The results indicate that HVAC filters may be a viable option for investigating indoor biological contaminants and could be used as surrogate for long-term air samples, as suggested by other researchers. The current study represents an exploratory investigation of the potential use of HVAC filter as sampling mechanism for indoor microbial communities. The results are promising and suggest that a more comprehensive investigation of this technique is warranted.

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REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990) Basic Local Alignment Search Tool. J. Mol. Biol., 215, 403–410.

Andersson, A.M., Weiss, N., Rainey, F. and Salkinoja-Salonene, M.S. (1999) Dust-borne bacteria in animal sheds, schools and children's day care centres. J. of App. Micr., 86, 622-634.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998. Prepared and published jointly with AWWA, WEF.

ASHRAE. 2007. ASHRAE Standard 52.2- 2007, Method of testing general ventilation air-cleaning devices for removal efficiency by particle size. Atlanta: American Society of Heating, Refrigerating and Air-conditioning Engineers, Inc.

Bouillard, L., Michel, O., Dramaix, M., and Devleeschouwen, M. (2005) Bacterial contamination of indoor air, surfaces and settled dust, and related dust endotoxin concentrations in healthy office buildings. Ann. Agric. Env. Med., 12, 187–192.

Brodie, E. L., DeSantis, T.Z., Moberg Parker, J.P., Zubietta, I.X., Piceno, Y.M. and Andersen, G.L. (20070 Urban aerosols harbor diverse and dynamic bacterial populations. PNAS, 104, 299-304.

Dales, R.E., D. Miller and McMullen, E. (1997) Indoor air quality and health: Validity and determinants of reported home dampness and moulds. Int. J. of Epid., 26, 120-125.

Fierer, N., Liu, Z., Rodriguez-Hernandez, M., Knight, R., Henn, M. and Hernandez, M. T. (2008) Short-term temporal variability in airborne bacterial and fungal populations. App. and Env. Micr., 74, 200-207.

Gynteleberg, F., Suadicani, P., Nielsen, J.W., Skov, P., Valbjorn, O., Nielsen, P.A., Schneider, T., Jorgensen, O., Wolkoff, P., Wilkins, C.K., Gravesen, S. and Norn, S. (1994) Dust and the sick building syndrome. Indoor Air, 4, 223-238.

Horak, B., Dutkiewicz, J. and Solarz, K. (1996) Microflora and acarofauna of bed dust from homes in Upper Silesia, Poland. Ann Allergy Asthma Immunol., 76, 41-50.

Hyvärinen, A., Vahteristo, M., Meklin, T., Jantunen, M., Nevalainen, A. and Moschandreas, D. (2001) Temporal and spatial variation of fungal concentrations in indoor air. AS&T, 35, 688-695.

Kelley, S.T., Theisen, U., Angenent, L.T., Amand, A.S. and Pace, N.R. (2004) Molecular analysis of shower curtain biofilm microbes. App. and Env. Micr., 70, 4187-4192.

Koch, A., Heilemann, K.-J., Bischof, W., Heinrich, J. and Wichmann, H.E. (2000) Indoor viable mold spores – a comparison between two cities, Erfurt (eastern Germany) and Hamburg (western Germany). Allergy 2000, 55:176-180.

Li, W.-H. and Li, C.-S. (1996) Size characteristics of fungus allergens in subtropical climate. AS&T, 25:2, 93-100.

Lozupone, C.A., Hamady, M. and Knight, R. (2006) UniFrac – An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics, 7, 371-384.

Lozupone, C.A., Hamady, M., Kelley, S.T. and Knight, R. (2007) Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. App. and Env. Micr., 73, 1576–1585.

Miteva, V. I., Sheridan, P.P. and Brenchley, J. E. (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. App. And Env. Micr. 70:202–213.

Nelson, N.A, Kaufman, J.D., Burt, J. and Karr, C. (1995) Health symptoms and the work environment in four nonproblem United States office buildings. Scand. J. Work Env. Health, 21, 51- 59.

Noris, F., Siegel, J.A. and Kinney, K.A. (2009) Biological and Chemical Contaminants in HVAC Filter Dust. ASHRAE Transactions, 115 part 2, 484-491.

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Molcalvo, J. and Vilgalys, R. (2005) Fungal Community Analysis by Large-Scale Sequencing of Environmental Samples. App. and Env. Micr., 71, 5544-5550.

Pakarinen, J., Hyvärinen A., Salkinoja-Salonen, M., Laitinen, S., Nevalainen, A., Mäkelä, M.J., Haahtela, T. and von Hertzen, L. (2008) Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. Env. Micr., 10, 3317-3325.

Park, J-H., Cox-Ganser, J., Rao, C. and Kreiss, K. (2006) Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building. Indoor Air, 16, 192-203.

Peat, J.K., Dickerson, K. and Li J. (1998) Effects of damp and mould in the home on respiratory health: a review of the literature. Allergy, 53, 120-128.

Pitkäranta, M., Meklin, T., Hyvärinen, A., Paulin, L., Auvinen, P., Nevalainen, A. and Rintala, H. (2008) Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture. App. and Env. Micr., 74, 233-244.

Ren, P., Jankun, T.M. and Leaderer, B.P. (1999) Comparison of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county. J. Exp. Analysis and Env. Epid., 9, 560-568.

Rintala, H., Pitkäranta, M., Toivola, M., Paulin, L. and Nevalainen, A. (2008) Diversity and seasonal dynamics of bacterial community in indoor environment. BMC Microbiology, 8, 56-69.

Ross, M.A., Curtis, L., Scheff, P.A., Hryhorczuk, D.O, Ramakrishnan, V., Wadden, R.A. and Persky, V.W. (2000) Association of asthma symptoms and severity with indoor bioaerosols. Allergy, 55, 705-711.

Smedje, G., Norback, D. and Edling, C. (1997) Asthma among secondary schoolchildren in relation to the school environment. Clin. Exp. Allergy, 27, 1270-1278.

Stamatakis, A., Ludwig, T. and Meier, H. (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics, 21, 456-463.

Stanley, N.J., Kuehn, T.H., Kim, S.W., Raynor, P.C., Anantharaman, S., Ramakrishnan, M.A. and Goyal, S.M. (2008) Background culturable bacteria aerosol in two large public buildings using HVAC filters as long term, passive, high-volume air samplers. J. Env. Monit., 2008, 10, 474–481.

Täubel, M., Rintala, H., Pitkäranta, M., Paulin, L., Laitinen, S., Pekkanen, J., Hyvärinen A. and Nevalainen, A. (2009) The occupants as a source of house dust bacteria. J. Allergy and Clin. Imm., 124-4, 834-840.

Tamura, K., J. Dudley, M. Nei, and Kumar, S. (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software. Version 4.0. Mol. Biol. and Evol. 24:1596–1599.

Terzieva, S., Donnelly, J., Ulevicius V., Grinshpun, S.A., Willeke, K., Stelma, G.N. and Brenner, K.P. (1996) Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. App. and Env. Micro., 62, 2264-2272.

Toivola, M., Alm, S., Reponen, T., Kolari, S. and Nevalainen, A. (2002) Personal exposures and microenvironmental concentrations of particles and bioaerosols. Journal of Env. Mon., 4, 166-174.

Tringe, S.G., Zhang, T., Liu, X., Yu., Y., Lee, W.H., Yap, J., Yao, F., Suan, S.T., Ing, S.K., Haynes, M., Rohwer, F., Wei, C.L., Tan, P., Bristow, J., Rubin, E.M. and Ruan, Y. (2008) The airborne metagenome in an indoor urban environment. PLoS ONE, 3, e1862.

U.S. Bureau of Census 2005. American housing survey. Washington, DC.

Verhoeff, A.P. and Burge, H.A. (1997) Health risk assessment of fungi in home environments. Ann. of Allergy Asthma & Imm., 78, 544-554.

Vesper, S.J, McKinstry, C., Haugland, RA., Iossifova, Y., Lemasters, G., Levin, L., Khurana Hershey, G.K., Villareal, M., Bernstein, D.I., Lockey, J. and Reponen, T. (2007) Relative moldiness index as predictor of childhood respiratory illness. J. Exp. Science and Env. Epid., 17, 88-94.

Yi, H., Yoon, H. I. and Chun, J. (2005) Sejongia antarctica gen. nov., sp. nov. and Sejongia jeonii sp. nov., isolated from the Antarctic. Int. J. Syst. Evol. Micr. 55:409–416.

Yu, Y., Breitbart, M., McNairnie, P. And Rohwer, F. (2006) FastGroupII: A web-based bioinformatics platform for analysis of large 16S rDNA libraries. BMC Bioinformatics 2006, 7:57.

Appendix C

PAPER III

FATE ANALYSIS OF INDOOR PARTICLES AND EVALUATION OF HVAC FILTERS AS SAMPLERS

(In preparation for submission to Building and Environment)

ABSTRACT

HVAC filters are in place for extended periods of time and could serve as long-term samplers. To assess the potential use of HVAC filters as passive indoor samplers in a typical residence, we conducted a scaling analysis to evaluate the removal of particles resulting from deposition, exfiltration, and capture in the HVAC filter. Experiments under controlled conditions were conducted in a full-scale test house to confirm the validity of the model. In the model, typical characteristic times for each removal mechanisms were applied to indoor airborne particles in the 0.001-100 μm range to assess the effectiveness of using filters as samplers. The results suggest that large particles are likely to deposit, particles in the 0.03-3 μm range are likely to be removed by exfiltration or, if a high efficiency filter is installed, to be captured by the HVAC filter. Ultrafine particles (< 0.1 μm) are also likely to be captured by filters, particularly for elevated recirculation air exchange rates. HVAC filter efficiency and the recirculation air exchange rate play a key role in the use of HVAC filters as representative samplers of indoor particles. High efficiency filters with elevated recirculation air exchange rates (>5.2 h^{-1}) are particularly effective for a wide range of particle sizes suggesting that HVAC filters may be a promising means for assessing indoor particulate contaminants.

KEYWORDS

Particulate matter, Filtration, Air sampling, Settled dust, Removal mechanisms.

INTRODUCTION

Indoor air quality investigations often focus on air and settled dust samples to assess chemical and biological contamination. Although the information provided by these techniques is useful, both types of sampling locations have limitations including the spatial variability of indoor contaminant concentrations. Additionally, air samples are short-term in nature providing only a snapshot of contaminant concentrations. The specific contaminants and concentrations found in particles suspended in air and in settled dust are often different (i.e., Rudel *et al.,* 2003) possibly due to the fact that air and settled dust sampling methods preferentially sample different particle size ranges. Settled dust may be biased toward larger particles that are likely to deposit by gravity onto surfaces. Additionally, floor dust may be influenced by tracked-in dust of outdoor origin. In contrast, air samples may tend to preferentially collect particles with sizes that are not effectively removed by other mechanisms such as deposition and filtration.

In order to compare the contaminant concentrations observed in samples collected from various indoor locations, the fate of indoor airborne particles and their likelihood to be removed by different mechanisms needs to be investigated. The fate of indoor airborne particles is a complex phenomenon with several competing mechanisms that are influenced by a variety of parameters, including the specific characteristics of the building and of the HVAC filtration system as well as by the particle size of interest. The main indoor particle removal mechanisms are deposition onto surfaces, exfiltration through the building envelope and, if the HVAC system is being operated, HVAC filtration. Particle deposition onto indoor surfaces as a function of particle size has been widely studied (Long *et al.* 2001; Riley *et al.*, 2002; Thatcher and Layton, 1995) mostly in chamber studies and in controlled environments. Riley *et al.* (2002) reported that loss processes vary with building conditions and operation and are strongly particle-size

dependent. Air exchange rate and exfiltration of particles affect indoor particle concentrations as reported by Abt *et al.* (2000). HVAC filters are capable of removing indoor airborne particles (Hanley *et al.*, 1994) and play a critical role in the decay of particle concentration in indoor environments (Fisk *et al.*, 2000). Wallace *et al.* (2004) investigated the impact of a central fan and mechanical filters and reported that filters can effectively reduce indoor air concentrations with increased particle removal rates by up to 2 h^{-1} for fine and ultrafine particles. Siegel and Waring (2008) observed the influence of HVAC filter efficiency, time of operation and particle size on the loading rates of HVAC filters. Zhao and Wu (2009) investigated particle fate in ventilation systems, including filters, for a range of different scenarios reporting a strong dependency on particle size. To evaluate the merits of utilizing HVAC filters as passive samplers, the current study expands on this particle fate analysis to assess the likelihood of particle capture on filters for a range of building and HVAC scenarios.

