A Side by Side Comparison of Three Allergen Sampling Methods in Settled House Dust

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The Boston Medical Center Institutional Review Board approved this study.

This study was funded by the U.S Department of Housing and Urban Development, Department of Healthy Homes and Lead Hazard Control (Grant #MALHH0163-07), but do not necessarily reflect the views of the federal government.

Abstract

Understanding allergen exposure and potential relationships with asthma requires allergen sampling methods, but methods have yet to be standardized. We compared allergen measurements from dust collected from 200 households with asthmatics and conducted side-by-side vacuum sampling of settled dust in each home's kitchen, living room and subject's bedroom by three methods (EMM, HVS4, AIHA). Each sample was analyzed for dust mite, cockroach, mouse, rat, cat and dog allergens.

The number of samples with sufficient dust mass for allergen analysis were significantly higher for EMM and HVS4 compared to AIHA in all rooms and surfaces tested (all $p<0.05$). The allergen concentration (weight of allergen divided by total weight of dust sampled) by the EMM and HVS4 methods was higher than those measured by the AIHA. Allergen loadings (weight of allergen divided by surface area sampled) were significantly higher for HVS4 than AIHA and EMM. Cockroach and rat allergens were rarely detected via any method. The EMM method is most likely to collect sufficient dust from surfaces in the home and is relatively practical and easy. The AIHA and HVS4 methods suffer from insufficient dust collection and/or difficulty in use.

Key Words: Asthma, Allergens, House Dust, Healthy Housing

Introduction

Indoor exposure to allergens is an important risk factor in asthma development,[1] and is associated with allergen sensitization, [2] although the relevant aspects of exposure (e.g., timing, dose) remain unclear. To elucidate the mechanisms involved in the onset and exacerbation of this disease, accurate and precise sampling and estimation of allergen exposure are needed. The National Academy of Sciences has called for the establishment of "effective mechanisms for medical professionals to acquire assessments of potential exposure to indoor allergens in residential environments."[3] Furthermore, two recent reviews have found that home-based multi-trigger multi-component interventions are effective.[4] [5] Further work is needed to help focus those interventions because there is not yet a standardized method of measuring allergens in settled dust.

Despite this lack of standardization, dust sampling is important in assessing exposure to allergens in the home.[6] Indeed, the proliferation of sampling and analytical methods used in different studies may be part of the reason why the observed associations between allergen exposure and asthma remain inconsistent across studies. There are well over two dozen sampling and analytical methods for various asthma triggers.[7, 8, 9, 10, 11, 12, 13, 14,] Yet only a few studies have attempted to compare the different methods in field studies to determine the strengths and weaknesses of each.[15, 16, 17, 18] For example, methods have been reported that use different sized vacuums of different makes with cyclone or mechanical separation of particles using filtration media of differing pore sizes. Other methods have included wiping with a variety of different media, adhesive (press) tape or plate samplers or using electrostatic media[12] Other methods use different locations for sampling such as intranasal samplers,[19] air sampling via filtration, impingement, or impaction through slits or sieves,[20] patch, tape and vacuum sampling of skin,[21], dust fall,[22] and simple visual assessment.[23] Most studies appear to measure one or at most three allergens, such as those from dust mites (Der f 1 or Der p

1), cats (Fel d 1), dogs (Can f 1), cockroaches (Bla g 1 or Bla g 2), mice (Mus m 1) and rats (Rat n 1). Results of home allergen sampling are impacted by the number and location of surfaces measured,[24, 25] including, but not limited to bedding, carpeted and uncarpeted floors in single or multiple rooms, furnishings, and others. Some studies have also examined the effect of the type of housing,[26] seasonal variability,[27] sampling over time,[28] use of occupants to collect dust samples compared to using trained technicians[6] and effect of city and rural areas.[29] One study of allergen sampling methods showed that a vacuum method correlated well with a wipe method, but a tape sampler did not.[12] Another study showed that a modified High Volume Small Surface Sampler (HVS4) recovered twice as much dust and four times more dust mite allergen that had been deposited onto carpets.[17]

In the few studies that compared differing allergen sampling methods, results have been inconclusive. We conducted this study to help overcome these earlier attempts by using a larger cohort with a wider range of exposures. We also increased the number of allergens analyzed, the size of the surface areas and the number of rooms sampled.. We also limited the methods assessed to the three leading ones.[15] One of our co-authors (Adgate) has also recently completed a laboratory-based study of the same three methods[30]. We also conducted this study to compare different units of measure for settled dust —, such as concentration (μg/g) and loading (μg/unit surface area), using the same sampling methods in different rooms and surfaces. To our knowledge very few studies have compared these two different measurements across different allergens and sampling methods side by side.[15]

This paper reports how three field sampling methods performed in different rooms and on different surface types and how they compare using two different metrics of concentration (ug/g) and loading (ug/surface area).

