



## Measurement of aeroallergens from furnace filters



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### ABSTRACT

**Background:** Exposure assessment is an important component of allergic disease diagnosis and management. Analysis for allergen content in vacuumed dust has been used traditionally.

**Objective:** To study allergen levels of dust taken from high-efficiency furnace filters in Midwestern homes. **Methods:** Furnace filters used were FQT12 1-inch disposable filters with high-efficiency media placed in homes enrolled in the Kansas City Safe and Healthy Homes Project. Dust was removed from the filters by vacuuming. Fungal culture was used to obtain counts of viable spores. Aeroallergens Fel d1, Can f1, Mus m1, Der f1, Der p1, and Bla g2 and antigenic material from *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species were measured using commercially available immunoassay materials.

**Results:** Sixty filters were recovered from 56 homes after an average 135 days in situ. Mean weight of dust recovered was 2.43 g and correlated well with the time the filter was in place. Viable spore counts ranged to  $4.8 \times 10^7$  per gram of dust. Mean fungal antigenic material ranged to 42  $\mu\text{g}$  per gram for *Cladosporium* species. Mean aeroallergen material ranged to 7  $\mu\text{g}$  per gram for Fel d1. Aeroallergen measurements were above the level of detection in 100% of houses for Fel d1 and 89% of houses for Bla g2. Levels of Fel d1 and Can f1 were strongly positively correlated.

**Conclusion:** Allergens from 5 common aeroallergen species and antigenic material from 4 common fungal taxa can be measured in dust taken from high-efficiency furnace filters.

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### Introduction

Exposure assessment is an important component of the diagnosis and management of allergic diseases.<sup>1,2</sup> Since allergen exposure measurements were initially explored, there has been a dichotomy concerning whether measurements of airborne allergen or dustborne allergen provide the best surrogate for exposure.<sup>3–7</sup> Much evidence has been presented that indicates various allergenic components partition into airborne and dustborne fractions<sup>8,9</sup> depending on the nature of the particles associated with specific allergens.<sup>10</sup> Many previously reported allergen measurements in house dust used vacuumed dust collected from house floors, carpets, and bedding.<sup>11</sup> However, most allergen exposure is by the inhaled route, and airborne allergen levels might provide a better indication of exposure and sensitization.<sup>12,13</sup>

It has generally been considered that heavier particles, or particles with increased density and less favorable aerodynamic characteristics, spend less time airborne, whereas smaller particles

or particles adapted toward airborne dissemination spend more time in the air. For practical purposes, house dust can be considered in 2 regions of the home: mobile (airborne and associated with hard surfaces) and non-mobile (reservoir embedded in carpet or permeable surfaces).<sup>14,15</sup> Because practical aspects of studying allergen exposures have dictated the use of vacuumed dust samples from houses to evaluate home allergen exposure, it has been presumed that the airborne exposure level is a constant function of the total amount of reservoir dust that can be collected.<sup>16</sup> However, clear differences can be shown in the dustborne and airborne levels of many important allergens.<sup>3,17</sup>

Methods for measurement of airborne dust allergen levels have ranged from the use of a high-volume pump and filtration device<sup>18</sup> to specially equipped Anderson samplers.<sup>19</sup> Also, several types of personal samplers have been used, including those worn in the nose<sup>6</sup> and pumped filtration devices worn on the collar of an individual<sup>19</sup> or placed on a pillow.<sup>20</sup> The routine use of house dust collected from furnace filters has not previously been reported. Before 2000, most furnace filters used in the United States would not have allowed sufficient collection of dust. They were of a loose-weave fiberglass design that functioned mostly to protect the fan of the furnace blower. However, near the turn of this century, the use of more effective furnace filtration for removing contaminant particles from breathable air began to be common in US homes. The

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authors' hypothesis was that indoor allergens could be effectively measured from high-efficiency filters routinely placed in the heating, ventilating, and air conditioning (HVAC) system of domestic houses.