HVAC filter dust has received little attention as a potential resource for indoor environment investigations. These filters are in place for extended periods of times and throughout their life can collect an integrated sample of particles present in the indoor environment. Analysis of HVAC filter dust may enhance our understanding of indoor occupant exposure by providing an integrated measure of indoor pollutant concentrations associated with particles. Recently, several researchers have utilized HVAC filters as a sampling mechanism for airborne particle-bound contaminants such as microorganisms and metals (Noris *et al.*, 2009; Stanley *et al.*, 2008; Tringe *et al.*, 2008). The objective of the current study was to validate a modeling approach capable of predicting the removal probabilities of indoor particles by the different mechanisms. The model was then applied to a typical residential scenario and the influence of filter efficiency, air recirculation rate, and air exchange rate on the size-dependent particle fate was evaluated.

The likelihood of HVAC filters to collect particles was evaluated in order to delineate the conditions and particle sizes for which HVAC filters are most likely to be effective samplers. Additionally, we wanted to compare the use of HVAC filters to more traditional sampling approaches such as periodic air measurements or of settled dust collection. The results from this analysis will be useful for assessing the effectiveness of using HVAC filters as an indoor sampling technique.

MODEL DESCRIPTION AND PARAMETERS

A scaling analysis was performed to estimate the probability that 0.001-100 μm particles would be removed from indoor air in a typical residence by deposition, exfiltration, or filtration through the HVAC filter. The volume of the residence was selected considering a typical floor area of 163.3 m^2 (US Bureau of Census, 2005) and assuming a ceiling height of 2.4 m for a total volume (V_T) of 391.9 m^3. To estimate the removal probability for each mechanism, we considered the size-dependent characteristic time for each removal process. The characteristic time for deposition was the particle size-resolved deposition loss rate coefficients (β), for exfiltration, the air exchange rate (λ) was utilized, while for filtration, the recirculation rate (λ_r) multiplied by the size-dependent removal efficiency (η) of the HVAC filter was used.

Model parameters were estimated from the literature. For deposition, the β values summarized by Riley *et al.* (2002) were utilized. For exfiltration, we utilized the 10^{th}, 50^{th} and 90^{th} percentile values of the λ distribution reported by Murray and Burmaster (1995). These values, corresponding to λ = 0.2, 0.5 and 1.3 h^{-1}, were used to evaluate how the tightness of the residence may affect the potential use of HVAC filters as passive samplers. For the loss rate due to filtration, the λ_r multiplied by the size-dependent removal efficiency (η) of the HVAC filter was utilized. We considered two different λ_r

values (5.2, 1.1 h^{-1}) by assuming either continuous operation for mechanical ventilation (λ_r= 5.2 h^{-1}) or cyclic duty operation (λ_r= 1.1 h^{-1}) for a typical 3-ton air conditioner operating 22% of the time (Noris *et al.*, 2009). Three different clean filters with minimum efficiency reporting values (MERV), as determined by ASHRAE Standard 52.2 (ASHRAE, 2007), of <5, 6 and 11 were considered using the filtration efficiencies employed by Waring and Siegel (2008). For each scenario, *j*, considered, the size-dependent characteristic times for each mechanism were then normalized by the sum of all the characteristic times, k_j, and the resulting fraction represented the relative removal probability of that mechanism for a given particle size. . The sum of the characteristic times, k_j, was calculated as follows:

$$k_j = \beta + \lambda_j + \lambda_{r,j}\eta_j \quad (1)$$

As a consequence, for a particular scenario, the size-dependent particle removal probability via each mechanism, $p_{r,m}$, was estimated with the model as follows:

$$p_{r,m} = \frac{r_{j,m}}{k_j} \quad (2)$$

where $r_{j,m}$ is the size-dependent characteristic time of each process for a particular scenario. Particle deposition in the HVAC system ducts and coil was neglected based on the results of previous studies (Sippola and Nazaroff, 2003; Waring and Siegel, 2008). We assumed isothermal conditions and ignored particle resuspension, coagulation and phase change.

METHODOLOGY

The validation of the modeling approach was performed in a 110 m^2 (volume of 250 m^3) unoccupied test house. The house was equipped with a 2.5-ton (8.8 kW) air

conditioning system that was continuously operated during the experiments. The system airflow was measured using a TrueFlow metering plate and DG-700 digital manometer (Energy Conservatory, Minneapolis, MN) connected to a pressure tap in the supply plenum. Approximately 50 g of Ultrafine Arizona Test dust (Powder Technology, Burnsville, MN) were dispersed into the house using a dust sprayer and mixing fans. Eight mixing fans and one ceiling fan were operated to improve the mixing of the injected particles. Six Aerotrak Handheld Particle Counters (TSI Inc., Shoreview, MN) were located in different indoor locations: living room, kitchen, upstream and downstream of the HVAC filter and one in each of two bedrooms. We measured particle concentrations in the following size bins: 0.3-0.5, 0.5-1, 1-3, 3-5, 5-7, 7-10 μm. Samples were recorded at 30-second intervals. Experiments with high (MERV 12) and low (MERV 2) efficiency fibrous filters were conducted in triplicate. Each experiment lasted approximately 120 min. To estimate the mass accumulated on each filter, we weighed the filters before and after the experiment using a balance (Sartorius B310S, Goettingen, Germany). Prior to the beginning of the experiments, particle decay tests were performed to estimate the removal of particles due to deposition onto surfaces when the HVAC system was off. For these particle decay tests, approximately 10 g of dust was sprayed and the concentrations were measured as explained below. In this way, the deposition loss coefficient (β) in the test house could be determined for conditions similar to those present during the tests.

At the beginning of each experiment, all surfaces and floors were cleaned and a clean filter was installed. Approximately 4 m^2 of selected high surfaces (horizontal surfaces > 1 m above the floor) and 6 m^2 of floor surface were sampled using a Dynamite Plus vacuum (Dirt Devil, Glenwillow, Ohio) equipped with a Duststream Collector (Indoor Biotechnologies, Charlottesville, VA). The particle mass deposited on high

surfaces and on the floor was measured by weighing the collectors before and after the sampling. During the tests, the house was pressurized using an Duct Blaster (Energy Conservatory, Minneapolis, MN) equipped with two MERV 11 filters and an activated carbon mat to remove outdoor particles and particle forming compounds. During each test, the air exchange rate was assessed by the best fit to exponential decay of CO_2 concentrations verses time, correcting for background CO_2. The CO_2 concentrations were monitored in several locations inside and outside the house using Telaire 7001 Carbon Dioxide Monitors (GE, Billerica, MA). The size-resolved number of particles removed by filtration, n_f, was estimated using the following equation for filtration:

$$n_f = \sum_{t=0}^{t=120} \left(C_{up} - C_{down}\right) Q_r t \qquad (3)$$

where C_{up} and C_{down} are the measured size-resolved concentrations (#/m^3) upstream and downstream of the HVAC filter, Q_r is the flow rate (m^3/h) through the filter, and t is the duration of each experiment (h). The size-resolved n_f was then multiplied by a characteristic volume for each size bin (assuming spherical particles) to obtain the volume of particles collected on the filter, v_f. Using the particle size distribution provided by the manufacturer for the Ultrafine Arizona Test dust, the size-resolved volume of particles injected, v_i, was calculated assuming a constant density across particle size ranges. The ratio between v_f and v_i represents the estimated probability of filter removal for each size bin during the experiments, $p_{f,e}$:

$$p_{f,e} = \frac{v_f}{v_i} \qquad (4)$$

RESULTS AND DISCUSSION

Results from validation experiments

The air exchange rates during the experiments ranged from 1.57 to 1.96 h^{-1}, while the HVAC flow rate (Q_r) was approximately 1530 m^3/h and 1630 m^3/h for the high and low efficiency filters, respectively. The majority of the injected particles were removed from the air in the first 10-15 minutes. With the exception of the submicron particles, 90% of the particles in the other four size bins, that were ultimately collected on the filter, deposited on the filter within the first 20 minutes after particle injection. Submicron particles tend to be removed more slowly and, as a consequence, HVAC filters may have limitations for an application that requires rapid detection of contaminants in this particle size range. Table 1 shows the comparison between the fraction of the injected dust mass that was collected on the filters and the particle volume fraction calculated using Equation 4. Since we assumed a constant density across particle size ranges, the mass fraction and the volume fraction represent the same metric and can be compared. The volume percentage estimated using Equation 4 matches the measured mass fraction on the filters within 10%, except for the Test 3 high efficiency filter and the Test 3 low efficiency filter. During these two experiments, the equation overestimated the fraction of the injected particles captured on the filter, possibly because of nonuniform mixing conditions throughout the house. For these two experiments, the standard deviations of the indoor concentrations measured in the house normalized by the initial concentrations were the highest values of all the experiments. This would lead to a greater variation (positively or negatively) between what is captured on the filter and what is predicted by the model which assumes a well-mixed condition. The particle mass collected on the floor and high surfaces varied more between tests than did the mass collected on the filters. During the experiments with high efficiency filters, we calculated that between 57% and 76% of the total mass of particles injected in each test deposited on surfaces, while for the low efficiency experiments this percentage was between 67% and 83%.

Table 3. Comparison between measured mass and calculated volume fraction of injected particles on HVAC filters

Test	Measured Mass Fraction (%)	Calculated Volume Fraction (%)[1]
High efficiency filter test 1	20.0	20.9
High efficiency filter test 2	19.2	19.9
High efficiency filter test 3	14.4	18.5
Low efficiency filter test 1	8.47	8.56
Low efficiency filter test 2	9.15	10.8
Low efficiency filter test 3	7.96	10.9

[1]This fraction is calculated using the upstream and downstream filter concentrations and Equations 3 and 4.

Figure 1 presents the comparison between the filter capture probabilities determined during the experiments (and calculated using Equation 4) and the model prediction for the low and high efficiency filters. The model utilized the actual λ, Q_r and V_T values measured during the experiments; it also employed the deposition loss rate (β) and the filter efficiency (η) values obtained from the literature (Riley *et al.*, 2002; Waring and Siegel, 2008) While the λ, Q_r and V_T are parameters relatively easy to obtain and measure, measuring β and η requires particle injection tests which are time consuming and more complex to perform. Thus, we were interesting in evaluating the applicability of the model if the β and η parameters are not measured directly but rather estimated from literature values. The model used the mean of the λ, Q_r measured during the three tests. These values corresponded for the high efficiency filter model predictions to λ= 1.74 $h^{-1}$and Q_r= 1529 m^3/h,, while for low efficiency filter model predictions we utilized λ= 1.71 h^{-1} and Q_r= 1628 m^3/h. The results shown in Figure 1 indicate that the observed values and the model predictions follow similar trends suggesting that the modelling

approach provides a reasonable prediction of the likelihood of particle capture by filters. However, for high efficiency filters, the model overestimated particle capture probabilities relative to that measured during the experiments with mean normalized percentage differences between the probabilities calculated during the experiments and the model predictions of 21%, 72%, 63%, 101%, 92%, and 99% for the six size bins considered.

The grey lines in Figure 1 present the model predictions if the measured β and η values are employed. If these two measured parameters are utilized, the model is in much better agreement with the measured filter capture probabilities, particularly for the high efficiency test, suggesting the strong influence of these two parameters in the predictions and the need to accurately estimate them. Table 2 presents the comparison between the β and η values obtained from the literature and those measured during the current experiments. Significant differences between the β and η values measured during our tests and the literature values (utilized in the model) exist, particularly for the β values for the smaller size bins. The deposition loss rate (β) has been reported to vary significantly depending on several factors including the structure of the house, building conditions, and mixing conditions (Nazaroff *et al.*, 1993; Riley *et al.*, 2002). During our particle decay tests to estimate the β values, eight fans and one ceiling fan were operated to increase the mixing and the obtain well-mixed conditions. This high level of mixing likely caused elevated average velocities in the house that could have increased the likelihood of particles to collide against a surface and remain attached to it. This phenomenon is likely to be more important for smaller particles that have lower deposition loss rate and tend to stay longer in air, and may be responsible for the elevated difference in β values between the experiments and the literature values for small particle size bins (Table 2). In Figure 1, we notice a greater difference between the predicted and

measured filter removal fraction for particles in the 0.3 to 3 μm size range, mainly because for that size range, the β values estimated during our tests were much greater than the values assumed in the model based on literature values (Table 2). Better agreement between the model predictions and the experimental observations was evident for particles greater than 3 μm, where the model may simulate effectively what was observed during the experiments. We also observed differences between the filter efficiencies (η) measured during the tests and the literature values used in the model (Waring and Siegel, 2008), particularly for the high efficiency filter. The efficiency values obtained from the literature were for clean new filters and were determined by ASHRAE Standard 52.2 (ASHRAE, 2007). Filters in real systems and conditions may perform differently than that estimated in the Standard 52.2 tests, particularly if they are challenged with particles of a different nature or if bypass occurs.

