Assessing allergenic fungi in house dust by floor wipe sampling and quantitative PCR

Abstract In the present study, we modified an existing surface wipe sampling method for lead and other heavy metals to create a protocol to collect fungi in floor dust followed by real-time quantitative PCR (qPCR)-based detection. We desired minimal inconvenience for participants in residential indoor environmental quality and health studies. Accuracy, precision, and method detection limits (MDLs) were investigated. Overall, MDLs ranged from 0.6 to 25 cell/cm^2 on sampled floors. Overall measurement precisions expressed as the coefficient of variation because of sample processing and qPCR ranged 6–63%. Median and maximum fungal concentrations in house dust in study homes in Visalia, Tulare County, California, were 110 and 2500 cell/cm^2, respectively, with universal fungal primers (allergenic and nonallergenic species). The field study indicated samplings in multiple seasons were necessary to characterize representative whole-year fungal concentrations in residential microenvironments. This was because significant temporal variations were observed within study homes. Combined field and laboratory results suggested this modified new wipe sampling method, in conjunction with growth-independent qPCR, shows potential to improve human exposure and health studies for fungal pathogens and allergens in dust in homes of susceptible, vulnerable population subgroups.

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Practical Implications

Fungi are ubiquitous in indoor and outdoor environments, and many fungi are known to cause allergic reactions and exacerbate asthma attacks. This study established—by modifying an existing—a wipe sampling method to collect fungi in floor dust followed by real-time quantitative PCR (qPCR)-based detection methodologies. Results from this combined laboratory and field assessment suggested the methodology's potential to inform larger human exposure studies for fungal pathogens and allergens in house dust as well as epidemiologic studies of children with asthma and older adults with chronic respiratory diseases.

Introduction

Type I hypersensitivity reactions including allergic asthma and allergic rhinitis are a major cause of illness and disability in industrialized countries, and exposure to allergens is a strong risk factor for asthma symptom prevalence and severity (Ring et al., 2001). Fungi are ubiquitous in indoor and outdoor environments, and many fungi are known to cause allergic reactions and exacerbate asthma attacks (Dharmage et al., 2001). Approximately 4.6 million people in the USA have asthma attributable to dampness and mold in homes, with a corresponding annual economic loss estimated at $3.5 billion in 2004 (Mudarri and Fisk, 2007).

Another important adverse health outcome caused by fungi is invasive fungal infection including invasive pulmonary aspergillosis (IPA), which is highly fatal for immunocompromised patients (Lin et al., 2001). Chronic obstructive pulmonary disease (COPD) patients, typically treated with antibiotics and corticosteroids, are likely a main risk group for invasive fungal infections such as IPA (Guinea et al., 2010). However, quantitative aspects of indoor air and surface-associated fungal allergens are largely unknown compared to well-studied dust mite allergens. For instance, while several threshold levels for sensitization and asthma symptoms are suggested for dust mite allergens Der p1 and Fel d1 (Platts-Mills et al., 1992), such values are not available for fungi, in part due to lack of standardized measurement methods.

To date, the current paradigm in measuring a diversity of indoor fungal allergens includes sample by vacuuming and culture-based methods to estimate the fungal allergen contents (Cho et al., 2008; Hicks et al., 2005). Culture-based methods, however, are often biased by the selection of the growth media (Takahashi, 1997) and are incapable of detecting noncultivable microorganisms (Lignell et al., 2008; Meklin et al., 2004) which are potential allergens and pathogens (Hirvonen et al., 1997). Additionally, growth-based methods cannot detect nonviable fungi, which still have clinical importance because they retain bioactive materials such as ergosterol and allergenic proteins (Kauffman and Van Der Heide, 2003). Furthermore, identification of fungal species by growth-based methods is based on the observation of their microscopic and macroscopic morphologies, and accuracy of the identification can thus be biased by an operator's experience and level of expertise. Some research, for example, has indicated growth-based methods underestimate concentrations of fungi in dust by 2–3 orders of magnitude compared to quantitative polymerase chain reaction (qPCR)-based measurements (Meklin et al., 2004). Consequently, traditional growth-based methods likely lead to less toxicologically relevant exposure assessments for epidemiologic analyses. In contrast, emerging molecular-based methods including qPCR are not dependent on fungal cultivability or viability and therefore appear to be toxicologically more relevant than growth-based methods.

In recent years, studies have started to use molecular-based methodologies to investigate the fungal contents in house dust (Amend et al., 2010; Cai et al., 2009; Lignell et al., 2008; Meklin et al., 2004; Pitkaranta et al., 2008; Vesper et al., 2004). Using the DNA sequences for qPCR probes and primers provided by the U.S. Environmental Protection Agency (EPA) (Haugland and Vesper, 2002), some studies also quantified fungi in house dust (Cai et al., 2009; Lignell et al., 2008; Meklin et al., 2004; Vesper et al., 2004, 2007). The majority of these studies (Lignell et al., 2008; Meklin et al., 2004; Vesper et al., 2004, 2007) used vacuum cleaner methods to collect house dust. In these methods, house dust is collected from a specified area of carpeted or hard floors, and sampled dust is sieved and weighed for subsequent qPCR-based detection methodologies. Although the methods are suitable to collect large quantities of house dust and allow for dust collection from carpeted areas, they are relatively intrusive upon participants during field monitoring, and for hard surfaces like wood floors as well as for carpets of varying pile, do not as efficiently remove surface-bound organisms compared to the moist wipe method standardized for lead sampling (Reynolds et al., 1997). Moreover, highly accurate and precise measurement methods to identify and quantify target fungal species are still needed.