Methods

We obtained consent to sample allergens in 200 homes in Boston. At all homes at least one person between the ages of 4-64 years had doctor diagnosed asthma and who had lived in their current residence for at least six months. Subjects were recruited either from past asthma study cohorts, Boston Medical Center asthma clinics, newspaper ads or referred by other subjects. The study was approved by the Boston University/Boston Medical Center Institutional Review Board.

We selected three leading methods of settled dust sampling for allergens, the Eureka Mighty Mite (EMM)[31], High Volume Surface Sampler (HVS4)[32] and a widely used method referenced in a publication from the American Industrial Hygiene Association (AIHA).[33]

Supplemental Figure 1 shows the EMM (model 3670, Electrolux Home Care Products, Inc., Peoria, IL), which collects dust using the DUSTREAM® Collector (Indoor Biotechnologies, Inc., Charlottesville, VA). The EMM has been used in several dust sampling studies[30 15 34], including a national survey of allergens in housing.[35]

Supplemental Figure 2 shows the HVS4 (CS_3, Inc.), which uses a specific nozzle and cyclone that is attached to a Nalgene bottle (model PP, Nalgene Nunc International, Rochester, NY). High volume small surface samplers have been used in multiple studies for the collection of dust[36] and various dust contaminants, including lead, [37 38] Polychlorinated Biphenyls,[39] Polyaromatic hydrocarbons,[31] pesticides,[40 41] and allergens. [15 18]ASTM has described a method for sampling floors using the HVS3[42], and this study used the HVS4, which is a slight modification of the HVS3[43]. At a pressure of 8 inches of water the flow rate was between 20-25 cubic foot per minute (566-707 liters per minute).

Supplemental Figure 3 shows the AIHA method, which uses a 37mm open-faced filter cassette (Model 738PC, Zefon International Inc., Ocala, FL) connected to an AirCon2 pump (P/N 801012, Sensidyne, Clearwater, FL) operating at a nominal flow rate of 17 L/min. [44] [45] We modified the AIHA method slightly by attaching a paper clip over the edge of each cassette when bare floors were sampled to enable the cassette to be moved over the surface without becoming immobilized from the vacuum suction.

Samples were collected from the floors of the living room and the kitchen and from the subject's bed. Samples were collected preferentially from carpets in locations within the living room and kitchen to collect the largest volume of dust possible to avoid insufficient dust collection, a problem seen in other studies.[15] [16] In the bedroom, the bedding was pulled back to expose only the fitted sheet or, in the absence of a fitted sheet, the surface on which the subject directly slept. If dust mite covers were present, they were not removed prior to sampling. Sampling surfaces were divided into longitudinal thirds using tape to demarcate the surface area. Two pillows on the bed were also divided into thirds and sampled.

Smooth, cleanable wooden templates were used to standardize the size of the floor surface area sampled. We used three templates in each room for each method to obtain 1.8288 m^2 (6 ft^2) of surface area in each room and alternated the three methods into left, middle and right side to avoid introducing sampling bias. Each 1 ft^2 was sampled with the appropriate method for 1 minute. Approximately 30 seconds were spent sampling by traveling in the east-west direction and another 30 seconds were spent sampling in the north-south direction.

After collection, all samples were refrigerated immediately at approximately 4°C. The dust and sample containers were then desiccated for at least 24 hours in a Secador™ Desiccator (Bel-Art Products, Pequannock, NJ) with Drierite (Anhydrous Calcium Sulfate, CAS 7646-79-9, W.A.

Hammond Drierite Company LTD., Xenia, OH) to prevent mold growth and reduce weight variability associated with moisture content. The samples were shipped to Indoor Biotechnologies, Inc., Charlottesville, VA for immunoassay analysis.