For low efficiency filters, the model and the experiments are in better agreement, particularly for larger particle sizes ($\geq$ 2 μm), than during the high efficiency tests. In the low efficiency filter scenario we observed mean normalized percentage differences between the probabilities calculated during the experiments and the model predictions of 81%, 89%, 18%, 31%, 19%, and 23% for the six particle size bins considered. The probabilities for the low efficiency filter scenario are relatively constant across the size range, around 0.15, revealing that these filters are not likely to oversample a particle size range and therefore show promise as samplers that are not biased towards a specific particle size.

Table 2. Comparison between literature and measured values for the β and η coefficients

Size bin (μm)	β (h^{-1})			η (-)					
				Low efficiency filter			High efficiency filter		
	Literature	Tests	% difference	Literature	Tests	% difference	Literature	Tests	% difference
0.3-0.5	0.03	1.00	97	0.01	0.13	92	0.43	0.33	30
0.5-1	0.05	1.77	97	0.01	0.12	92	0.66	0.58	14
1-3	0.92	4.01	77	0.08	0.11	27	0.86	0.77	12
3-5	3.37	6.88	51	0.13	0.29	55	0.96	0.90	7
5-7	7.10	9.95	28	0.11	0.06	83	0.98	0.92	7
7-10	14.45	14.3	0.8	0.13	0.07	85	0.98	0.96	2

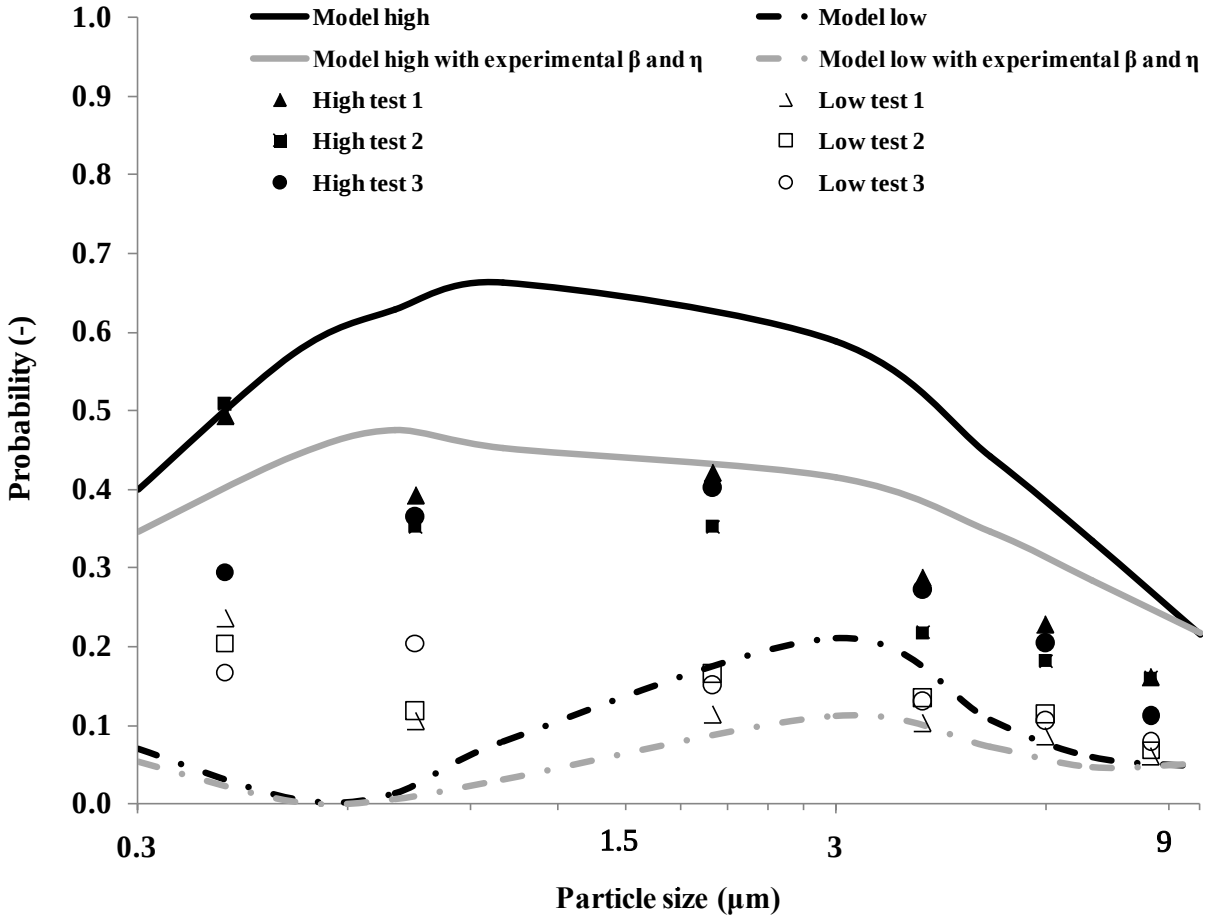


Figure 1. Comparison between model predictions and the observed capture probabilities during the experiments with the low and high efficiency filters.

The variations between the model predictions and the experimental results confirm the complexity of the phenomena and suggest that several factors are important and play a role in the fate of particles in an indoor environment. For instance, even with the high level of mixing present during the tests, the assumption of perfectly well-mixed conditions in the house may not have been met and this will affect the predicted particle concentrations and capture efficiencies. If the well-mixed assumption is not met, there are lower (or greater) particle concentrations near the return and the particles have actually a smaller (or greater) probability of being captured on the filter than what the

model predicts with the assumption of perfectly mixed conditions. Another factor that could have influenced the results is the assumption of constant density across the particle size range. It is possible that larger particles may have greater density because they are more likely to contain crustal material (Seinfeld and Pandis, 1998). The fact that we assumed a lower density for larger particles would lead to an over prediction in the volume (or number) of particles injected, v_i, which would lead to an under prediction in the probability of filter capture, $p_{f,e}$. Moreover, the particles were assumed to be spherical and a characteristic size for each bin was utilized to estimate the volume of the particles measured on that bin. This assumption could have also affected the filter capture probabilities because a different volume of particles could have been injected.

Filter Capture Probabilities

Even with the discrepancies described above, the experiments in the full-scale test house indicate that the modeling approach can be utilized to estimate the likelihood that particles are collected on filters or are removed from the air via other mechanisms such as deposition or exfiltration. Subsequently, in order to evaluate a broader application of filters as samplers, the model was applied to more realistic cases and conditions using typical characteristic times for each removal mechanism reported in the literature. Figure 2 shows the removal probability via different mechanisms for the baseline scenario (MERV 6 filter, $\lambda_r = 1.1\ h^{-1}$ and $\lambda = 0.5\ h^{-1}$). The results indicate that, for a mid-efficiency filter, large particles (> 3 μm) are likely to deposit on surfaces and are unlikely to get captured on filters. The deposition probability increases with size due to the increase in the deposition loss rate (β) for larger particles. Particles in the 0.03-3 μm range are likely to be removed by exfiltration or, if a high efficiency filter is installed, captured on the filter (Figure 3). Particles in this range have a greater residence time in air and, therefore,

have a greater opportunity to be captured by the filter. The filter removal probability increases for ultrafine particles (< 0.1 µm) since the filter efficiency for these particles is greater for all three MERV ratings. Sippola *et al.* (2003) and Zhao *et al.* (2009) reported that large particles (> 1 µm) are likely to deposit on surfaces, while small (submicron) particles tend to be exfiltrated. Figure 2 shows two peaks in the filter capture probabilities, at approximately 0.01 and 3 µm. For the baseline scenario conditions, filtration is about 18 times less likely to capture a 0.1 µm particle before it is removed by exfiltration and 12 times less likely to capture a 5 µm particle before it deposits on a surface. For an HVAC system operating only 22% of the time, particles between 0.03 and 1 µm are most likely to be removed by exfiltration and are less likely to be found in settled dust or in the dust that collects on a mid efficiency filter. From Figure 2, we notice how HVAC filters capture particles over a wide size range and are likely to be effective overall samplers. In contrast, settled dust may be biased toward larger particles that have greater mass, while air samplers may oversample those particles (0.03 - 3 µm range) that have a longer residence time. In addition, although the filter capture probability illustrated in Figure 2 does not seem particularly elevated relative to deposition or exfiltration, it is important to note, despite the higher removal probability for these two mechanisms, it may be more difficult to obtain a representative sample since settled dust and air samples only sample a very small fraction of the total surface or volume in a building at a particular time. In contrast, HVAC filters are typically in place for several weeks and, during this period, a great volume of air is filtered and a significant portion of the filter dust cake can be analyzed easily.

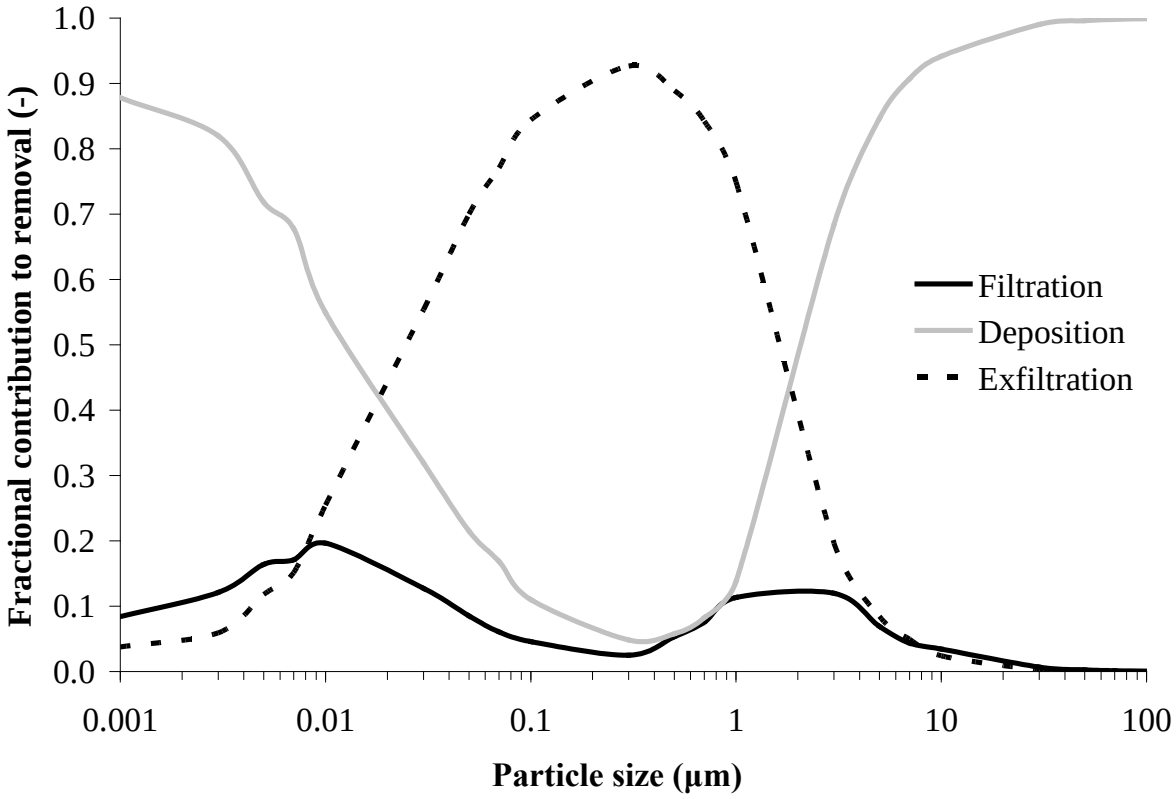


Figure 2. Removal probability curves for filtration, deposition and exfiltration for the baseline scenario (MERV 6, λr=1.1 h^{-1} and λ=0.5 h^{-1}).

