

Variability and repeatability of house dust mite allergen measurement: implications for study design and interpretation

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Summary

Background To interpret individual measurements of house dust mite (HDM) allergen and to design and analyse HDM studies it is necessary to quantify the variability which is inherent in the measurement of this exposure.

Objective To estimate the repeatability of one method of HDM allergen measurement.

Methods We analysed data from one or more HDM allergen measurements in 215 houses included in four previous studies conducted in Sydney (a high allergen environment) and Busselton, Western Australia (a moderate allergen environment). Samples were collected from the bed by vacuuming above and below the sheets and inside the pillow case and from the bedroom and living room floors by vacuuming a 1 m² area for 1 min. Extracts from aliquots of fine dust from each sample were assayed for HDM allergen *Der p 1* using a monoclonal antibody enzyme linked immunosorbent assay (ELISA). The values for HDM allergen were positively skewed and the suitability of a log transformation was established by the resulting normal distribution and stable within-site variance.

Results The range of single determination (within which the true value lies with 95% certainty) was 3.1-fold for samples from the bed and 3.5-fold for samples from the floor. The coefficient of repeatability (the ratio beyond which a change between two estimates is established with 95% certainty) was 4.9 for the bed and 5.8 for the floor.

Conclusion We estimate that, to detect a twofold difference or change in allergen levels, 35 houses per group will be required in cross-sectional studies and 30 houses per group in parallel-group, randomized controlled trials. We recommend that beds be sampled by collecting dust from the layer of bedding below the bottom sheet. A single site within the bedroom floor may be taken as representative of this site but this is not true for the living-room floor.

Keywords: *Der p 1*, risk, repeatability, precision, sample size

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Introduction

The role of house dust mite (HDM) allergen exposure in the induction and maintenance of allergic diseases is now

well established [1,2]. Studies to characterize the exposure–response relationship [3–12] and to evaluate avoidance strategies [13–31] have been undertaken but much remains unknown in both these fields and it is likely that further studies involving the measurement of house dust mite allergen will be conducted. The publication of thresholds for risk of allergic sensitisation and

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symptoms [1] offers the prospect that the measurement of HDM allergen levels will have a role in the management of individual patients with allergic diseases.

In designing HDM studies decisions about the sites to sample and the number of samples to collect are required. The efficiency of the study design will be enhanced if these decisions are based upon knowledge of the expected repeatability or variability of the measurements. The interpretation of an individual's exposure to HDM allergen relative to a proposed risk threshold or relative to a previous measurement also requires a knowledge of the repeatability or precision of the estimate. There are no published data on the repeatability of the measurement of HDM allergen in houses.

In the course of conducting two population studies (one accepted for publication and one unpublished) and two trials of allergen avoidance [27,32] we have had the opportunity to evaluate one method of assessing the HDM allergen concentration of surface dust in houses. We have assessed the repeatability of HDM allergen measurements and the relation between concurrent measurements made at nearby sites in the floor and the bed. We have estimated sample size requirements for studies designed to compare groups with respect to change within subjects (i.e. parallel group randomized controlled trials) and for studies designed to analyse cross-sectional differences between subjects.

Study designs

Data from four studies involving the measurement of HDM allergen have been used in this analysis. Relevant features of the design of each study will be described.

Study A

Nineteen houses in Sydney, Australia, were selected, without reference to the clinical status of the occupants, for a study to assess the efficacy of an acaricidal/allergen denaturing spray [27]. Only data for dust collected prior to the use of the spray are presented here. Samples of dust were collected from 10 sites in three rooms: the main bedroom, a second bedroom (usually a child's room), and the living room. From the main bed, samples were collected from below the lower sheet (mattress), above the upper sheet (upper bedding) and inside the pillow case (pillow). A further sample was collected from all of these sites collectively (whole main bed). A similar whole bed sample was collected from a bed in the second bedroom (second bed). Two samples were collected from different areas of the main bedroom floor, one from the second bedroom floor and two from different areas of the living room floor. Floor samples were taken

from carpet or rugs in all cases. Two weeks later further samples were collected from five items: the whole main bed, the second bed, and one floor area in both bedrooms and the living room.