## Methods

Furnace filters used in this study were FQT12 (Minimum Efficiency Reporting Value [MERV] 12) 1-inch disposable furnace filters with high-efficiency media provided by Allergy Zone (Louisville, Kentucky). Allergens, including Fel d1, Can f1, Mus m1, Der f1, Der p1, and Bla g2, were assayed using standards and monoclonal and polyclonal antibodies obtained from Indoor Biotechnologies (Chantilly, Virginia). Fungal allergens were analyzed using whole-species antigen assays for fungi, including *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium notatum*. Whole-species determinations used polyclonal antibodies and standards obtained from Greer Laboratories (Lenoir, North Carolina).<sup>21</sup> Petri plates for determination of fungal growth were obtained from Remel Laboratories (Lenexa, Kansas). Other chemicals and buffers were obtained from Sigma (St Louis, Missouri).

## Sampling Protocol

Environmental health specialists placed and removed furnace filters in a subset of homes enrolled in the Kansas City Safe and Healthy Homes Project (KCSHHP). Homes were typically visited several times throughout the KCSHHP for assessment, Healthy Homes Education, and evaluation of remediation results. Typically, sequential visits were scheduled 90 to 180 days apart. During sequential visits to the home, whenever possible, furnace filters that had been installed at prior visits were removed, sealed in a large plastic bag, and returned to the laboratory. Typically, only 1 filter was collected from a home but a second filter was collected from some homes at a subsequent visit. All human subject aspects of the KCSHHP were approved by the internal review board of Children's Mercy Hospital that reviewed and approved informed consent and assent documents. Enrollment criteria for participants in the KCSHHP included income guidelines of less than 80% of the median income for the area, housing not of historic significance, housing not situated in a flood plain, and at least 1 child with asthma resident in the home for a minimum of 4 days per week. Homes in the program were assessed by environmental health professionals for indoor hazards, general repair, indoor air quality problems, allergen levels, mechanical systems, and general clutter and condition. Based on the assessment, a series of targeted interventions designed to improve safety and indoor air quality of the home was recommended. Homeowners typically received a new vacuum cleaner and furnace filters at the beginning of the study, in addition to an assortment of useful cleaning supplies. Financial support was provided by the Department of Housing and Urban Development as a Healthy Homes Initiative Demonstration Grant awarded to Children's Mercy Hospitals and Clinics. Each home environment was assessed using a specific set of onsite and laboratory sampling and analyses.<sup>22</sup>

## Dust Collection and Analysis Procedures

In the laboratory, filters were vacuumed to collect dust using a HEPA-rated vacuum unit and a dust collection sample bag (x-cell-100, Midwest Filtration, Cincinnati, Ohio). All dust samples were sieved to remove large material, weighed to estimate recovery, and extracted in phosphate buffered saline. One hundred microliters of a 1:10,000 dilution of the extracted dust was spread onto Inhibitory Mold Agar (Remel IMA with gentamycin) to estimate fungal load. Dilutions up to 1:1,000,000 were performed, if necessary, to obtain estimates. Levels of Fel d1, Can f1, Mus m1, De f1, Der p1, and Bla g2 were determined using monoclonal-based immunoassays. Levels

of Der f1 and Der p1 were presented as the sum of dust mite allergens. Levels of fungal antigens from *A alternata*, *A fumigatus*, *C herbarum*, and *P notatum* were evaluated by polyclonal assays.<sup>22,23</sup> Results of immunoassays were displayed as nanograms per gram of sifted dust for analysis. The published lower level of detection for the polyclonal based fungal assays is 100 ng per gram of dust,<sup>24</sup> and for the monoclonal-based assays the published level of detection is 12 to 50 ng per gram of dust.<sup>25</sup>

## Data and Statistics

Descriptive statistics were produced with an Excel spreadsheet (Microsoft Corporation, Redmond, Washington) and comparative statistics were generated with SPSS software (IBM Corporation, Armonk, New York). Correlation coefficients were by the Pearson correlation equation as performed in Excel or the Spearman rank method for dust immunoassay results as performed in SPSS.

## Results

Furnace filters were collected from July 1, 2011 to December 31, 2012. Three hundred eighty-two families were enrolled in the Healthy Homes Project overall. General home characteristics for homes in Healthy Homes Studies have been presented previously.<sup>21</sup> For this study, 60 filters were recovered from 56 homes. A sufficient amount of dust to allow analysis was removed from 58 filters, and 2 filters had no dust on them and gave the appearance of not having been used. The average number of days these filters were installed in the HVAC system was 135. The longest time a single filter was in place was 579 days and the shortest time was 13 days. The mean surface area of the filters was 408 in<sup>2</sup>, varying from 280 in<sup>2</sup> (14 × 20) to 500 in<sup>2</sup> (20 × 25). The mean weight of dust recovered from the filters was 2.42 ± 2.87 g. There was a good positive correlation between dust recovered and days a filter was in place ( $r = 0.38$ ,  $P < .01$ ).