Additional results of the analysis are presented in Figures 3, 4, and 5; in each case, one parameter at a time was modified from the baseline scenario. Figure 3 shows that the filter efficiency is a more important variable for sampling particles in the range between 0.03 and 3 µm than for particles below 0.03 µm or above 3 µm. These results are due to the fact that the HVAC filter efficiency is particle-size dependent especially in the range from 0.01 to 3 µm (Hanley *et al.*, 1994). The high efficiency filter probability curve has a minimum around 0.1 µm due to the reduced efficiency of the filter for particles around this dimension. Low and mid efficiency filters have similar probabilities for a wide range of particles, although a greater variation is observed in the range

between 0.3 and 10 µm. A MERV 11 filter is approximately 9 times more likely to capture a 0.5 µm particle than a MERV 6 filter and about 150 times more likely than a MERV <5 filter. If a high efficiency filter is used, there is an elevated probability of capturing particles in the 0.3 – 3 µm and 0.005 – 0.03 µm ranges. In the size range of 0.3 – 3 µm, the model predicts that more than one third of the particles should be captured on a high efficiency filter. As a consequence, high efficiency filters are likely to be reasonable samplers for particles in these size ranges. For larger particles (> 3 µm), deposition onto surfaces is the dominant removal mechanism and filters are less likely to be good samplers.

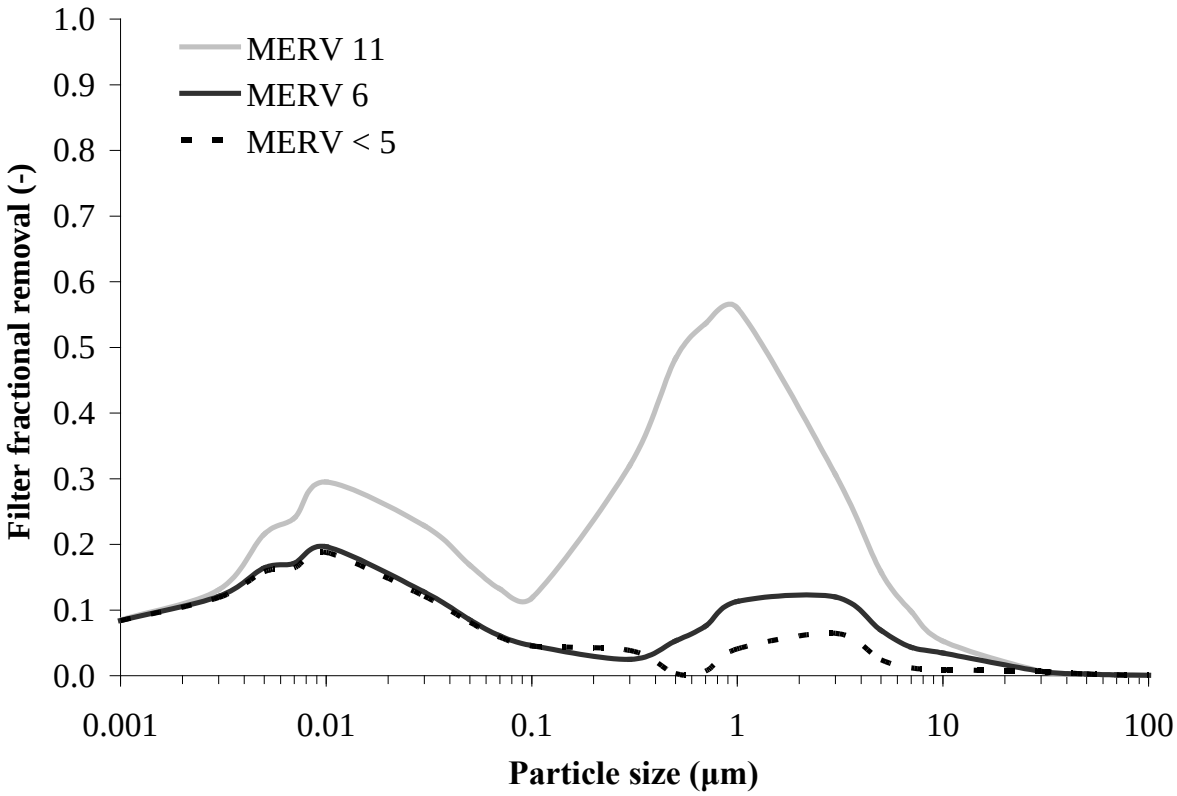


Figure 3. Filter capture probability curves for different filter efficiency scenarios.

Figure 4 illustrates the influence of the HVAC system duty cycle on the filter capture probability curve. The profiles for the two λ_r values investigated follow similar

patterns, with the probability for removal via filtration for normal (cycling) use reduced approximately by the duty cycle fraction (0.22) relative to the continuous operation case. The greatest difference between the two duty cycle scenarios is evident for particle sizes that are more likely to be captured on the filter, 0.01 and 3 µm. The results suggest that, if a mid efficiency filter is used, the HVAC system would need to operate with an elevated duty cycle in order for the filter to be an effective sampler. However, high efficiency filters with elevated recirculation air exchange rates (> 5.2 h^{-1}) are particularly effective, with more than 30% capture probability up to 7 µm and often above 60% (data not shown). Filters are more effective particle samplers if they have either high removal efficiency or if the HVAC system has an elevated air recirculation rate. If both of these two conditions are met filters, the filters are more likely than air or settled dust samples to capture particles in a wide size range. In particular, for these conditions, the particle capture probabilities onto the filter are predicted to be as high as 85% for particles around 1 µm.

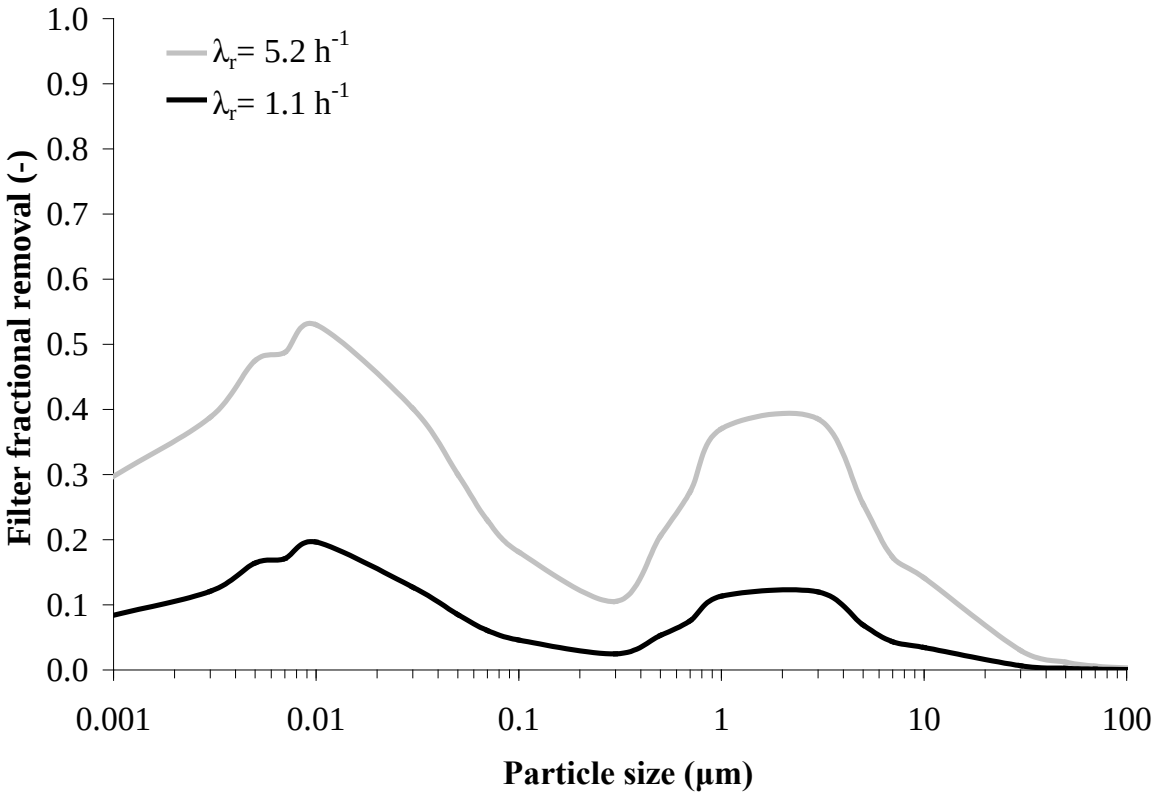


Figure 4. Filter capture probability curves for different air recirculation rate scenarios.

Figure 5 presents the filter probability curves for residences with varying tightness. Abt *el al.* (2000) found that air exchange rate has a significant effect on particle removal. As evident in Figure 5, this parameter does have an influence on the probability of particle capture on the filters; however, this parameter does not seem to be as important as the filter efficiency or the λ_r with similar patterns and capture probability among the three scenarios investigated. For instance, the difference in filter capture probability between a residence with $\lambda = 0.2$ and one with $1.3\ h^{-1}$ is, typically, below 10%. As a consequence, filters could potentially be used as samplers independently of the tightness of the residences investigated. This is an important consideration since the current trend is to move toward tighter and more energy efficient buildings.

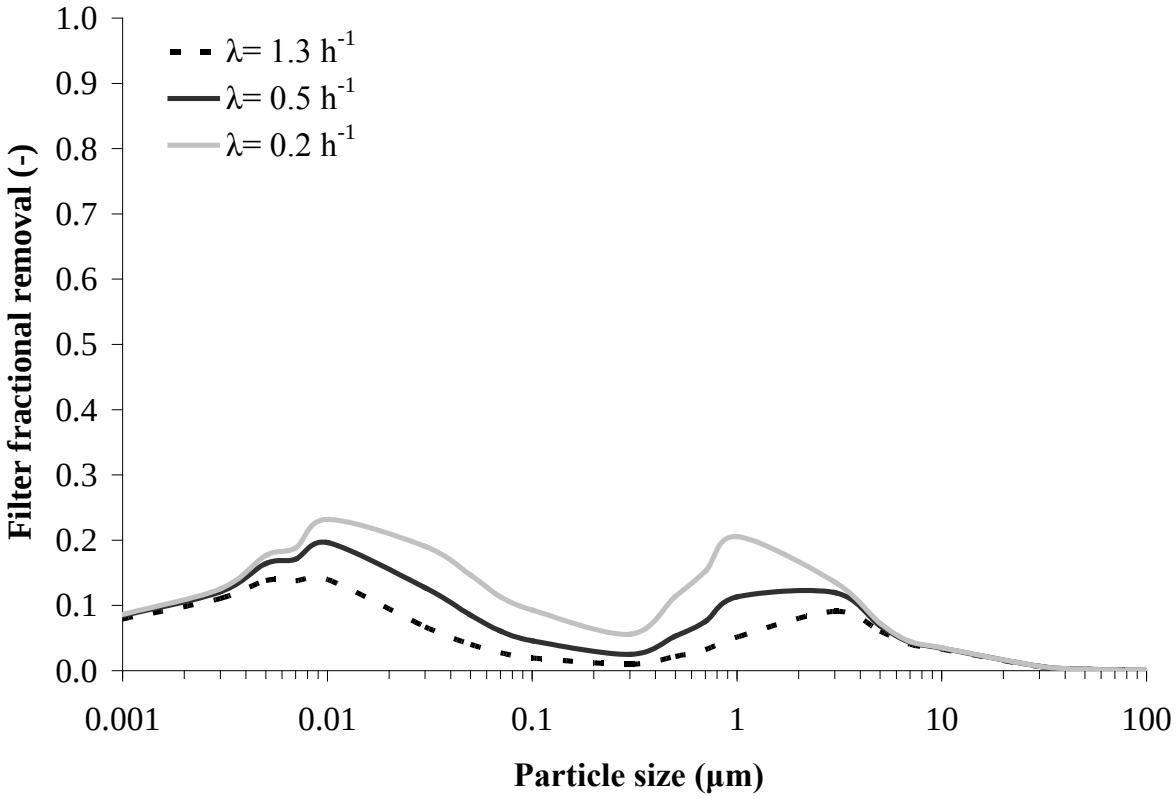


Figure 5. Filter capture probability curves for different air exchange rate scenarios.