Upon arrival at the laboratory, each sample was sieved using a No. 45 mesh screen, 355μm diameter (VWR No. 57332146), weighed and extracted with phosphate-buffered saline. Eight common allergens were quantified in the dust samples using *Multiplex ARay for Indoor Allergens (MARIA®):* Bla g 2, Der f 1, Der p 1, Mus m 1, Rat n 1, Fel d 1, Mite 2 and Can f 1. Samples were analyzed in three dilutions: neat (undiluted), 1:10 and 1:1000. When diluted, the detection limit was 0.012 μg/g for Der p 1 and Der f 1, 0.012 μg/g or 0.048 μg/g for Can f 1, 0.004 μg/g for mite group 2 and Rat n 1, 0.012 or 0.048 μg/g for Mus m 1, 0.004 or 0.01 μg/g for Fel d 1 and 0.098 or 0.20 μg/g for Bla g 2. When undiluted, the detection limit was 0.0012 μg/g for Der p 1, Der f 1, and Can f 1, 0.0004 μg/g for mite group 2, Fel d 1 and Rat n 1 and 0.0196 μg/g for Bla g 2. Results for dust samples were reported in both loading and concentration; loading is allergen mass per surface area and concentration is allergen mass divided by total dust mass.

Statistical Analysis:

Models for Allergen Measurement:

We used SAS version 9.3 for all analyses.[46] For each allergen, metric, room and surface type, we used a repeated measures Tobit model for left censored measurements[47] under the assumption of log-normality to determine if the geometric mean (GM) allergen levels were significantly different for the 3 sampling methods. These models were also employed to estimate the GM and Geometric Standard Deviation (GSD). If the quantity of dust was not sufficient for analysis, allergen concentrations were not used in the models but we determined upper bounds for allergen loadings and used them in the models. A repeated measures Tobit model under the assumption of log-

normality was also used for total sieved loading. We used a Cochran-Mantel-Haenszel test to determine if the percents of detectable allergen concentrations were different for the three different sampling methods. Comparisons of GM allergen and loadings were not considered for Bla g 2 and Rat n 1 because the vast majority of measurements were below detection limits.

Floor surfaces were classified as bare if the entire room was uncarpeted. Floor surfaces were classified as carpeted if any carpeted areas, including area rugs, walk-off mats or wall to wall carpeting were vacuumed.

RESULTS

Residents of homes samples were largely of low-income (86% participating in Medicaid insurance) African-American (54%) people with doctor-diagnosed asthma living in the metropolitan Boston area, many participating in subsidized housing programs. Fourteen percent of households were occupied by the owner and 15% identified their housing as federally assisted (public housing or Section 8 voucher) though more than half were in other subsidized programs.. Demographics are summarized in Table 1. Of 200 homes, 198 beds (one sample was lost and one subject refused) and 95 carpeted and 104 bare living room floors (defined as the area where the subject spent the most time awake, other than the bedroom) were sampled. Also, 30 carpeted and 171 bare kitchen floors were sampled (one home had separate kitchen samples from bare and carpeted areas). A total of 1,790 samples were collected from living room floors (597 samples), kitchens (597 samples), and beds (596 samples). Each sample was analyzed for 8 allergens (see methods section), resulting in a total of 14,325 analyses (200 homes x 3 rooms x 3 samplers x 8 allergens/sample ≈ 14,325 total analyses). Of these 14,325 analyses, 8,381 had results that were below the laboratory-reported detection limits, either due to insufficient dust collected during the sampling (1,735) or insufficient allergens in the dust (6,646). The AIHA method had by far the highest percentage of insufficient

quantity of dust for analysis, possibly due to a low flow rate and its tendency to stick to floors. (Figure 1 and Supplemental Table S-1). This was true for all rooms and surfaces (all $p<0.05$; Supplemental Table S-2). EMM had significantly more samples with insufficient dust than HVS on beds, and bare kitchen and bare living room floors (all $p<0.05$), but the difference was small (for beds 2% of EMM samples vs. 0% of HVS4 samples had insufficient dust amounts; for bare kitchen and living room floors 2% of EMM samples vs. 1% of HVS4 samples had insufficient dust). Nonetheless, all carpeted floors vacuumed with the EMM or the HVS4 had enough dust to permit allergen analysis.

Geometric Mean sieved dust weights were not significantly different between any methods on kitchen carpets (Supplemental Table S-2). In living rooms, GM sieved dust amounts were significantly higher for HVS4 than AIHA and EMM except on bare living room floors. GM sieved dust amounts were significantly higher for EMM than AIHA for beds (all $p<0.05$) (Supplemental Table S-2). Other comparisons were not significant.