## Fungal Colonies

The estimate of viable fungal colonies recovered from the furnace filters ranged from  $2 \times 10^4$  to  $4.8 \times 10^7$  per gram of dust and calculated to an average of  $3.9 \times 10^6$  (SD  $1.1 \times 10^7$ ) colonies per filter. The number of viable colonies collected did not correlate with the weight of material collected (Correlation = 0.21,  $P = .15$ ) but did correlate with the time the filter was in place (Correlation = 0.36,  $P = .01$ ) and correlated inversely with the surface area of the filter (Correlation =  $-0.31$ ,  $P = .03$ ).

## Fungal Antigens

The fungal antigenic material was measured from the furnace filter dust. Values ranged from 100 ng per gram of dust, which was the lower level of detection of the assay, to more than 100,000 ng per gram for *A fumigatus* in 1 sample. The mean fungal antigen load varied from 42,347 ng per gram of dust for *C herbarum* to 8,284 ng per gram of dust for *A alternata* (Table 1).

There was a positive correlation between all specific fungal antigens and the number of colonies per milligram of dust except for *A fumigatus*. The most significant correlations with number of

**Table 1**  
Antigen concentration in nanograms per gram of dust

|               | <i>Alternaria alternata</i> | <i>Aspergillus fumigatus</i> | <i>Cladosporium herbarum</i> | <i>Penicillium notatum</i> |
|---------------|-----------------------------|------------------------------|------------------------------|----------------------------|
| Average       | 8,283                       | 34,017                       | 42,347                       | 12,253                     |
| SD            | 4,931                       | 34,342                       | 25,951                       | 9,226                      |
| Median        | 8,355                       | 12,545                       | 35,504                       | 10,147                     |
| Detectable, % | 90.7                        | 92.6                         | 94.4                         | 94.4                       |

colonies were for *C herbarum* and *P notatum* ( $P < .001$ ), but there was no significant correlation between any of the fungal antigens and the weight of the dust collected or the days the filter was in place.

A correlation analysis of the fungal antigen values for the filters (Table 2) indicated that *A alternata* and *C herbarum* were weakly positively correlated with each other and strongly correlated with *P notatum*, whereas *A fumigatus* was weakly correlated with *A alternata* and negatively correlated with *C herbarum* and *P notatum*. There was a positive correlation between the total fungal antigens measured and the total colonies ( $r = 0.26$ ,  $P = .07$ ).

The summary of allergenic material measured for each filter dust, including Fel d1, Can f1, Bla g2, and Mus m1 and the sum of De f1 and Der p1, is listed in Table 3. Values for Fel d1 ranged over 4 orders of magnitude, whereas values for Mus m1 ranged over a little more than 2 orders. The highest mean allergen level in the furnace filter dust was for Fel d1 at 7.4  $\mu\text{g}$  per gram of dust and the lowest was for dust mite at 689 ng per gram of dust for the sum of De f1 and Der p1 (Table 3). The allergen assays detected allergen levels above the assay level of detection in a large percentage of houses, ranging from 100% for Fel d1 to 89% for Bla g2. There was a positive correlation between allergens from individual filters for dog, cat, mite, and roach ( $r = 0.03$  to  $0.63$ ). There was a poor correlation between any of the other measured allergens with Mus m1 ( $r = -0.16$  to  $0.10$ ; Table 4).

## Discussion

In this study, the authors found that allergens from 5 common aeroallergen species and antigenic material from 4 common fungal taxa could be measured in dust taken from high-efficiency home furnace filters. The amount of allergen or antigen collected by the filters was quite large for Fel d1 and the 4 fungal taxa evaluated. Moreover, the amounts of Can f1, De f1, Der p1, Bla g2, and m1 were easily within the range measured by the assays used. Even for Mus m1, the average levels should be sufficient to produce sensitization; for Fel d1, the average levels are easily adequate to produce symptoms in already sensitized individuals.<sup>26–29</sup>

Dust for this study was from high-efficiency furnace filters taken from houses participating in the Kansas City Healthy Homes Demonstration Project during a 19-month period. The entire 3-year Healthy Homes Project study screened many potential subjects and enrolled 382 families. Enrollment in the overall Healthy Homes Project study was limited by the family income criteria of the funding agency to be less than 80% of median family income. The collection period for furnace filters encompassed 1 air-conditioning season and slightly more than 1 heating season. Homes were chosen by convenience when 2 home visits were scheduled at the same house within a period of 6 months. The mean duration filters were in place (135 days) was a function of the study design and does not represent the frequency with which individual families would typically change filters. One of the filters was in place for longer than 1 year because of scheduling difficulties with the family. Nearly all the filters contained sufficient dust for analysis.