This analysis suggests that HVAC filters may be used as passive samplers that are in place for long periods of time and can overcome the short-term sampling limitations of traditional air samplers. In particular, HVAC filters capture particles over a wide size range and can be considered effective overall samplers. In contrast, as can be seen in Figure 2, settled dust samples are biased toward larger particles, while conventional air samples may oversample those particle sizes that are not removed effectively by other mechanisms and tend to stay longer in the air (0.03 - 3 µm range). The best way to increase the probability that a broader size range of indoor particles will be captured by the HVAC filter is to increase the filter efficiency. This variable has a greater predicted effect than increasing the λ_r or decreasing the λ. High efficiency filters, in particular, could develop into a less intrusive and effective way to obtain information regarding the

indoor contamination in homes. This information could be integrated with conventional indoor air sampling strategies or, depending on the data being sought, it may provide an alternative, more efficient mechanism for collecting samples during large-scale investigations of multiple residences. However, as with all sampling methodologies, using HVAC filters as samplers has limitations that must be considered when interpreting the data collected. For instance, the influence of filter location relative to potential particle generation sources is a variable that should be considered and investigated further. In an investigation related to the effectiveness of portable air cleaners, Novoselac and Siegel (2009) reported the importance of device location with respect to the particle source. Similarly, we expect that the locations of the filter and return vent will be important factors that affect particle capture on the HVAC filter.

An additional limitation of using HVAC filters to sample the indoor environment is that in certain geographical regions or among specific socioeconomic groups, a significant fraction of the residences may not have a centralized air conditioning system, or the system is not used for certain seasons and, therefore, filters are not a sampling option. Even for the buildings that have a centralized air-conditioning system with built-in filtration, the occupants have significant control over several important factors including the efficiency of the installed filter and the duty cycle of the HVAC system. Filters have a possibility of collecting particles only when they are operated and, as a consequence, when there is limited need for conditioning, filters are unlikely to be effective samplers. Additionally, even if HVAC systems have elevated air recirculation rates, the use of filters are samplers will be affected by when the system is operated relative to when the contamination occurs and therefore the importance of HVAC system cycling may required further investigation. Filters located in systems with elevated return side leakages may not be representative samplers of indoor particle-bound

contaminants, particularly if the ducts and system are located in the unconditioned space. Filters capture particles and, therefore, can only be used for investigating particle-bound contaminant concentrations. Specifically, they are effective samplers for those particles that are not likely to deposit on surfaces and tend to stay suspended in air, thus increasing their chances to be captured on the filter. Finally, another factor to be considered is how to obtain a representative sample of what is captured on the filter. This is an important aspect that requires a careful sampling procedure because certain particles may tend to stay attached to the filter fibers, leading to biases in the particles that are actually analyzed.

CONCLUSIONS

The effectiveness of HVAC filters as passive samplers was investigated for a range of typical residential scenarios. A model based on the characteristic time of each process was applied to evaluate the importance of the main removal mechanisms for indoor airborne particles. Experiments in a full-scale test house corroborated the validity of the model, although some discrepancies exist between the experimental results and the model predictions, confirming the complexity of the phenomena involved. Typical values from the literature were used to investigate the influence that filter efficiency, air recirculation rate and air exchange rate have on the predicted filter capture probability for a range of particle sizes with the objective of evaluating the use of HVAC filters as long-term samplers. Large particles are likely to deposit, while particles in the 0.03-3 μm range stay suspended in air longer. The fate of particles with different sizes also has implications on the particles likely to be collected by conventional air and settled dust sampling techniques. Our scaling analysis indicates that filter efficiency is more important parameter than air recirculation rate or air exchange rate. High efficiency

filters have an elevated probability of capturing a wide range of particle sizes and could potentially develop into an attractive sampling alternative especially if the recirculation air exchange rate is elevated and the HVAC system is operated frequently. However, filters may not be good samplers for large particles or if the system operates with a reduced duty cycle. Other critical parameters including the location of the return vent and filter with respect to the emission source, the zoning of the building and the frequency of the HVAC cycles, could also play a significant role and should be investigated further.

REFERENCES

Abt, E., Suh, H.H., Catalano, P. and Koutrakis, P. (2000) Relative contribution of outdoor and indoor particle sources to indoor concentrations. ES&T, 34, 3579-3587.

Fisk, W.J., Faulkner, D., Palonen, J., and Seppanen, O. (2002) Performance and costs of particle air filtration technologies. Indoor Air, 12, 223-234.

Hanley, J.T., Ensor, D.S., Smith, D.D., and Sparks, L.E. (1994) Fractional aerosol filtration efficiency of in-duct ventilation air cleaners. Indoor air, 4, 169-178.

Liu, D.L., and Nazaroff, W.W. (2001) Modeling pollutant penetration across building envelopes. Atmospheric Environment, 35, 4451-4462.

Long, C.M., Suh, H.H., Catalano, P.J., and Koutrakis, P. (2001) Using time- and size-resolved particulate data to quantify indoor penetration and deposition behavior. ES&T, 35, 2089-2099.

Murray, D.M., and Burmaster, D.E. (1995) Residential air exchange rates in the United States: empirical and estimated parametric distributions by season and climatic region. Risk. Anal., 15, 459–465.

Nazaroff, W.W., Gadgil, A.J., and Weschler, C.J. (1993) Critique of the use of deposition velocity in modeling indoor air-quality. Modeling of indoor air quality and exposure, 1205, 81-104.

Noris, F., Siegel, J.A., and Kinney, K.A. (2009) Biological and Chemical Contaminants in HVAC Filter Dust. ASHRAE Transactions, 115 part 2, 484-491.

Novoselac, A., and Siegel, J.A. (2009) Impact of placement of portable air cleaning devices in multizone residential environments. Building and Environment, 44, 2348-2356.

Riley, W.J., McKone, T.E., Lai, A.C.K., and Nazaroff, W.W. (2002) Indoor particulate matter of outdoor origin: importance of size-dependent removal mechanisms. Environmental Science Technology, 36, 200–207.

Rudel, R.A., Camann, D.E, Spengler, J.D., Korn, L.R., and Brody, J.G. (2003) Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environmental Science Technology, 37, 4543-4553.

Seinfeld, J.H., and Pandis, S.N. (1998) Atmospheric Chemistry and Physics, New York, Wiley.

Sippola, M. R., and Nazaroff, W. W. (2003) Modeling particle loss in ventilation ducts. Atmospheric Environment, 37, 5597-5609.

Stanley, N.J., Kuehn, T.H., Kim, S.W., Raynor, P.C., Anantharaman, S., Ramakrishnan, M.A., and Goyal, S.M. (2008) Background culturable bacteria aerosol in two large public buildings using HVAC filters as long term, passive, high-volume air samplers. J. Env. Monit., 2008, 10, 474–481.

Tringe, S.G., Zhang, T., Liu, X., Yu., Y., Lee, W.H., Yap, J., Yao, F., Suan, S.T., Ing, S.K., Haynes, M., Rohwer, F., Wei, C.L., Tan, P., Bristow, J., Rubin, E.M., and Ruan, Y. (2008) The airborne metagenome in an indoor urban environment. PLoS ONE, 3, e1862.

US Bureau of Census. 2005. American housing survey. Washington, DC.

Wallace, L.A., Emmerich, S.J., and Howard-Reed, C. (2004) Effect of central fans and in-duct filters on the deposition rates of ultrafine and fine particles in an occupied townhouse. Atmospheric Environment, 38, 405-413.

Waring, M. S., and Siegel, J. A. (2008) Particle loading rates for HVAC filters, heat exchangers, and ducts. Indoor Air, 18, 209-224.

Zhao, B., and Wu, J. (2009) Modeling particle fate in ventilation system-Part II: Case study. Building and Environment, 44, 612-620.

Appendix D

DNA-Based Methodology

There are several steps in the microbial community analysis. First the DNA needs to be extracted, then amplified, cloned and finally sequenced. Details are provided below.

DNA EXTRACTIONS

DNA extraction is the most critical step. To amplify the DNA, it is necessary to extract a sufficient amount of DNA while minimizing the concentration of inhibitors that can prevent amplification. Several different DNA extraction protocols were investigated for the HVAC dust samples as described below. Based on the results of these preliminary experiments, a modified Power Soil DNA extraction procedure was ultimately used to extract the DNA from the dust and air samples collected in the study.

Direct Extraction. In this set of experiments, the author attempted to extract DNA from the microorganisms directly from the dust by immersing 0.5g of dust into the extraction tubes and the solutions provided by the Power Soil DNA kit. The quantity of inhibitors (visible on the peaks of the Nanodrop) present in the dust prevented the PCR amplification from taking place. The Wizard DNA Clean-up kit was subsequently used to try to purify the DNA extracted. Similarly, an ethanol precipitation step (Frank, 1997) was utilized to try to improve DNA recovery. In both cases, the DNA obtained could not be amplified via PCR. One of the main challenges is to obtain a balance between a sufficient amount of DNA and good purity of the DNA extracted. During every clean up, some DNA is lost, therefore, it is important to start from elevated DNA concentrations to account for potential losses during the purification steps. In an attempt to increase the DNA yield, thaw and freeze cycles (3 cycles) for a range of times and temperatures was attempted but this protocol did not improve the results. The problem was partially overcome by using a MP FastPrep-24 (QBiogene) to lyse the cells instead of using a vortexing step. The use of this device increased the DNA yield. However, DNA

amplification still did not occur due to the inhibition caused by substances present in the dust. The presence of inhibitors in the dust was verified during tests in which positive controls (*Pseudomonas putida*) were spiked with different aliquots of DNA extracted from dust samples. In these tests, the inhibitors present at elevated concentrations in the dust prevented the amplification of the positive control, while the positive controls with no dust DNA spike were amplified.

Separation Followed by Extraction. To work around the inhibitor problems mentioned above, the author decided to separate the microorganisms from the particles before extracting DNA from them. This was based on the protocols used by Colorado University at Boulder researchers (Dr. Norm Pace's lab group) that we have been collaborating with as well as the methodology utilized by Tringe *et al.* (2008). As discussed in the main text of the executive summary and in Appendix B, there were two filtration steps: in the first filtration step, the large particles were separated from small particles and microorganisms; in the second step, the microorganisms were separated from the liquid and captured on the filter. This methodology proved much more effective at removing inhibitors while yielding elevated DNA concentrations.

PCR AMPLIFICATION

After the DNA was extracted from microorganisms, the PCR reaction could be performed. Initially, there were problems amplifying the DNA extract due to the inhibition problems mentioned above and due to the reduced number of cycles (i.e., 16-20 cycles) at which the PCR reactions were performed. However, for cloning and sequencing techniques, a greater number of PCR cycles are typically employed and once the number of cycles was increased to 35, amplifications were able to occur. Another