For allergens other than cockroach and rat, all three samplers were able to collect enough dust to have measurable allergen levels (Figure 2; Supplemental Tables S-3 and S-4). Cockroach allergen (Bla g 2) and rat allergen (Rat n 1) were detectable in no more than 20% of samples for any methods or locations, consistent with the report by study participants that cockroaches and rats were only observed in 15% and 8% of homes, respectively. There were no significant differences in percent of samples with detectable allergen concentrations among the three methods on carpeted surfaces of kitchens and living rooms for any allergen (all $p>0.05$) (Supplemental Table S-3). However, for beds, EMM and HVS4 had significantly more samples with detectable allergens than AIHA for cat (Fel d 1), dog (Can f 1), and the three dust mite allergens (Der f 1, Der p 1, Mite2) (all

$p<0.05$). For kitchen and living room bare floors, allergen levels varied by allergen type and collection method. (Supplemental Table S-3).

For kitchen carpets or living room carpets for any allergen, there were no significant differences in GM concentrations among the three methods (all $p>0.05$) (Figure 3; Supplemental Table S-3). For beds, HVS4 GM concentrations were significantly higher than EMM for Fel d 1, Der p 1 and Mus m 1. For other locations (bare floors of kitchens and living rooms), results varied by sampling method and allergen type (Figure 3; Supplemental Table S-3). In general, allergen loadings were highest for HVS4 and lowest for AIHA (Supplemental S Figures 5-10; Supplemental Table S-4). For example, AIHA loadings were significantly lower than those for the EMM and HVS4 methods on carpeted kitchen floors for all allergens except Der p 1.

DISCUSSION

The choice of which sampler to use depends on a number of factors, including ease and practicality of use, performance on different surfaces, cost, and how well the results can be used to target actual home-based interventions.

Both the AIHA and the HVS4 suffer from several practical considerations. The AIHA method tends to "stick" to smooth surfaces due to the smoothness of the filter cassette and the suction created by the pump, making it difficult to fully cover the entire surface to be sampled. While this was partially overcome in this study by creating a paper-clip shunt to slightly separate the cassette from the surface, this and the lower flow rate likely explain why this method had the highest prevalence of non-detectable allergens. This method may also suffer from significant sample loss due to electrostatic charges on the plastic filter cassette, making quantitative transfer of dust from

the filter in the laboratory problematic. The HVS4 has a long sampling arm that makes its use in cramped spaces, such as bedrooms, difficult. (See Supplemental Figure 3) The cyclone also requires cleaning after each sample to avoid cross-sample contamination and is difficult to disassemble in the field, limiting the number of home visits that can be conducted in a day. Often the AIHA method did not pick up enough dust, but this was not a major issue for the EMM or HVS4 methods. In general, the HVS4 picks up more dust than the AIHA and EMM, while the EMM picks up more than the AIHA (See Figure 1).

Settled dust allergen is often considered a proxy of exposure, and the distribution of allergen in the settled dust likely reflects that which becomes airborne after disturbance. One limitation of this study is that we did not measure airborne allergens. Even when enough settled dust was collected for analysis, many allergen concentrations were below the limit of detection, especially for the AIHA method on bare kitchen floors and beds. For carpeted kitchen floors, and bare and carpeted living rooms, there were not significantly more detectable allergens for the AIHA method compared to the EMM and HVS4 methods.

If allergen concentrations are not detectable, it is likely because inadequate dust was picked up or that allergen concentrations are very low. While the latter indicates a low allergen concentration, the former indicates a clean sampled surface but no useful results about the allergen concentration. This is one of the reasons that allergen loadings are appealing, because they are not as dependent on household cleaning practices. However, the variation between methods in loadings is much greater than concentrations, indicating that concentration may be a better measure. Because most of the existing literature has shown associations between allergen expressed as a concentration, not loading, this suggests that concentration can be retained as the principal metric of choice. When sampling

from a smooth surface (e.g., kitchen floor or living room floor without any carpeting), all three methods collect essentially the same amount of dust. Only when some woven textile covering is involved does the greater suction of EMM and especially the HVS4 affect the amount of collected dust. Unexpectedly, the amount of dust collected from the bed was similar across all three methods. The light fluffy composition of the bed dust (mainly skin scales) is most likely the reason for the similarity, even though this is also a woven textile covering. It is important to keep in mind that all of the dust was sieved at the lab, and although very light-weight lint does not usually pass through the sieve, very heavy particles (e.g., sand, dirt, salt, sugar) still could. Therefore, when allergen levels are expressed as a concentration (mass of allergen per unit dust mass collected), the allergen concentrations can be severely underestimated if heavier particles (even those that make it through the sieve) are included in the denominator.