**Table 2**  
Spearman rank correlation ( $\rho$ ) of antigen concentration among the fungal taxa evaluated

|                              | <i>Alternaria alternata</i> | <i>Aspergillus fumigatus</i> | <i>Cladosporium herbarum</i> | <i>Penicillium notatum</i> |
|------------------------------|-----------------------------|------------------------------|------------------------------|----------------------------|
| <i>Alternaria alternata</i>  | 1                           | 0.13                         | 0.14                         | 0.55 <sup>b</sup>          |
| <i>Aspergillus fumigatus</i> |                             | 1                            | −0.38 <sup>a</sup>           | −0.29 <sup>a</sup>         |
| <i>Cladosporium herbarum</i> |                             |                              | 1                            | 0.55 <sup>b</sup>          |

<sup>a</sup> $P < .01$ .

<sup>b</sup> $P < .001$ .

**Table 3**  
Allergen concentration in nanograms per gram of dust

|               | Fel d1    | Can f1 | Dust mite | Bla g2   | Mus m1 |
|---------------|-----------|--------|-----------|----------|--------|
| Mean          | 7,407.24  | 622.06 | 239.87    | 689.75   | 342.59 |
| SD            | 18,917.79 | 798.39 | 751.31    | 2,734.06 | 457.43 |
| Median        | 842.94    | 179.97 | 63.34     | 99.29    | 178.53 |
| Detectable, % | 100       | 96     | 100       | 89       | 98     |

Only 2 filters did not have sufficient dust and these appeared not to have been used. The authors presume that for these homes the HVAC system was not functioning or the family did not use the HVAC system during the time the filters were in place.

Furnace filters have evolved dramatically over the past several years. Early filtration devices served mostly to protect the fan and fan motor used in the HVAC systems. With the development of American Society of Heating, Refrigerating and Air-Conditioning Engineers Standard 52.2, air filters were ranked on the MERV scale.<sup>30</sup> The filters used in this study were MERV 12, which is rated for particles as small as 1  $\mu$  and is suitable for most dust and nebulized droplets.

The filters were standard sizes for residential homes, varying from small (14 × 20 inches) to large (20 × 25 inches). The filters typically yielded 2 g of dust when they were vacuumed. Experiments were performed that attempted liquid extraction of furnace filter material directly, but yields and recovery rates for known amounts of allergens were not satisfactory (data not shown). Therefore, the most satisfactory method for obtaining dust from the filters was to vacuum the filters much like carpet is vacuumed. Because the number of grams of dust recovered was moderately well correlated with the time the filter was in place but not with the size of the filter, on average the filters were not saturated with dust during the 4 to 5 months they were in use. The weight of dust recovered from the filter was positively and significantly correlated with the amount of time the filter was in place, as would be expected if the filters were not saturated and still actively filtering the air.

The evaluation of viable fungal colonies present in the dust was essentially as complex as evaluating fungal colonies present in house dust. Viable fungal colonies were extremely numerous in all collections, and fungal colony estimations often involved dilutions of 4 to 6 logs. The range of results for colonies per gram of dust varied over 3 orders of magnitude. In addition, when calculated per filter, these values ranged from  $10^3$  to  $10^7$ . Specific identification and enumeration of all viable fungi present in the collection was not possible, but close observation indicated the taxa to be dominated by the *Pelospora* and in agreement with the airborne studies of Meng et al<sup>29</sup> and the fungal biome studies of Rittenour et al<sup>31</sup> who evaluated house dust and airborne fungi from similar homes.