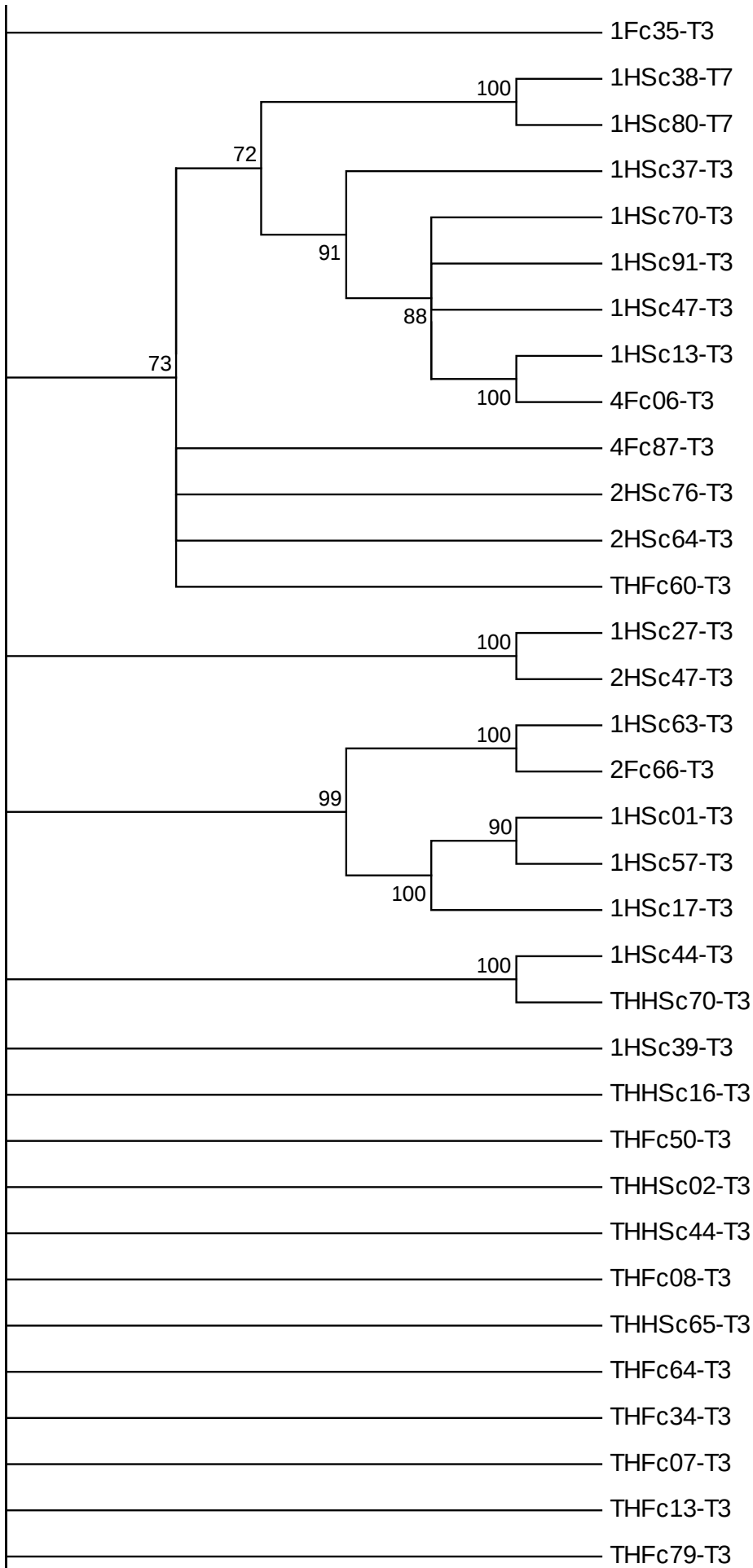
important parameter to optimize was the aliquot of DNA extract to use in every PCR reaction. The author performed tests with different aliquots and observed the best amplification for PCR reactions was achievable with 2µl aliquots.

GEL PURIFICATION AND CLONING

Once the DNA was extracted and amplified, it was important to remove the DNA fragments that belonged to unused primers due to partial amplification. Therefore, a gel extraction step was employed using a QIAquick Gel Extraction Kit. In this step, it is critical to perform the gel extraction under the UV lamp very quickly and to use fresh and effective ethidium bromide to enhance the visibility of the band.

Subsequently, the DNA fragments could be cloned using the TOPO TA cloning kit for sequencing. For this step, there were several important aspects to keep in mind during the procedure. First, it was important to increase the incubation time to the maximum recommended time for all the steps. In addition, the competent cells had to be thawed in ice and after the temperature shock step in the water bath, they needed to be transferred immediately into ice. Finally, the volume of PCR product introduced into the cloning reaction is a key parameter that must be considered. The author obtained the best results using an elevated volume (i.e., 4µl), but always performed two cloning reactions using different volumes; the the reaction that yielded the greatest number of colonies on the plate, ideally at least one hundred colonies for each plate was used. Subsequently, approximately one hundred clones were picked, isolated, and the plasmid extracted following the protocol described in the Fast Plasmid Mini Kit 5 Prime Inc. (Gatheirburg, MD). Finally, after adjusting the concentration to 50 ng/µl by dilution, the clones were sent to sequencing on ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA).

