

Original article

Four methods of sampling for dust mite allergen: differences in 'dust'

Background: Measurement of exposure to the dust mite allergen Der p 1 is important in asthma research and is potentially useful in managing asthma. As no single measure can capture all characteristics of an exposure, it is important to recognize differences in the available methods of measuring exposure to Der p 1.

Methods: Fourteen bedrooms and living rooms were sampled using four methods for 1 week. Airborne allergen was sampled by static Institute of Occupational Medicine samplers. Settling dust was collected on Petri dishes and an adhesive-membrane system (A-book). Vacuumed reservoir dust samples were collected from floors at the end of 1 week. Der p 1 was measured in all samples by enzyme-linked immunosorbent assay, except A-books, in which it was measured by Halogen immunoassay.

Results: All four methods intercorrelated moderately (r range = 0.40–0.64, $P = 0.04$), except between allergen in reservoir dust (as $\mu\text{g}/\text{m}^2$ and $\mu\text{g}/\text{g}$ dust) and settling dust by Petri dishes ($P = 0.2$). Reservoir allergen, expressed as $\mu\text{g}/\text{m}^2$, did not correlate with any measure, except reservoir allergen expressed as $\mu\text{g}/\text{g}$ ($r = 0.39$, $P = 0.04$). No differences in these associations occurred between bedrooms and living rooms.

Conclusions: While the four methods examined correlated moderately, all have practical advantages and difficulties. No method can be considered as ideal for measuring individual exposure. For practicality, use of vacuum cleaner and Petri dish methods are recommended.

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Allergy to environmental allergens such as those from house dust mites is a common feature of childhood asthma, and the level of environmental allergenic exposure may play a critical role in determining clinical outcomes of allergic diseases (1, 2). While associations have often been found between allergen concentrations in house or bed dust and the prevalence of allergic sensitization in the residents (3–5), relationships for the prevalence or severity of asthma are more complex and have only been found in some studies (2) and not others (6). More accurate measures of allergen exposure would provide greater insights into the nature of allergic disease.

Some asthma outcomes, such as the triggering of acute episodes (7) and the worsening of airway hyper-responsiveness (8) are associated with increases in aeroallergen exposure, and conversely improvements in airway hyper-

responsiveness are associated with reducing exposure (9). To better understand these and other asthma outcomes, it would be preferable to adopt measurements of exposure that sample airborne dust rather than reservoir dust. In the indoor environment, the distribution of aeroallergen is constantly dynamic, which is a product of the extent and timing of disturbance of allergenic dusts. As the amounts of allergen involved are often relatively small it is difficult to collect in measurable quantities. Additional constraints are whether the methods are practical to use, particularly in large epidemiological studies which employ repeated home visits, costly or delicate equipment and the need to collect samples in many houses simultaneously.

In this study, we examined four methods of sampling airborne house dust mite allergen (Der p 1) and compared them with each other in the same houses. To understand their variation, we also examined the relationship between paired aeroallergen samples in the same room. The methods explored here were evaluated in the context of what would be practical to perform in an intervention study in houses where a minimal number of visits were permitted.

Abbreviations: IOM, Institute of Occupational Medicine; HDM, house dust mite; PVDF, polyvinylidene fluoride; IgE, immunoglobulin E; BSA/PBST, 0.2% bovine serum albumin in phosphate-buffered saline, 0.05% Tween 20.

Methods

Sampling was performed in 14 homes of researchers employed by the Cooperative Research Centre for Asthma and the Woolcock Institute of Medical Research in metropolitan Sydney, Australia, during the winter months of 2000. All samples were collected by the resident researcher. A bedroom and a living area were sampled in each house. Because of differences in houses and apartments, the term 'living area' was used to include 10 lounge rooms, two kitchens and two sunrooms ($n = 14$) – rooms in which people spent most time outside of their bedroom. Aeroallergen sampling was conducted over a given week for each home and reservoir dust samples were collected at the end of that week.

Airborne allergen

Volumetric air sampling. An Institute of Occupational Medicine (IOM) filter sampler (SKC Ltd, Dorset, UK) connected to a modified diaphragm pump, operating at 2 l air/min, was placed in each room. The sampling head was fitted with a polyvinylidene fluoride (PVDF) membrane (0.45 μm , 25 mm diameter; Millipore Corp., Bedford, MA). The IOMs were placed with the membrane face in a vertical plane, at approximately 1 m above the floor, away from drafts, electrical or heating devices and open windows or doors. The IOM sampler was attached to a timer that was programmed to operate for 4 h/day over the 7-day sampling period (i.e. total IOM sampler operation time was 28 h). The samplers were run intermittently for the 7-day period to avoid overheating of the motor and to prevent oversaturation of the membranes. The timer was set so that the IOM sampler operated for 1 h from 2 a.m., 7 a.m., 2 p.m. and 7 p.m., to coordinate with the timing of expected maximum and minimum human activity within the room.