The goal of this study was to establish a wipe sampling method for quantifying fungi concentrations of hard flooring surfaces in homes. The method includes a novel modification of an existing wipe sampling method developed by the American Society for Testing and Materials (ASTM) originally designed to determine lead content on floor surfaces followed by a laboratory-based analytical procedure for identifying and quantifying fungi by real-time qPCR. Accuracy, precision, and method detection limits (MDLs) associated with extraction and qPCR-based quantification of fungi from sampling wipes were investigated. The method was demonstrated in a field study conducted in Visalia, Tulare County, California, USA in 2009–2010. Correlations among fungal species as well as seasonal and spatial variations in fungal concentrations in house dust were investigated.

Material and methods

Collection of house dust

Floor dust samplings were performed as a part of a community-based participatory research pilot project (CBPR) conducted in Visalia, Tulare County, California, USA in July 2009 (representing summer cooling season) and January 2010 (representing winter heating season) (Shendell et al., 2011a,b). Samples were taken from homes of older adults with asthma and COPD. For further details about the targeted community, CBPR process, recruitment of participants, and results from using three questionnaires and one technician walk-through survey, see the study by Shendell et al. (2011a). In the present study, we summarize these key details. A modification to an existing wipe sampling method developed by ASTM (Method E 1728-03) (ASTM, 2003) was used to collect floor dust. Pre-wetted wipes with 6% isopropyl alcohol (23 × 23 cm, polyester, TX1086 Vectra QuanSat, ITW Texwipe) were used to collect dust on a 30 × 30 cm floor area.

The selected surface area was wiped side to side several times while applying pressure with the fingertips. Nine homes were sampled in the cooling season, of which six homes were selected for repeated measurements in the heating season. Samples were taken in the kitchen on hard flooring that was relatively smooth and nonporous (e.g., tile and sheet vinyl floors). Within individual homes, we also identified and sampled other locations with flooring similar to that in the kitchen (e.g., another room if continuous with the kitchen, like a home office or computer workspace, or the den/living room and/or family room). In five of the homes considered during the cooling and heating seasons, two sampling locations (i.e., kitchen and other location) were selected. Overall, 30 samples across study homes and both seasons, including two field blanks and six duplicates, were collected. For duplicates, sampling locations were adjacent to one another, i.e., primary and duplicate samples were side-by-side. A field blank was a wipe handled in the same manner as both the field and duplicate samples except no sample was conducted, i.e., no surface was wiped. Sampled wipes were stored in screw-top glass containers (USEPA class 1/2, purchased from Cole-Parmer) in a −20°C freezer until subsequent analysis. We also note that a technician walk-through survey, composed of previously validated questions in specific sections from a federal agency tool, was conducted in each study home each season. Topics included potential exposure agents, including known environmental asthma triggers, ventilation—both natural and mechanical—and the observed conditions of bathrooms, ceilings, floors and walls, doors, the water heater, the heating, ventilation and air conditioning system, kitchen, laundry area, lighting, patio/porch/deck/balcony, smoke and carbon monoxide detectors, stairs and windows. For a summary of the content and results from using this survey, please see the study by Shendell et al. (2011a).

Preparation of the standard fungal DNA

These experiments considered the quantitation of five medically important allergenic fungi including *Alternaria alternata* (PEM 01043; Prestige Enviro-Microbiology), *Aspergillus fumigatus* (ATCC 34506), *Cladosporium cladosporioides* (ATCC 16022), *Epicoccum nigrum* (TU BL-3; The University of Tulsa), and *Penicillium chrysogenum* (CAES PC-1; The Connecticut Agricultural Experiment Station). To prepare standards for qPCR, spores of *A. fumigatus*, *C. cladosporioides*, and *P. chrysogenum* were generated by growth on malt extract agar. The spores were harvested by cotton swabs and suspended in 10 ml of 70% ethanol. The suspensions were divided into 1.5-ml microtubes to obtain several subsamples. These subsamples were centrifuged at 10,000×g for 3 min to pellet the spores, and the ethanol supernatant was removed

from the tubes prior to DNA extraction. To generate the standard curves based on cell counts, the numbers of spores in the pellets were enumerated by direct microscopy (Hospodsky et al., 2010). Unlike fungal species forming unicellular amerospores, such as *A. fumigatus*, *C. cladosporioides*, and *P. chrysogenum*, accurate cell enumeration is difficult for *A. alternata* and *E. nigrum*, which produce multicellular dictyospores. Therefore, tissues of approximately 4 cm^2 areas of the colonies were collectively isolated and used to prepare the DNA standards for these two species, and the original numbers of fungal cells were back-calculated based on the recovered DNA quantities and genome sizes of these two fungal species. Then the theoretical mass concentration of DNA per cell for each *A. alternate* and *E. nigrum* organism was estimated by Equation 1 (Dolezel et al., 2003):

$$\text{DNA mass in one cell [pg]} = \frac{\text{genome size [bp]}}{0.978 \times 10^9 \text{[bp/pg]}}. \quad (1)$$