The analysis of fungal antigens in the dust collected from furnace filters is in some agreement with the viable colony counts and indicates there is a large amount of antigenic fungal material in the furnace filter dust. Large amounts of airborne *aspergillus* and other fungal antigens have been reported previously in agricultural facilities.<sup>32</sup> The mean *A alternata* antigen level of approximately 8.3  $\mu\text{g}$

**Table 4**  
Spearman rank correlation ( $\rho$ ) of antigen concentration among aeroallergens evaluated

|                | Fel d1 | Can f1            | De f1 + Der p1 | Mus m1 | Bla g2            |
|----------------|--------|-------------------|----------------|--------|-------------------|
| Fel d1         | 1      | 0.63 <sup>b</sup> | 0.11           | −0.16  | 0.03              |
| Can f1         |        | 1                 | 0.21           | 0.06   | 0.31 <sup>a</sup> |
| De f1 + Der p1 |        |                   | 1              | 0.10   | 0.20              |
| Mus m1         |        |                   |                | 1      | 0.16              |

<sup>a</sup> $P < .01$ .

<sup>b</sup> $P < .001$ .

per gram in the furnace filter dust was slightly above the mean *A alternata* allergen level of approximately 5.7  $\mu\text{g}$  per gram of house dust reported in lead and allergen studies.<sup>33,34</sup> Of the 4 fungal taxa tested by immunoassay, *A alternata*, *C herbarum*, and *P notatum* correlated positively with one another. Surprisingly, *A fumigatus* levels did not correlate well with other fungal taxa, having strong negative correlations with *C herbarum* and *P notatum*. Because of the well-recognized cross-reactivity between fungal allergens,<sup>35,36</sup> the authors had expected there to be good correlation among all 4 of the fungal taxa measured by polyclonal immunoassay. However, *A fumigatus* could represent an altered fungal biome related to damp housing. Several *Aspergillus* species appear on the Environmental Relative Moldiness Index list of fungi associated with damp housing.<sup>37</sup> Alternatively, not all indoor fungal related material might become airborne or deposit under the same conditions as has been modeled for the outdoor environment.<sup>38</sup>

The levels of 5 common non-fungal aeroallergens measured in dust from furnace filters were different from those expected in house dust. There was little dust mite allergen and the Der f1 and Der p1 values correlated well, so these were treated as a single number. Low dust mite levels have been reported previously when low humidity conditions persist.<sup>39</sup> Very few of these homes had pets (only 2 had cats and 3 had dogs). Nevertheless, the mean Fel d1 level was much higher than the mean vacuumed dust levels for houses without cats in previous studies<sup>40</sup> and higher than levels in vacuum dust from homes with similar demographics (0.73 mg/g) to this study.<sup>29</sup> Levels of dog allergen also were slightly higher than expected. Conversely, levels of dust mite and roach were lower than expected based on reported levels in house dust vacuumed from floors. The authors did recover allergen above the level of detection in nearly all houses. This was not unexpected because these collections represent more than 1,000 times the air collected by previous studies.<sup>17</sup> Correlation between these aeroallergens was positive except for mouse, which correlated negatively with cat and weakly with dog, dust mite, and roach. The correlation of these aeroallergens was not unexpected because they have common facilitative factors.

This study has several limitations in its applicability to the general US housing stock. By necessity, all the homes sampled can be defined as lower income in that families were below the median income level for the area. Because of the financial resources available to these families, the HVAC units might not be used as much as they would have been used by families with greater resources. In addition, these houses were typical for the Midwestern United States in that most contained basements and used forced air heating and cooling, but they are quite different from houses in other geographic areas where different heating and cooling methods are common. Also, the authors cannot at this time say how the dust from furnace filters compares with floor dust that is typically collected for allergen exposure assessment. Also, the use of furnace filter dust might represent the kind of long-term sampling that has been shown to produce relevant values in other studies.<sup>41</sup>

In conclusion, the authors measured levels of significant aeroallergens from dust collected from improved furnace filters installed in the HVAC systems of houses in the Midwestern United States. Significant levels of cat, dog, mouse, dust mite, roach, *A alternata*, *A fumigatus*, *C herbarum*, and *P notatum* allergens were found on filters installed in the homes tested. Allergens from the 5 common aeroallergen species and antigenic material from 4 common fungal taxa were measured using assays derived from commercially available material in dust taken from high-efficiency home furnace filters. The allergens expected to be airborne (Fel d1 and fungi) appeared at highest levels, but other allergenic material also was detected. Further work remains to be done to determine the significance of these findings and their relation to traditional vacuumed floor dust in determining indoor allergen exposure.

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