We conducted preliminary modeling for each allergen, metric and method to determine if GM allergen concentrations and loadings were significantly different on the beds, bare kitchen floors, carpeted kitchen floors, bare living room floors, and carpeted living room floors. We found that nearly all pairs of rooms/surfaces were significantly different across the allergens, metrics and methods. Thus we presented results separately for the 5 rooms and surface type combinations. When sampling a specified room's floor, one does not have control over the surface type. If allergen concentration or loading health-based exposure limits are identified, they may need to be different for carpets and bare floors due to the wide variability we discovered.

Generally, the AIHA method allergen concentrations were significantly lower than the EMM and HVS4 methods, but there were not as many significant differences in concentrations between the EMM and HVS4. However for loading, most method and location differences were significant.

It is surprising that non-carpeted kitchens were a good location for testing dust mites, which are typically most often located in the bedroom or living room. This result may be because most of the dust actually present on a non-carpeted floor would be collected; however carpeting and bedding are more heterogeneous surfaces and the dust collected likely depends on the composition and wear of those surfaces, i.e., their degree of "smoothness."

While five homes had replicate samples collected, the number was too small to assess the precision of each method. Adgate et al. recently evaluated precision and collection efficiency in a laboratory study where known quantities of cockroach, cat, and dust mite allergens were deposited on surfaces. Although the same samplers were used (AIHA, EMM, and HVS4), the lab study had some major differences that were not or could not be replicated in the field (e.g., controlled temperature and humidity, particle size fraction). In the lab study, the samplers were compared by exploring two concepts: mass collection efficiency (CE), which was derived from collected dust mass/ applied dust mass); and concentration ratio (CR), which was derived from allergen concentration in the sample divided by allergen concentration in test dust).

In a laboratory study the AIHA sampler collected little dust in the large (212-90 μm) size fraction, and about half the typical mass of dust of the other samplers for the medium and small size fractions. Obtaining enough dust is a crucial requirement of successful allergen sampling. The AIHA and HVS4 had less variable CRs compared to the EMM method. This implies that the higher CE of the EMM understates measured concentrations for the three allergen types tested in the lab, and this feature is likely also true for field samples. As a consequence, health based standards that use allergen concentration as the basis for their recommendations need to either specify the method or

adjust for this variability, which can range up to 2X for the EMM. Health-based exposure limits almost always specify a sampling and analytical method to be used, although the allergen field has not yet done so. Therefore, health studies that use concentration as an exposure metric should consider the implications of sampler performance when interpreting links to health outcomes and development of health-based standards.

Finally, most existing allergen sampling procedures do not control for moisture content in the dust, which can affect the total sample weight and could affect allergen loading value. We attempted to control for moisture content by desiccation of sample media prior to sample collection and desiccation of sampling media and dust after sample collection. It is not known whether this desiccation process affects the amount of allergen detected in the MARIA laboratory analysis. Further research may be needed to determine influences from moisture on both the concentration and loading metrics and whether it should be controlled or accounted for in the future.

This study gives limited results for cockroach and rat allergens. Few homes observed cockroaches or rats (15% and 8%, respectively) so it isn't surprising that most of these allergens were not detected by any method or in any location (<20% for cockroaches and <11% for rats across all methods and locations). Given that previous literature has shown cockroach allergens are mostly associated with larger particles and rat allergen can be found on large and small particles, our results for dust mite and mouse allergens might be proxies for cockroach and rat allergens, respectively[48]. Future studies with high rat and cockroach allergen exposures should explore this hypothesis.

Our study may be of limited generalizability. This study was conducted among a low-income, minority population in a US Northeast urban area, many of whom were sensitized to one or more

allergens studied. It is not known to what degree our findings (i.e., the value of sampling and analyzing dog and cat allergen on bare floors using the EMM or HVS4 sampler) would have been different had the study been conducted in other settings.

Conclusions

Both the AIHA and HVS4 methods suffer from insufficient dust collection (depending upon surface sampled) and/or difficulty in use. Concentration appears to be a superior metric in expressing allergens in settled house dust instead of loading. Further research is needed to establish health-based exposure limits for allergens in the home environment

ACKNOWLEDGMENTS

We want to extend our gratitude to the many residents who welcomed the research team into their homes. We thank Suzanne Gaynor and J Kofi Berko Jr. of the U.S Department of Housing and Urban Development. We also thank Karen Dannemiller, Emily Suther, Vanessa Vasquez, Madeline Abrams and Salwa Masud for data collection and Stephanie Filep and Eva King at Indoor Biotechnologies, Inc. for their help with laboratory analysis.

Supplementary information is available at the Journal of Exposure Science and Environmental Epidemiology website.

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