The recovered DNA was quantified by PicoGreen (dsDNA Reagent and Kits; Invitrogen), and the genome size of *E. nigrum* was assumed to be 30.0 Mbp [same as *A. alternata* (Masunaka et al., 2005)] because no data were available at the time of this study. We assumed a 10% DNA extraction efficiency for these two species based on levels typically observed for fungal spore DNA extraction in our laboratory (Hospodsky et al., 2010). For all five fungal species considered and in general for fungi, the quantities of rRNA gene copies are known to be highly variable by fungal strain (Herrera et al., 2009). As a result, in this study, qPCR standards were based on the total numbers of spores harvested from the five above-mentioned fungal strains rather than from the gene copies. For universal fungal qPCR, standard curves were generated using the *A. fumigatus* DNA standard.

DNA extraction from floor samples

Each wipe sample was loaded in a sterile 250-ml tissue culture flask (Product Number 353134; BD Biosciences, Sparks, MD, USA) with 150 ml of Tween 20 solution (0.1%). The flasks were vigorously shaken by hand for 1 min and then agitated overnight by a titer plate shaker (Model 4525; Lab-Line Instruments, Melrose Park, IL, USA). The resultant suspension was introduced into a sterile 100-ml disposable filter funnel (MicroFunnel Plus filter funnel with 47 mm membrane filter with 0.45 μm pores, Pall) to collect recovered fungal cells on the membrane filter. DNA extraction was performed for pure culture standards as well as the membrane filter samples containing recovered fungal spores using the Mobio PowerSoil DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA,

USA). One-quarter of each membrane filter was placed in 2-ml microtubes with Mobio power beads (1.0 g) and lysis solution (750 ml) supplemented with 0.1-mm-diameter glass beads (300 mg) and 0.5-mm-diameter glass beads (100 mg). Cell walls were lysed by bead beating for 5 min at 3450 rpm (Model 607; BioSpec Products, Bartlesville, OK, USA). After bead beating, DNA was purified in accordance with the manufacturer's protocols and eluted into 50 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH = 8.0).

Real-time quantitative PCR

The TaqMan method was used for species-specific, genera-specific, and universal fungal detection and employed Afumi, Aaltr, Cclad2, Enigr, PenGrp3, and PenAsp1mgb assays (Table 1) reported by the U.S. EPA (Haugland and Vesper, 2002). Selectivity of each qPCR assay, i.e., the extent of detectable fungal species, is summarized in Table 1. A 25-μl reaction mixture containing the template DNA (1 μl sample extract), 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA), 1 μM of each primer, and 0.08 μM of the probe was used. The SYBR Green method was used for universal fungal detection with the primers FF2 and FR1 reported by Zhou et al. (2000). A 25-μl reaction mixture containing the template DNA

(1 μl sample extract), 1× SYBR Green Master Mix (FastStart Universal SYBR Green Master (ROX); Roche Applied Science, Indianapolis, IN, USA), and 0.3 μM of each primer was used. A real-time PCR system (ABI 7500 Fast Real-time PCR System; Applied Biosystems) was used with the following cycle condition: 50°C for 2 min, 95°C for 15 min of initial denaturation, and 45 cycles of 95°C for 15 s of dissociation and 60°C for 1 min of annealing and extension. Cycle thresholds were calculated by using the auto function in ABI 7500. In this study, qPCR measurements were performed in triplicate. For PenAsp1mgb and FF2/FR1, standard curves were generated using the *A. fumigatus* DNA standard. To test for PCR inhibition, seven randomly selected extracts from the wipe samples were added to subsets of diluted *E. nigrum* DNA, and standard curves were produced. No significant inhibition was observed in this study.

Quality control

Accuracy, precision, and MDLs associated with the wipe sampling method, in conjunction with qPCR detection methodologies, were investigated. In floor dust samples collected by the wipe sampling method, accuracy in sample analysis is potentially limited by efficiencies in DNA extraction from cells and whole-cell recovery from sampling wipes. Therefore, spiked experiments were performed to investigate recovery efficiencies from the wipes. Aqueous suspensions containing known numbers of *A. fumigatus, C. cladosporioides*, and *P. chrysogenum* spores were spiked on clean wipes ($n = 3$ for each species) (Table 2). Fungal spores spiked on clean wipes were recovered and quantified according to the methodologies described earlier.