Following collection, the flow rate was re-measured to ensure a constant 2 l air/min had been maintained and allergen was eluted from the filter using 0.2% bovine serum albumin in phosphate-buffered saline, 0.05% Tween 20 (BSA/PBST) with an elution time of 2 h. Der p 1 content was quantified using a monoclonal enzyme-linked immunosorbent assay (ELISA) (Indoor Biotechnologies, Cardiff, UK) (10) incorporating an amplification step where the product of the alkaline phosphatase conjugate used in the ELISA acts as a cofactor in a cycling reaction, producing the coloured product formazan (Ampak; DAKO Ltd, Cambridgeshire, UK). Allergen results were expressed as ng Der p 1/m³ air.

Settling dust

Petri dishes. Two 15-cm diameter Petri dishes (settling plates) and the inside of their lids, which had been pretreated with 2% w/v teleostean gelatine/H₂O to block protein binding (11) were left open on a horizontal surface in each room. One set was placed near the IOM sampler and the other on the opposite side of the room. The dishes were located by the same criteria as for the IOMs (except being placed horizontally) and were left undisturbed for 7 days.

Petri dishes were washed and scraped with a rubber blade for 1 min in 1 ml of BSA/PBST and the resulting suspension allowed to elute for 1 h. Der p 1 content of the eluates was measured by amplified ELISA, as above. The allergen settling rate was calculated as ng Der p 1/m²/day.

A-books. The 'A-book', developed in our laboratory, is another method of collecting settling allergenic particles from the air. A-books were constructed from a 5 × 10 cm piece of cardboard folded over as a small booklet. On one-half of the interior of the book, a 5 × 5 cm piece of adhesive tape (Inhalix Pty Ltd, Sydney,

Australia) was affixed, with sticky side facing upwards. On the facing half there was a 5 × 5 cm piece of PVDF membrane (NEN Life Science Products Inc., Boston, MA). Each A-book was opened so that both the adhesive and the membrane were exposed to collect settling allergenic particles. Two A-books per room were opened and positioned adjacent to the two Petri dishes (i.e. one A-book near the IOM and one on the opposite side of the room). A-books were left to collect settled dust for 1 week, then closed to sandwich the dust particles on both surfaces between the PVDF membrane and the adhesive. A-book samples were then immunostained for Der p 1 using the Halogen assay (Inhalix Pty Ltd) (12). This technique uses monoclonal antibodies and a chromogenic enzyme to detect an antigen associated with individual particles. The number of particles staining positive for Der p 1 was counted by light microscopy from an area of 1 cm² on each A-book. As each sample was sandwiched, a count of 1 cm² represented a collection surface of 2 cm². Data were converted to the number of allergenic particles/m²/day.

Reservoir allergen

Vacuum sampling. Reservoir dust was collected from the floor using a handheld vacuum cleaner (Model 4071D; Makita Corp., Anjo, Japan) (13). Four nonadjacent 0.25 m² areas were vacuumed so that an area of 1 m² was sampled for 1 min (14). The sampling area was increased to four areas of 0.5 × 1.0 m where the floor was uncarpeted in order to obtain sufficient dust for analysis. The vacuum cleaners were fitted with a 500- μm pore nylon prefilter, and fine dust passing through this was collected in a 25- μm pore nylon bag. The weight of fine dust in vacuum samples was noted and 1 ml of BSA/PBST was used to extract the allergen. Extracts were assayed for Der p 1 by ELISA and allergen content was expressed as a concentration (μg Der p 1/g fine dust) and as amount per square metre (μg Der p 1/m²).

All collected samples were above the assay detection limit. Of the 120 samples, six data points were missing as samples were not collected (three settling dishes, one IOM and two vacuum samples).

Statistical methods

As the distributions of allergen levels in reservoir dust and settling plates were positively skewed, the values were log₁₀-transformed prior to analysis.

The agreement between pairs of settling dishes and pairs of A-books within the same room was estimated as the coefficient of repeatability, that is, two times the standard deviation of the differences (15). The mean of the paired values within each room was used in all subsequent analyses.