Appendix E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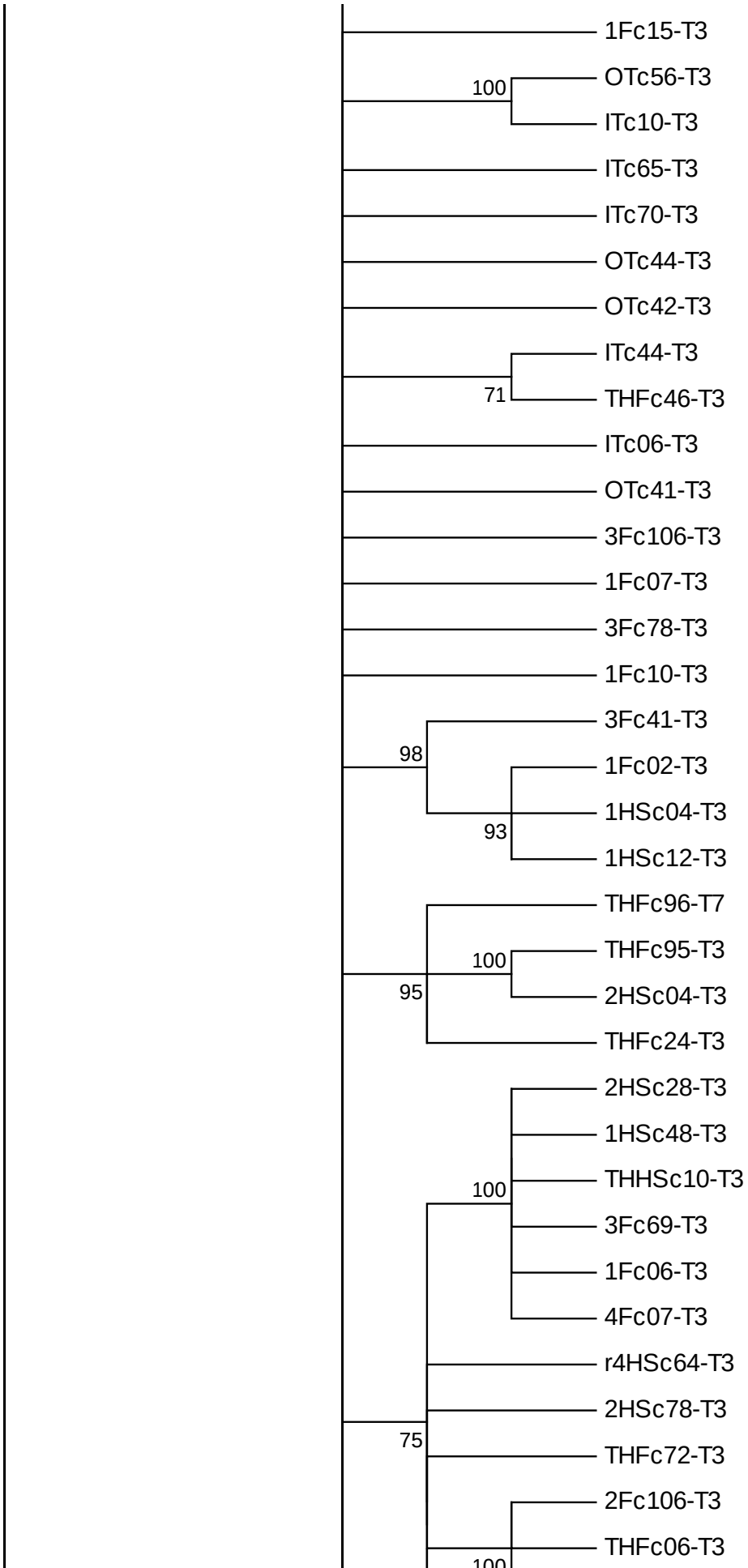
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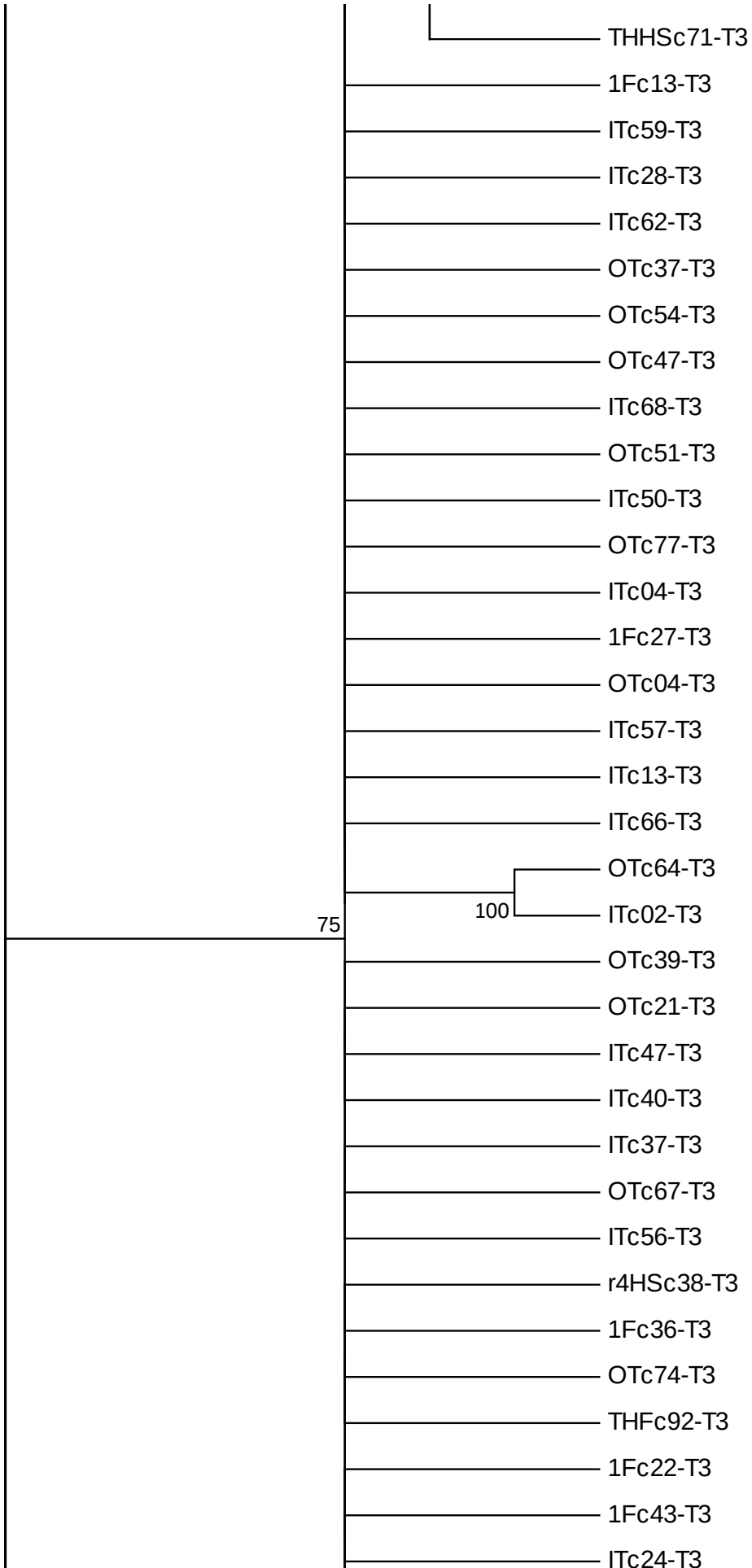
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ITc50-T3
OTc77-T3
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ITc57-T3
ITc13-T3
ITc66-T3
OTc64-T3
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ITc02-T3
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OTc39-T3
OTc21-T3
ITc47-T3
ITc40-T3
ITc37-T3
OTc67-T3
ITc56-T3
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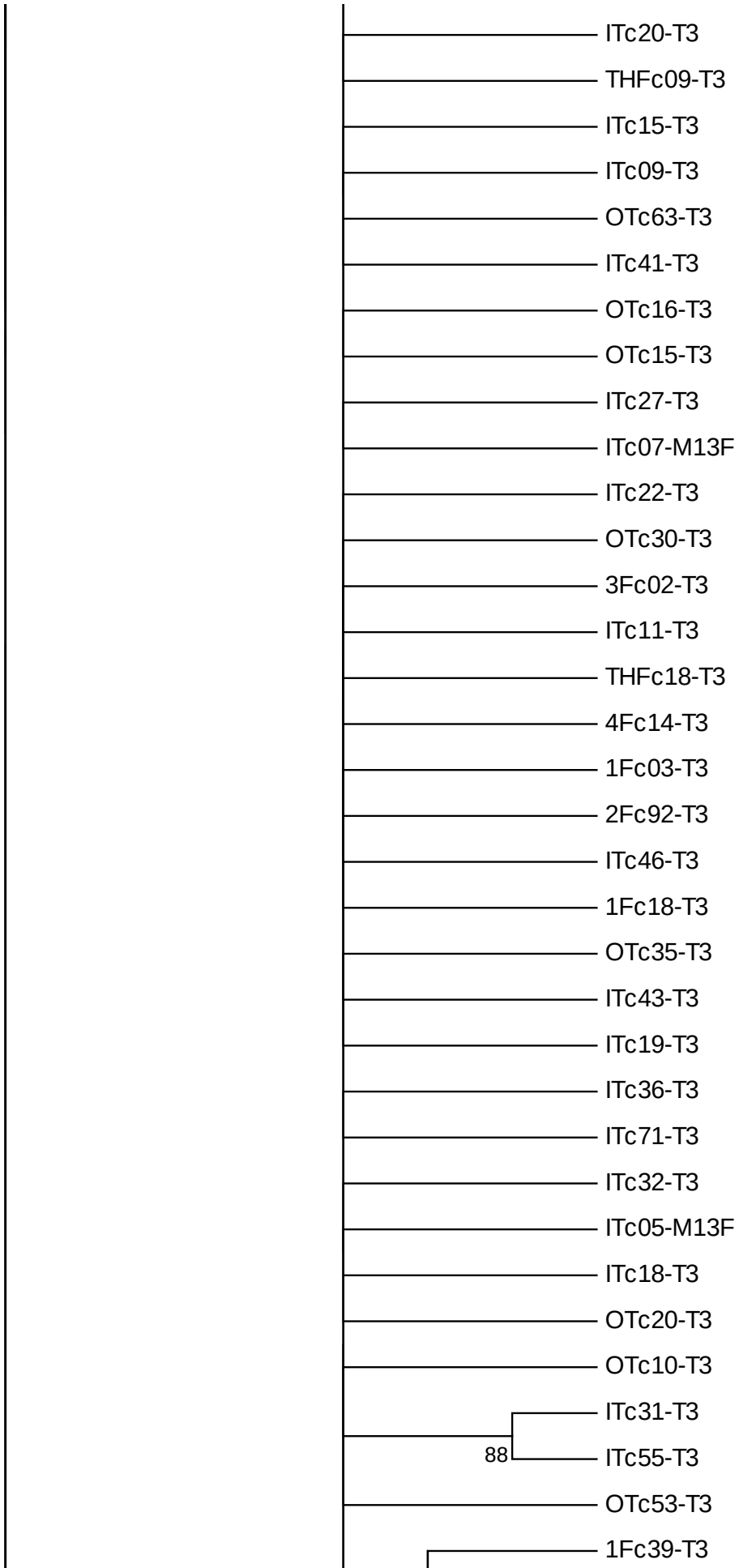
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ITc27-T3
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ITc22-T3
OTc30-T3
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ITc11-T3
THFc18-T3
4Fc14-T3
1Fc03-T3
2Fc92-T3
ITc46-T3
1Fc18-T3
OTc35-T3
ITc43-T3
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ITc36-T3
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ITc32-T3
ITc05-M13F
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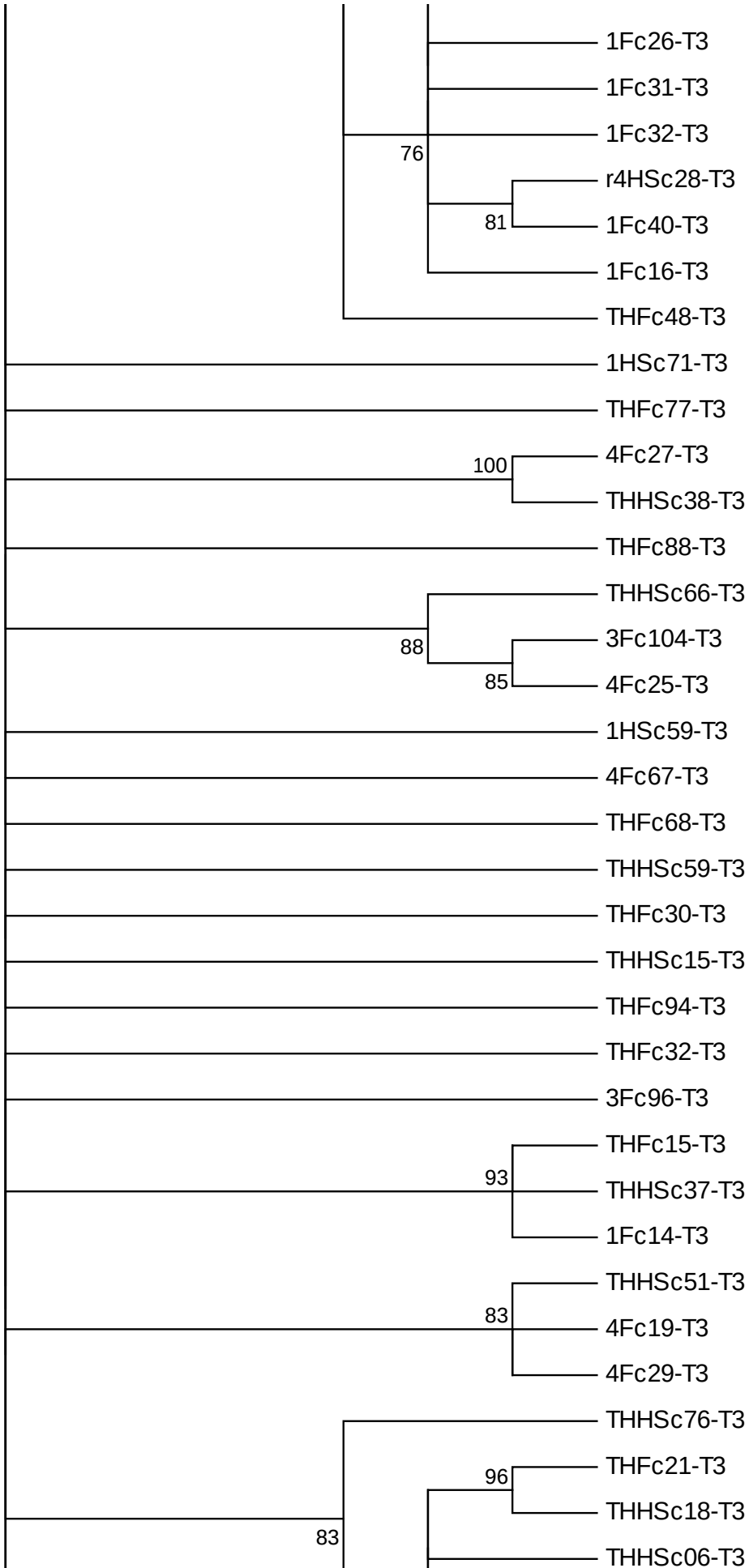
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THFc77-T3
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THHSc38-T3
THFc88-T3
THHSc66-T3
3Fc104-T3
88
4Fc25-T3
85
1HSc59-T3
4Fc67-T3
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THHSc59-T3
THFc30-T3
THHSc15-T3
THFc94-T3
THFc32-T3
3Fc96-T3
THFc15-T3
93
THHSc37-T3
1Fc14-T3
THHSc51-T3
83
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96
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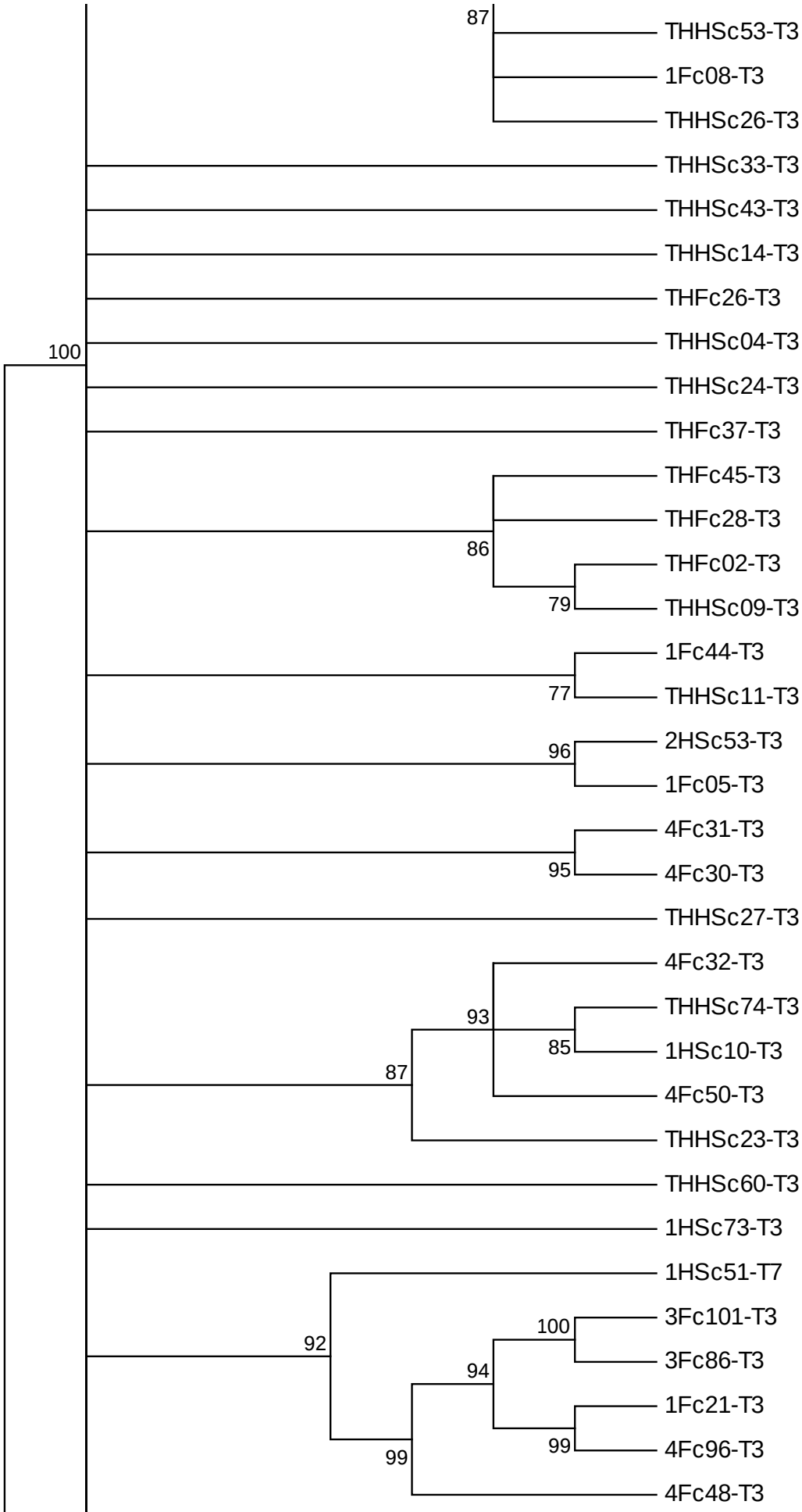
83
THHSc06-T3