Overall precision was characterized in terms of the cumulative coefficient of variation (COV) for six pairs of field duplicates, i.e. (x_{11}, x_{12}), (x_{21}, x_{22}), $(x_{31}, x_{32}) \sim (x_{n1}, x_{n2})$, calculated according to Equation 2 (Hyslop and White, 2009):

$$\text{Cumulative COV} = \sqrt{\frac{1}{n}\sum_{i}^{n}\left[\frac{(x_{i1}-x_{i2})/\sqrt{2}}{m_i}\right]^2} \qquad (2)$$

Table 1 Primers and probes used for the qPCR assays

Assay name	Target fungal species	Primer (probe) name	Sequence 5'–3'
FF2/FR1[a]	All	FF2	GGTTCTATTTTGTTGGTTTCTA
		FR1	CTCTCAATCTGTCAATCCTTATT
Aaltr[b]	*Alternaria alternata*	AaltrF1	GGCGGGCTGGAACCTC
		AltrR1-1	GCAATTACAAAAGGTTTATGTTTGTCGTA
		AaltrP1	TTACAGCCTTGCTGAATTATTCACCCTTGTCTTT
Afumi[b]	*Aspergillus fumigatus, Neosartorya fischeri*	AfumiF1	GCCCGCCGTTTCGAC
		AfumiR1	CCGTTGTTGAAAGTTTTAACTGATTAC
		AfumiP1	CCCGCCGAAGACCCCAACATG
Cclad2[b]	*Cladosporium cladosporioides,* svar. 2	Cclad2F1	TACAAGTGACCCCGGCTACG
		CcladR1	CCCCGGAGGCAACAGAG
		CcladP1	CCGGGGATGTTCATAACCCTTTGTTGTCC
Enigr[b]	*Epicoccum nigrum*	EnigrF1	TTGTAGACTTCGGTCTGCTACCTCTT
		EnigrR1	TGCAACTGCAAAGGGTTTGAAT
		EnigrP1	CATGTCTTTTGAGTACCTTCGTTTCCTCGGC
PenGrp3[b]	*Penicillium chrysogenum/ griseofulvum/ glandicola/ coprophilum/ expansum* and *Eupenicillium*	PchryF1	CGGGCCCGCCTTAAC
		PchryR1-1	GAAAGTTTTAAATAATTTATATTTTCACTCAGAGTA
		PenP2	CGCGCCCGCCGAAGACA
PenAsp1 mgb[b]	Universal *Penicillium, Aspergillus* and *Paecilomyces varioti*	PenAspF1	CGGAAGGATCATTACTGAGTG
		PenAspR1	GCCCGCCGAAGCAAC
		PenAspP1 mgb	CCAACCTCCCACCCGTG

Table 2 Recovery efficiencies of fungal spores from the wipes. Fungal spores were spiked on the clean wipes, and the recovered spores were quantified by qPCR

Fungal species	Spiked amount (cell)	Recovered amount (cell)		Recovery efficiency (%)	
		Mean	s.d.[a]	Mean	s.d.[a]
Aspergillus fumigatus	1.3×10^7	2.3×10^6	1.1×10^6	19	9
Cladosporium cladosporioides	1.7×10^6	1.7×10^5	3.7×10^4	10	2
Penicillium chrysogenum	6.9×10^6	1.7×10^6	3.0×10^5	25	4

where m_i is the arithmetic mean (average) of ith paired data. As shown in Equation 2, the cumulative COV is a root mean square of multiple COV values obtained by a series of duplicated experiments.

Overall MDLs were determined by qPCR sensitivities and the intensity of background signal. Detection by qPCR is based on a logarithmic signal amplification and is binary, i.e., either positive or negative. Thus, a probability distribution was used to estimate the qPCR MDL associated with sensitivities of qPCR (Hospodsky et al., 2010). The qPCR MDL was defined as the smallest fungal concentration where seven successful PCR amplifications out of seven trials ($P = 1/128$) could be observed to detect a microorganism concentration with 99% confidence. DNA standards serially diluted by 10 fold (from 0.0001 to 100 cells per reaction) were used to determine qPCR MDLs. Besides qPCR sensitivities, overall MDLs are limited by background signal caused by amplification of contaminant DNA or fungal spores. Here the intensity of this background signal was characterized by measurements of field blank. Thus, for this study, the overall MDLs were defined as the higher of the values characterized either by the qPCR MDLs or the degree of field contamination.

Results and discussion

Accuracy, precision, and MDLs

The spike experiments showed approximately 20% of *A. fumigatus, C. cladosporioides*, and *P. chrysogenum* spores were recovered from the wipes (Table 2). Recovery efficiency values were applied to field sample enumeration to present a more accurate representation of the absolute surface fungal concentrations. These quantities, however, still likely represent an underestimation of surface contamination as there may also be an efficiency associated with the transfer of fungal spores from the surface to the wipes. Although the spike experiment for *A. alternata* and *E. nigrum* was not performed because of difficulty in spiking accurate quantities of multicellular dictyospores produced from these two species, the recovery efficiency for these species was assumed to be 20% for subsequent analyses.