The correlations among rates of allergen accumulation on settling plates, concentration of airborne allergen measured by IOM samplers and concentrations of allergen in reservoir dust were tested by Pearson's correlation coefficient. The association between airborne allergen concentration and measurements of allergen concentration in reservoir dust and the rate of allergen accumulation on settling plates was tested by linear regression. Differences in this association between living rooms and bedrooms were tested by use of interaction terms.

Results

The geometric means and ranges of data collected by the four sampling methods are given in Table 1.

Table 1. Geometric mean and range of the four measures of Der p 1

Sampling method	<i>n</i>	Geometric mean	Range
Petri dish (ng/m ² /day)	57	113.4	20.7–1442.3
IOM (ng/m ³)	29	16.5	1.4–35.1
vacuum (µg/g dust)	28	7.9	0.4–70.1
A-book (particles/m ² /day)	55	8668.6	714.3–70 000

Examples of correlations between three sampling methods are shown in Fig. 1. The majority of the correlations between sampling methods were significant (Table 2), the most notable exception being reservoir allergen when expressed as an amount per area (µg/m²). The only method that correlated with reservoir allergen expressed as an amount per area was reservoir allergen expressed as per gram of dust (µg/g). Analysis of data from pairs of samples collected by Petri dishes and A-books on opposite sides of the same room demonstrated these to be highly related, although variable, within the same room. For the 28 pairs of settling plates in individual rooms ($r = 0.89$, $P < 0.0001$), the standard deviation of the difference in log₁₀-transformed values was 0.29. The coefficient of repeatability (that is the range within which 95% of pairs of repeated observations in the same room would be expected to lie) was 3.8-fold. For the 26 pairs of A-books ($r = 0.68$, $P < 0.001$), the standard deviation of the difference in log₁₀-transformed values was 0.35, giving a coefficient of repeatability of 4.9-fold.

There was no difference between living rooms or bedrooms in the associations between airborne allergen

concentrations and settling dust concentration, number of allergenic particles or reservoir dust concentration. Using a stepwise selection procedure, allergen concentrations in settling dust and reservoir dust were found to be significantly and independently associated with airborne allergen concentration, explaining 22 and 13% of the variance, respectively. A-book content did not make a significant contribution to explaining variation in airborne allergen concentration. After adjusting for changes in allergen concentration in reservoir dust, it was estimated that a doubling in the rate of accumulation of allergen on settling plates was associated with an increase in airborne allergen concentration of 2.6 ng/m³ (95% confidence interval: 0.2–5.1). After adjusting for changes in accumulation of allergen on settling plates, a doubling in the concentration of allergen in reservoir dust was associated with an increase in airborne allergen concentration of 1.4 ng/m³ (95% confidence interval: 0.1–2.8).

Discussion

In this study, we found a number of significant interactions between airborne mite allergen collected by air filtration or as settling dust, and reservoir samples collected with a vacuum cleaner, providing the latter were expressed as µg/g and not as µg/m².

There are several possible reasons for why this study found interactions between allergen concentrations in reservoir dust and in air, when other studies have only found variable, low or no correlations [see tables of

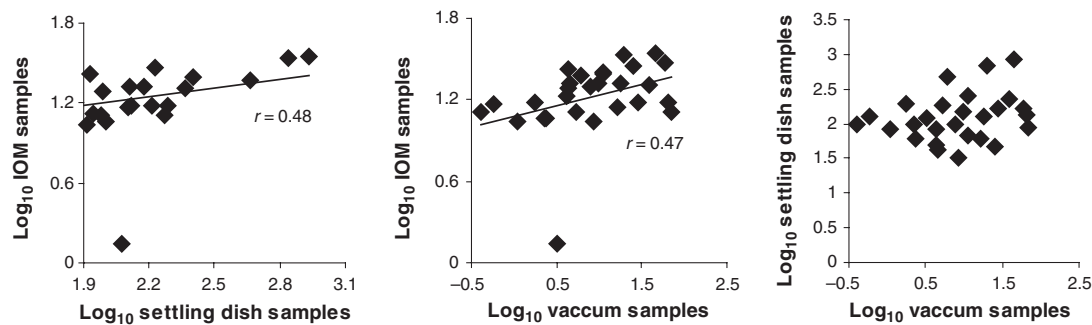


Figure 1. Correlations between three sampling methods for Der p 1; air sampling using IOM, reservoir vacuum samples and settling dishes. Correlation between vacuum samples and settling dish samples was not significant.