87
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THHSc04-T3
THHSc24-T3
THFc37-T3
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THFc28-T3
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THFc02-T3
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THHSc09-T3
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THHSc11-T3
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95
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THHSc27-T3
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93
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85
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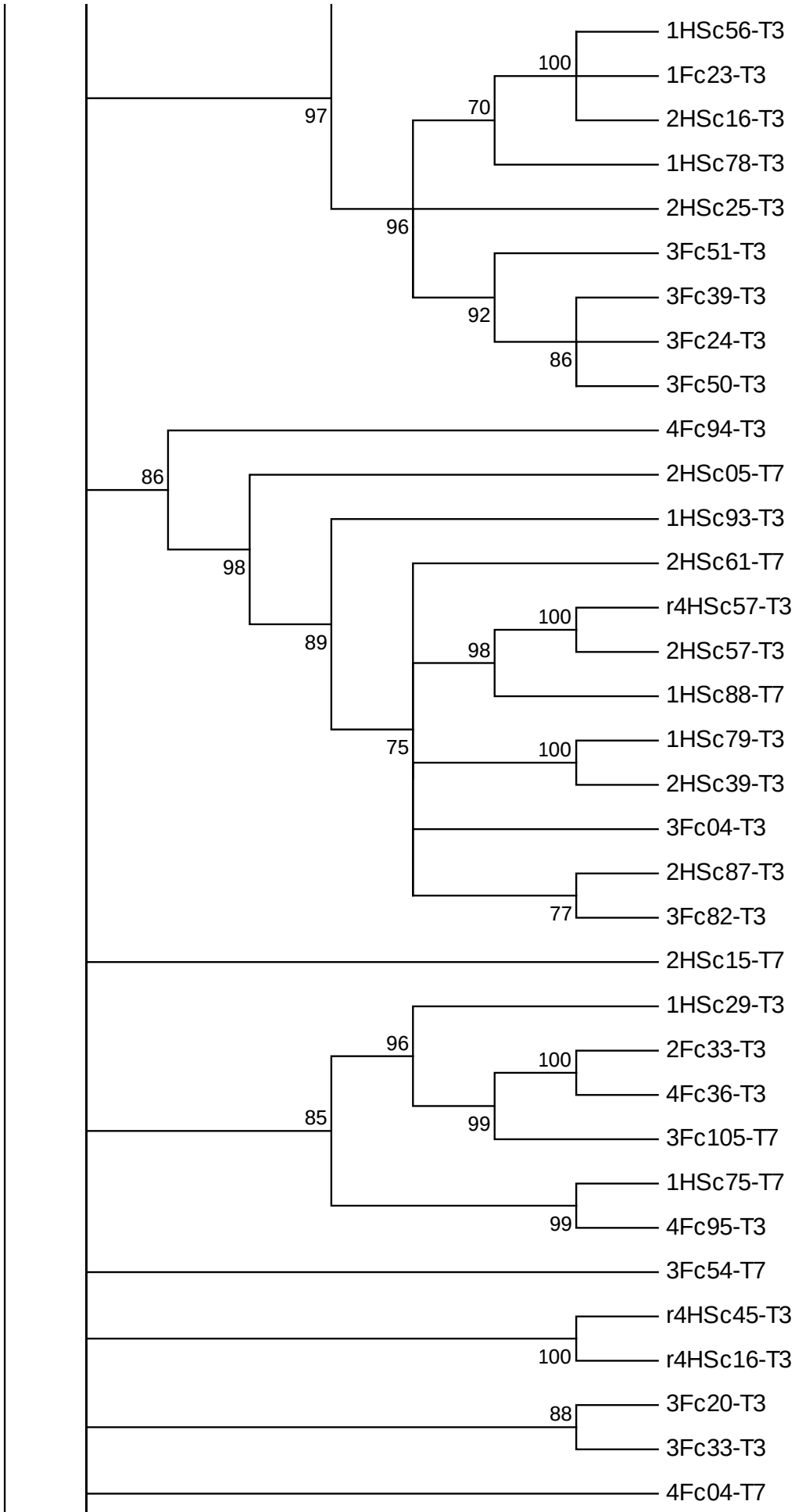
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3Fc24-T3
86
3Fc50-T3
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86
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1HSc93-T3
98
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89
98
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75
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77
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1HSc29-T3
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4Fc95-T3
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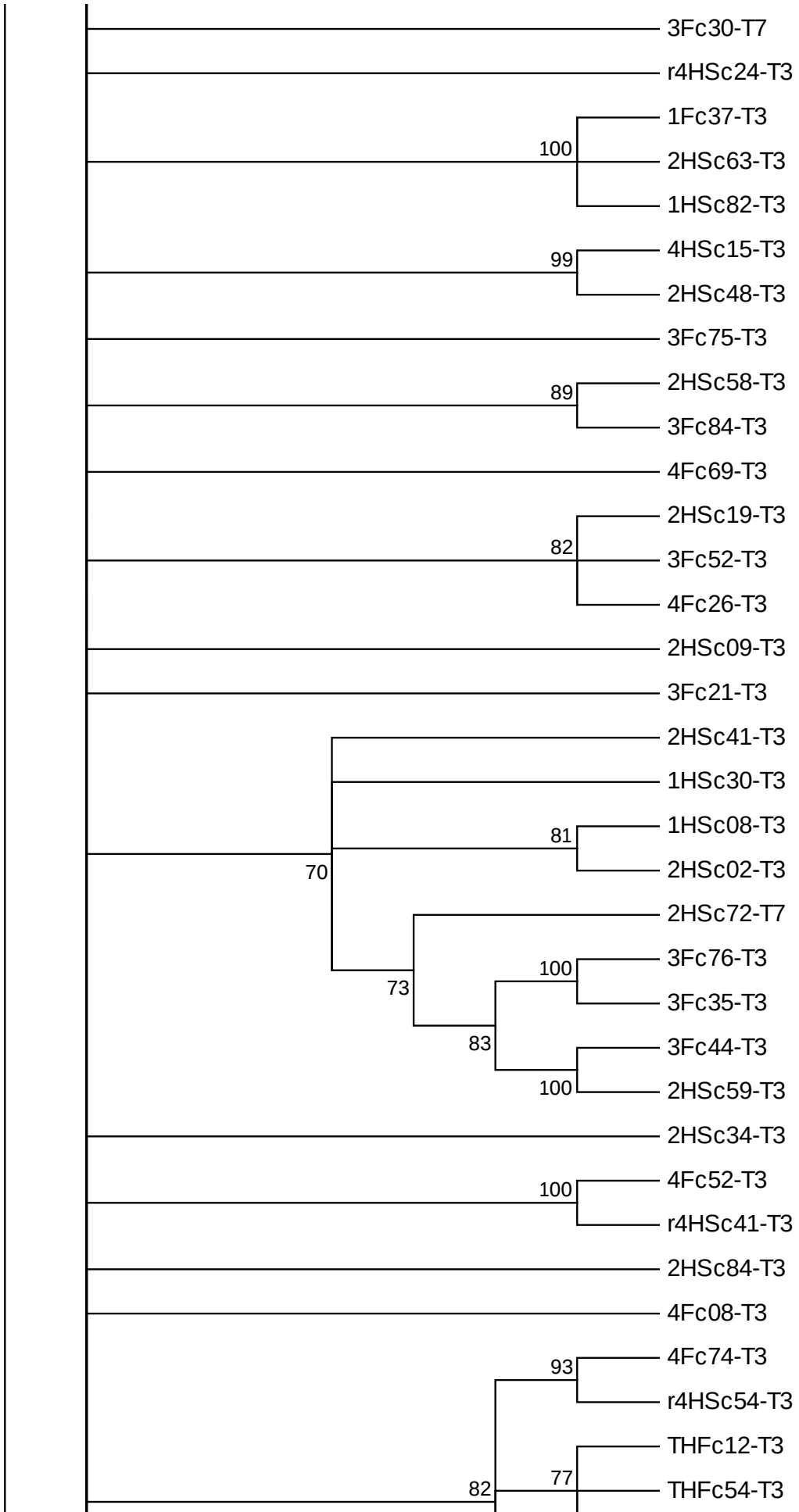
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3Fc75-T3
2HSc58-T3
89
3Fc84-T3
4Fc69-T3
2HSc19-T3
82
3Fc52-T3
4Fc26-T3
2HSc09-T3
3Fc21-T3
2HSc41-T3
1HSc30-T3
1HSc08-T3
81
70
2HSc02-T3
2HSc72-T7
3Fc76-T3
100
73
3Fc35-T3
83
3Fc44-T3
100
2HSc59-T3
2HSc34-T3
4Fc52-T3
100
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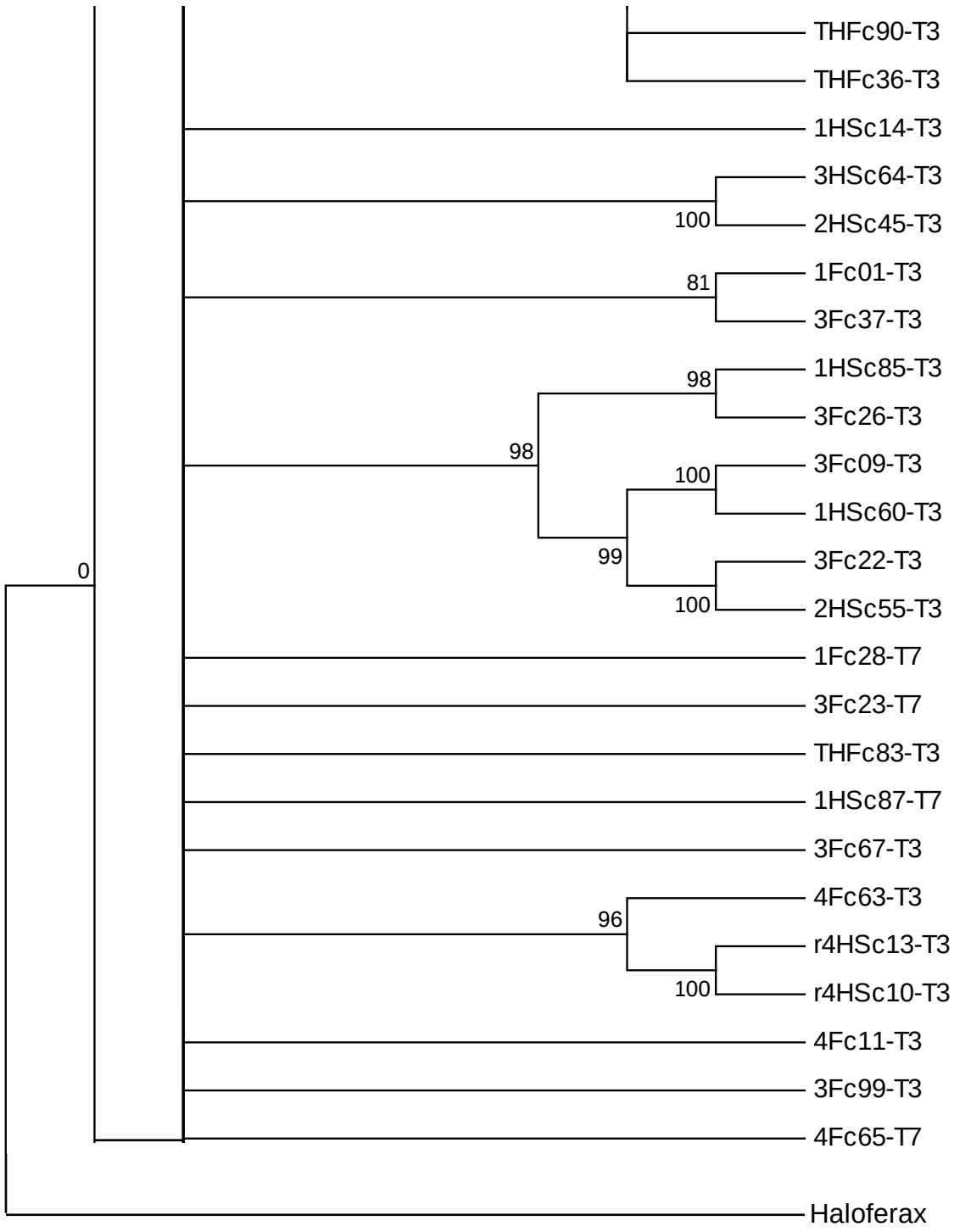
77
82
THFc54-T3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98
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3Fc09-T3
1HSc60-T3
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100
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1Fc28-T7
3Fc23-T7
THFc83-T3
1HSc87-T7
3Fc67-T3
4Fc63-T3
96
r4HSc13-T3
100
r4HSc10-T3
4Fc11-T3
3Fc99-T3
4Fc65-T7
Haloferax

Vita

Federico Noris obtained his Bachelor's and Master's degrees in Environmental Engineering from Politecnico di Milano, Italy in 2003 and 2006, respectively. During the final year of both degrees, he came to The University of Texas at Austin to work with Dr. Kerry Kinney on vapor phase biofilter and bioaerosol emissions released from these devices. Subsequently, Mr. Noris entered the Graduate School at The University of Texas at Austin and became a doctoral student in the Department of Civil, Architectural, and Environmental Engineering working with Dr. Kerry Kinney and Dr. Jeffrey Siegel on the evaluation of HVAC filters as a sampling mechanism of the indoor environment.

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This manuscript was typed by Federico Noris.