Overall precision was characterized by six field duplicates (Table 3). The cumulative COVs including nondetected duplicate pairs ranged from 6% to 63%. The cumulative COVs tended to be smaller for the qPCR assays with larger fractions of the nondetected samples because differences between nondetected duplicate pairs were 0%. To account for this bias, the cumulative COVs excluding the nondetected duplicate pairs were also calculated. The duplicate pairs with one detected and the other not detected were included in

Table 3 Precision of the qPCR measurements of fungi in house dust collected by the wipe sampling method. Six duplicates were employed in Visalia, Tulare County, California, USA

Assay name	Detection ratio[a]	Cumulative COV including nondetected pairs (%)	Cumulative COV excluding non-detected pairs (%)
FF2/FR1	10/12	60	67
Aaltr	3/12	21	46
Afumi	2/12	11	ND[b]
Cclad2	7/12	44	57
Enigr	7/12	47	60
PenGrp3	2/12	6	ND[b]
PenAsp1mgb	9/12	63	71

COV, coefficient of variation.
[a]Ratio of the detected samples to the total samples. Six duplicates included a total of 12 samples.
[b]Not determined.

the cumulative COV excluding nondetected pairs. A second COV was also defined that excluded nondetect pairs (Tables 3–5). The cumulative COVs excluding the nondetected pairs ranged from 46% to 71%. The overall precision can be divided into variation associated with the reproducibility of the qPCR measurements, variation associated with wipe sample processing, and variation associated with the wipe sampling process at study homes. Hospodsky et al. (2010) observed COVs of 28–79% for qPCR measurements of bacteria and fungi artificially spiked onto air sampling filters, and noted the majority of the variation was because of qPCR instrument repeatability, especially when the quantities were near the qPCR detection limit. Given the similar precision level estimated for this study, the measurement variation was likely due to the qPCR reproducibility and sample processing in the laboratory, rather than the variation associated with the wipe sampling process in the field.

For MDL, Figure 1 illustrates the detection ratios of the standard DNA with concentrations ranging from

Table 4 Seasonal variations in the qPCR measurements of fungi in house dust collected by the wipe sampling method from homes in Visalia, Tulare County, California, USA. Samples were taken twice in the same home in different seasons ($n = 6$ homes)

Assay name	Detection ratio[a]	Cumulative COV including nondetected pairs (%)	Cumulative COV excluding nondetected pairs (%)
FF2/FR1	11/12	72	72
Aaltr	3/12	35	77
Afumi	2/12	51	ND[b]
Cclad2	8/12	53	53
Enigr	7/12	84	94
PenGrp3	1/12	16	ND[b]
PenAsp1mgb	11/12	113	113

COV, coefficient of variation.
[a]Ratio of the detected samples to the total samples. Samples were taken twice in each six home, producing a total of 12 samples.
[b]Not determined.

Table 5 Spatial variations in the qPCR measurements of fungi in house dust collected by the wipe sampling method from homes in Visalia, Tulare County, California, USA. Two sampling locations (i.e., kitchen and another location) were selected within individual homes ($n = 5$)

Assay name	Detection ratio[a]	Cumulative COV including nondetected pairs (%)	Cumulative COV excluding nondetected pairs (%)
FF2/FR1	10/10	50	50
Aaltr	1/10	6	ND[b]
Afumi	0/10	0[c]	ND[b]
Cclad2	7/10	47	54
Enigr	8/10	45	45
PenGrp3	0/10	0[c]	ND[b]
PenAsp1mgb	10/10	94	94

COV, coefficient of variation.

[a]Ratio of the detected samples to the total samples. For five homes, two sampling locations within each individual home were selected, producing a total of 10 samples.
[b]Not determined.
[c]No sample was detected by qPCR. The cumulative COVs for qPCR assays with no sample detected were 0% as differences between nondetected duplicate pairs were 0%.
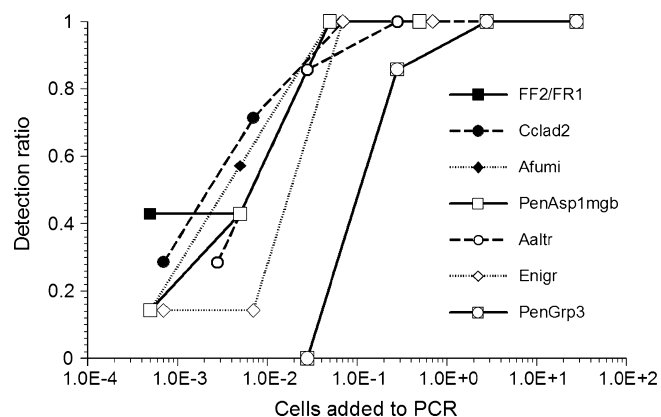
Fig. 1 Detection ratios of the DNA standards by qPCR. Seven replicates were performed for each concentration. The detection ratio was defined as a ratio of the number of successful PCR amplifications out of seven trials