Table 2. Correlation between measures of Der p 1 in houses: Pearson's r (P -value). Significant values ($P < 0.05$) are in bold

Method	Airborne allergen concentration	Settling allergen concentration	Reservoir allergen per area	Reservoir allergen per gram dust	A-book particle concentration
Airborne allergen concentration	1	0.48 (0.01)	0.29 (0.14)	0.47 (0.01)	0.41 (0.03)
Settling allergen concentration		1	0.00 (0.99)	0.25 (0.2)	0.64 (0.0002)
Reservoir allergen per area			1	0.39 (0.04)	0.18 (0.4)
Reservoir allergen per gram dust				1	0.40 (0.04)
A-book particle concentration					1

studies in reviews (16, 17)]. It may reflect the power associated with the higher concentration of Der p 1 in houses in Sydney compared with other locations [mean = 2.02 µg/g, Detroit, MI (18); 3 µg/g, Rochester, MN (19); 1.1 µg/g, Manchester, UK (20)]. Other possible differences include the longer sampling times involved in this study and the degree of disturbance of reservoirs.

Petri dishes have previously been evaluated as a means of collecting allergen. Bollinger found no correlation for Petri dish samples for cat allergen and reservoir concentrations ($r = 0.2$, $P = 0.29$) (21), although Mahmic (22) ($r = 0.206$, $P = 0.03$) and Oliver (23) ($r = 0.32$, $P = 0.012$) observed weak but significant correlations for mite allergen. Carswell et al. (24) observed differences in the relative proportions of cat and two types of mite allergen collected by Petri dish and air sampling, cautioning that there were insufficient data to find out what method was the more appropriate to represent 'exposure'. In the most detailed assessment of Petri dish sampling, Karlsson et al. (25) found a significant correlation between Petri dishes open for 5 days and aeroallergen samples collected for 3 h with personal air samplers when used to collect cat allergen in schools ($r = 0.66$, $P < 0.0001$). Duplicate sampling with Petri dishes was also performed in these experiments, with the finding that paired Petri dish samples were significantly correlated. Interestingly, the total amount of allergen collected in five daily samples in five separate Petri dishes was 30% more than five identical samples using one dish repeatedly, indicating nonlinear allergen detection during the sampling period.

In considering aeroallergen as a determinant of clinical indices of asthma, aeroallergen concentration as measured with a filter and pump can be taken as the 'gold standard', as it has in occupational studies (26). Devices such as the IOM samplers used in our study are designed to collect a sample of particles representative of what a person would inhale in a given situation. While discontinuous monitoring with pumps and filters revealed a greater range of exposure between the houses and may be more sensitive to such differences, it would be expensive to perform on a large scale.

The absence of a linear and proportional relationship for two different aeroallergen sampling systems, IOM samplers and Petri dishes, suggests that one or both systems may not have accurately reflected the aeroallergen concentration over the sampling duration used here. In particular, Petri dishes were not as sensitive in picking up differences in aeroallergen. This may be the result of the sensitivity of the Petri dish method changing over time as shown previously (25), or dishes being more biased towards collecting settling large particles than a filter that is positioned vertically.

Alternatively, intermittent sampling by IOM samplers (performed here for practical reasons) may not accurately represent the amount of allergen that was airborne over the entire time. The use of A-books and the Halogen assay is novel, but presently offers few advantages over Petri dishes with ELISA. A-books were more tedious to analyse and were less quantitative. Nonetheless, such assays may have applications when the morphology of particles carrying allergen is of interest, or when polyclonal immunoglobulin E (IgE) is used in the assay to identify allergenic particles relevant to a particular individual.

Correlations identified with reservoir levels, expressed as a function of dust weight and not the area sampled, further support the proposal that reservoir Der p 1 concentrations should be reported as µg/g dust (27, 28), particularly when dose-response relationships are based on this unit of measure. Such levels should be expressed as a concentration (µg/g) for most applications, although for some studies, for example, where allergen is diluted by an intervention, µg/m² is more appropriate.

The objective of studies comparing sampling methods is to validate measures of allergen exposure that will best correlate with clinical outcomes and thus can be used predictively. It is not practical to sample personal exposure continuously for long periods and whatever summary measure is chosen, the criteria needs to include its practicality. While reservoir measures may be suitable for studies of sensitization between subjects in communities, it is probable that measures that relate to asthma severity may need to include aspects that reflect the quantities that are more likely to play a role in clinical responses and as such need to represent allergen airborne over a long period of time (7, 29, 30). Given the absence of such a method, we believe that collection of reservoir dust remains worth performing. To measure short-term or acute 'exposure', Petri dishes with ELISA are preferred as these were simple, practical and capable of detecting allergen. The relationship between allergen exposure and related symptoms will only be accurately examined when the method of measuring allergen truly reflects exposure. The development of techniques that accurately quantify allergen exposure is essential for advancing the understanding of allergic disease.

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