0.0001 to 100 cells per reaction. The detection ratio was defined as a ratio of the number of successful PCR amplifications out of seven trials. The qPCR MDLs for FF2/FR1, Aaltr, Afumi, Cclad2, Enigr, PenGrp3, and PenAsp1mgb were 0.05, 0.28, 0.05, 0.07, 0.07, 2.8, and 0.05 cells per reaction, respectively. The qPCR MDLs for Aaltr and PenGrp3 were higher and reflect the lower PCR amplification efficiencies (75–85%) for these two primer sets compared to efficiencies for the remaining primers, which averaged > 95%. The qPCR MDLs are typically smaller than one cell because 18S rRNA genes usually occurred as a multiple tandem repeat. For instance, *A. fumigatus* has an average 54 copies of 18S rRNA gene per cell (Herrera et al., 2009). One field blank was employed per sampling season to identify degrees of field contamination. Few fungi were identified with the exception for FF2/FR1 showing a higher field blank value (0.49 cell) than the qPCR

MDL (0.05 cell). Consequently, the qPCR MDL for FF2/FR1 was defined to be 0.49 cell. Incorporating factors associated with accuracy in sample analysis including DNA extraction efficiencies from cells (10%) and whole-cell recovery from sampling wipes (20% for Aaltr and Enigr, and the values listed in Table 2 for the remaining species), fractions of DNA extract (1 μl out of total 50 μl extract) and membrane filter analyzed (one-quarter), and the fact that 900 cm^2 was the sampled area in study homes, the overall MDLs per unit surface area of sampled floor for FF2/FR1, Aaltr, Afumi, Cclad2, Enigr, PenGrp3, and PenAsp1mgb were calculated to be 5.7, 3.1, 0.6, 1.6, 0.8, 25, and 0.6 cell/cm^2, respectively.

Field measurements

Using the established method, fungal concentrations were characterized on floor surfaces collected from homes of older adults with asthma and/or COPD in Visalia, Tulare County, California, USA. Fungal concentrations were reported in the number of fungal cells per unit surface area of the floor (cell/cm^2) rather than in the number of fungal cells per unit mass of collected house dust (cell/mg) used in most previous studies (Lignell et al., 2008; Meklin et al., 2004; Vesper et al., 2004, 2007). Reporting fungal concentrations in cell/cm^2 is more relevant in human exposure assessments because it provides an absolute metric for microorganisms for a unit area of a home. In contrast, a fungal concentration in cell/mg is a relative metric that can be potentially biased if the same quantities of fungi, but different quantities of other dust particles, are on the floor.

Figure 2 illustrates the cumulative frequency distributions of fungal concentrations in floor dust collected from each home. Large variations were observed among study samples. The median concentrations for FF2/FR1, Aaltr, Afumi, Cclad2, Enigr, PenGrp3, and PenAsp1mgb were 110, < 3.1, < 0.6, 2.2, 1.3, < 25, and 2.9 cell/cm^2, respectively. The maximum concentrations were 2,500, 210, 7.3, 54, 160, 42, and 310 cell/cm^2, respectively. Cai et al. (2009) reported the fungal concentrations with a swab method for collection of surface dust of the upper part of the doorframe followed by qPCR-based detection methodologies. They reported the arithmetic mean and maximum values for PenAsp1mgb were 2.5 and 30.7 cell/cm^2, respectively, and a range of concentrations of total fungi from < 1 to 8610 cell/cm^2 with universal fungal primers 5.8F1 and 5.8R1. Although different methodologies were used in different sampling locations in homes, our results showed relatively good agreement with values reported by Cai et al. (2009).

Figure 3 demonstrates correlations among fungal species found in house dust collected from each home. Positive correlations were found among fungal species
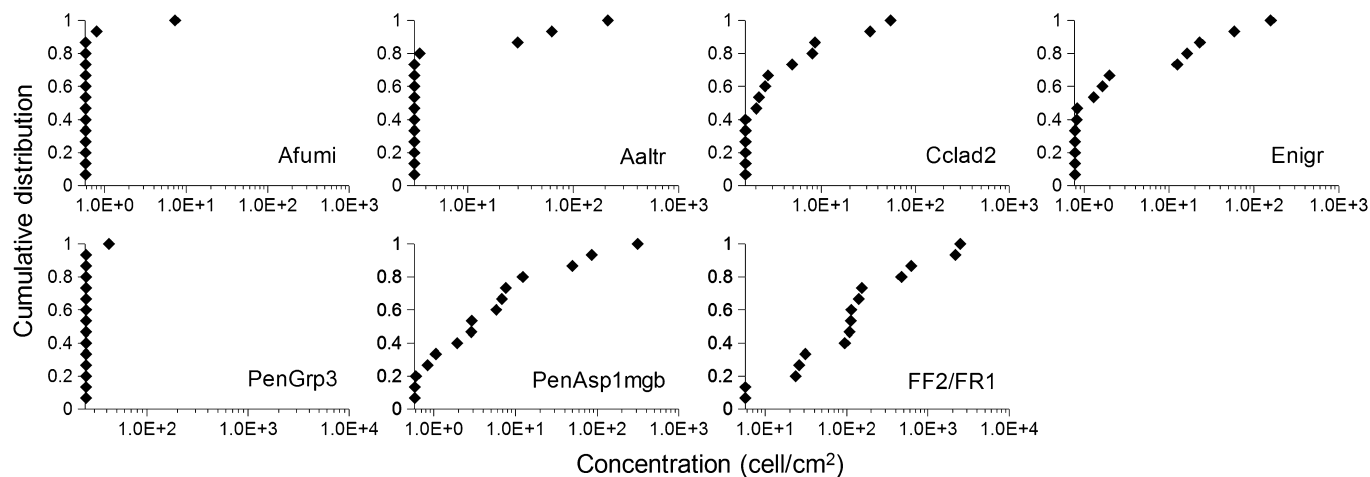
Fig. 2 Cumulative frequency distributions of fungal concentrations in floor dust collected from homes in Visalia, Tulare County, California, and USA ($n = 15$). Nine homes were sampled in the cooling season, of which six homes were available for the repeated measurements in the heating season. The results were averaged if samples were taken at multiple locations in the same homes. The samples taken in the same home in different seasons were represented as different data points
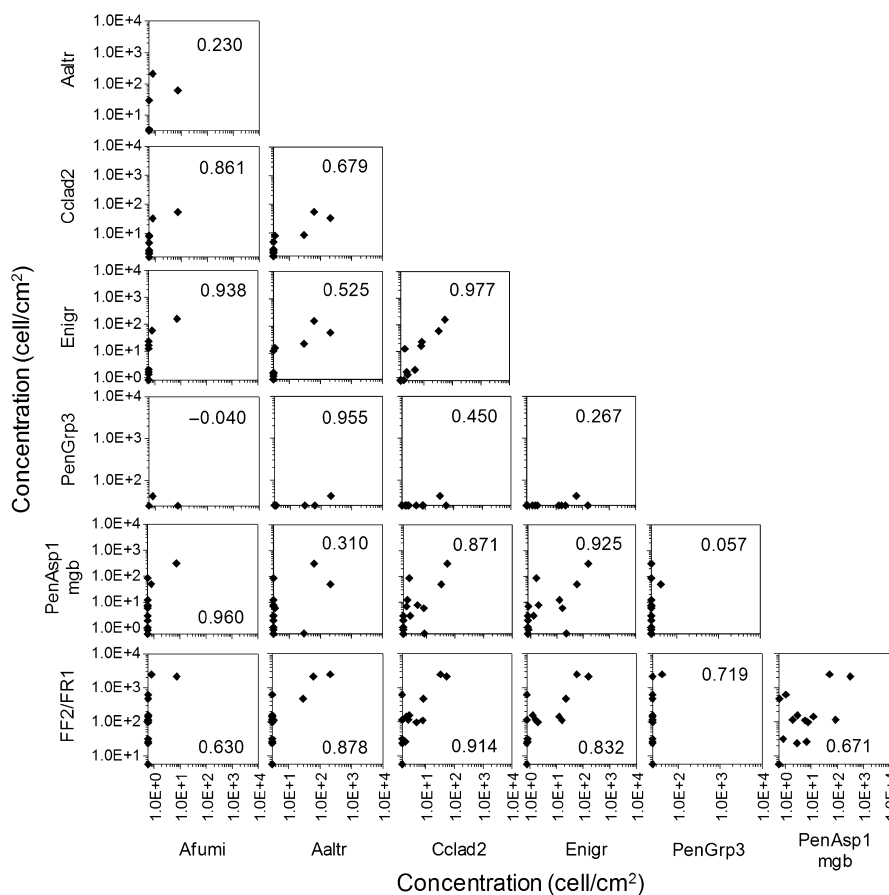

Fig. 3 Scatter-plot matrix and linear-scale correlation coefficients of fungal concentrations in floor dust collected from homes in Visalia, Tulare County, California, USA ($n = 15$). Nine homes were sampled in the cooling season, of which six homes were available for the repeated measurements in the heating season. The results were averaged if samples were taken at multiple locations in the same homes. The samples taken in the same home in different seasons were represented as different data points

with the exception being Afumi and PenGrp3 ($r = -0.040$), for which majorities of the samples were below MDLs. The greatest correlation was found between Cclad2 and Enigr ($r = 0.977$). Vesper et al. (2004) classified 32 species of fungi based on association with water damage of house, i.e., group 1 species

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associated with water damage and group 2 species not associated with water damage. Concentrations of group 1 species including *A. fumigatus* and *P. chrysogenum* were below MDLs in most of the present study's samples (Figure 2), which suggested water damage was not so severe, i.e., observed less frequently in the present sample population. This was confirmed through use of a technician walk-through survey conducted by community partners and researchers, which included questions to identify leaks, water-damaged flooring, wall and other interior finish materials, and any visible signs of mold (Shendell et al., 2011a). We also found positive correlations between the universal fungal detections (FF2/FR1 and PenAsp1mgb) and the remaining species-specific detections (Aaltr, Afumi, Cclad2, Enigr, and PenGrp3). This result suggested that a single universal fungal qPCR may be a strong indicator for multiple species-specific qPCR to characterize degrees of fungal contaminations in homes, although species-specific fungal detection may be desirable to better understand links with pathologies associated with each specific fungal species.

Seasonal variations in fungal concentrations in house dust were relatively large (Table 4) compared to variations associated with the reproducibility of the qPCR measurements and variation associated with house dust sampling (Table 3), and the concentrations were generally higher in the heating season than in the cooling season (Figure 4). The tendency was particularly true for Cclad2 and Enigr ($P < 0.05$; Wilcoxon signed-rank test). Some research has suggested concentrations of fungal species or assay groups in house dust varied significantly between different seasons (Kaarakainen et al., 2009). Although underlying causes of this tendency remains unclear, sampling in multiple seasons appears recommended to characterize representative whole-year

concentrations in a home. We note in the study community, in general, the summer cooling season demands air conditioning systems—homes vary in terms of added humidity to supply air—with windows and doors remaining closed most of the time, whereas in the winter heating season, space heating is typically needed late night through the morning until ambient temperature rises and morning fog dissipates. Future research should examine the potential role of air exchange rates and of particle filtration affecting measured concentrations of fungi of outdoor origin in home surface dust.

Finally, spatial variations in fungal concentrations in floor dust collected at multiple locations for a single study home were investigated. The spatial variations in terms of cumulative COVs were calculated according to Equation 2 and presented in Table 5. Cumulative COVs excluding nondetected pairs ranged 45–94%, which were comparable to cumulative COVs for the measurement precision (Table 3). Given the similar variation level estimated here, the observed spatial variation was likely due to measurement reproducibility, which is large when compared to variation associated with the spatial inhomogeneity of fungi in a single sampling site (study home). The observed tendency was consistent with findings on spatial variation of airborne fungi reported by previous literature (Ren et al., 2001; Reboux et al., 2009). However, in a moisture-damaged residence, some studies have also indicated the airborne fungal concentrations were different between rooms (Hyvarinen et al., 2001). Therefore, in future, larger field studies could help confirm this finding, especially for homes with historical and/or present moisture damages as well as larger and/or multistory homes. In the present field study, the study homes were one floor ranch style or apartments/condominiums with windows and/or sliding doors to the outdoors.
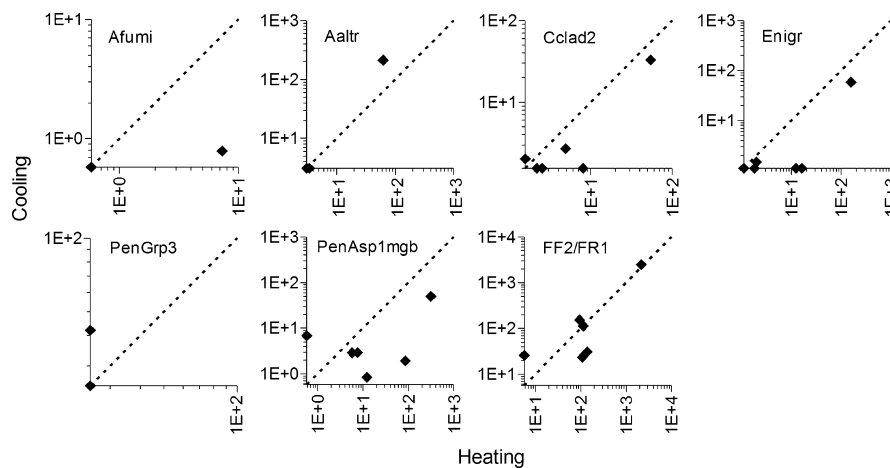

Fig. 4 Relationships between fungal concentrations in floor dust (cell/cm^2) collected in the same homes but in different seasons ($n = 6$). Floor dust samples were collected from homes in Visalia, Tulare County, California, USA. The dashed line represents the 1:1 line

Conclusions

This study established—by modifying an existing—a wipe sampling method to collect fungi in floor dust followed by qPCR-based detection methodologies. Accuracy, precision, and MDLs were investigated. The method was further validated by field investigation, which also suggested samplings in multiple seasons were desirable to characterize representative whole-year concentrations given the temporal variations observed in study homes. Spatial variations in fungal concentrations for a single sampling site (home) in the same seasons were relatively small. This wipe sampling method was less intrusive in homes of susceptible, vulnerable populations like immunocompromised older adults, i.e., less disruptive for participants and not noisy compared to vacuums used in previous studies of environmental asthma triggers and heavy metals. Therefore, this method, in conjunction with growth-independent qPCR methodologies, shows potential to improve human exposure studies for fungal pathogens and allergens in house dust as well as epidemiologic studies of older adults with chronic respiratory diseases and other potential comorbidity.

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