Study B

Thirty-five subjects with asthma were enrolled in a placebo-controlled clinical trial of allergen avoidance [32]. The study was conducted in Sydney between June 1989 and December 1990. Dust samples were collected from the subject's bed, the bedroom floor and the living room floor immediately before and 2 weeks after the allergen avoidance or placebo intervention was implemented. Only data from the 18 houses in the placebo group were used in this analysis.

Study C

Four hundred and forty schoolchildren aged 8–10 years living in western Sydney were enrolled in a cross-sectional study of asthma and allergy in 1988 [33]. Of these, 74 subjects were selected for a further study in 1989, to include equal numbers with and without airway hyperresponsiveness (manuscript accepted for publication). Households were matched for the presence of carpets and household pets. Dust samples were collected from the subject's bed and living room floor in autumn (April), winter (July) and spring (October) and from the bedroom floor in winter (July) and spring (October).

Study D

In November, 1990, a cross-sectional study was conducted among subjects aged 18 or over living in Busselton, Western Australia to measure the prevalence of asthma. HDM allergen was measured in dust collected from 107 subjects' houses. Subjects were selected to include approximately equal numbers with and without asthma. Dust samples were collected from the bed and from the living room (one chair and adjacent floor).

Materials and methods

Sampling from the bed entailed vacuuming the layer of bedding below the lower sheet, the layer above the upper sheet and inside the pillow case. An area near the subject's bed or lounge was vacuumed to collect bedroom and living room floor samples. In study A an additional sample was collected from a peripheral floor area in both of these rooms. At each site dust was collected by vacuuming a 1 m square area for 1 min.

Dust samples were collected using a portable vacuum

cleaner (Makita, model 4071D) modified to include an oblique wire mesh pre-filter to remove coarse dust and allow collection of fine dust into conical shaped nylon bags with 25 μm pore size. The collection apparatus was placed at the front of the vacuum cleaner.

In studies B, C and D weighed samples of fine dust (generally about 50 mg) were removed from the collection bags and extracted in 1 mL of phosphate buffered saline, 0.05% Tween 20 buffer in Eppendorf tubes. The dust extract was stored at -20°C until analysis. A similar method was used in Study A except that the whole sample was extracted by immersing the collection bag in the solution [27]. HDM allergen *Der p* I was assayed using a double monoclonal antibody ELISA (University of Virginia, Charlottesville, VA, USA) [34] as described in Tovey *et al.* [27]. The HDM allergen concentration was expressed as μg of *Der p* I per g of fine dust.

Analysis

Analysis was conducted using SAS Version 6.04 (SAS Institute, Cary, NC, USA). As the distribution of *Der p* I levels was positively skewed, values were $\log_{10}$ -transformed prior to analysis. Distributions were compared to the standard normal distribution using the Shapiro-Wilk statistic [35].

Absolute repeatability was measured by the method of Bland and Altman [36,37]. After graphically demonstrating that the difference between two collections from a single site was not related to the mean of these two observations (Fig. 1), the single determination standard deviation was estimated, in log units, as the square root of the error (residual) mean square from an analysis of

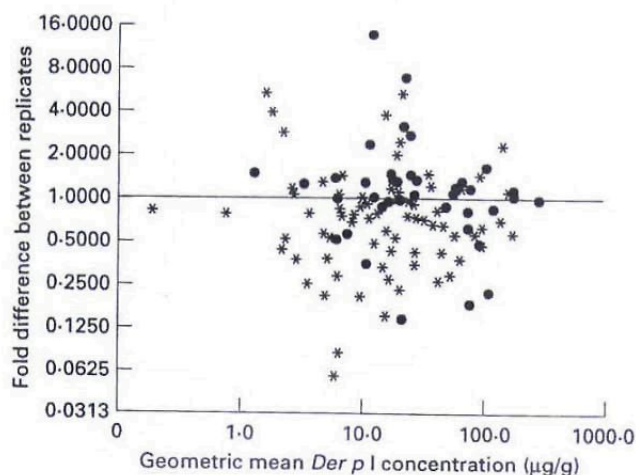


Fig. 1. Relation between mean of *Der p* I measurements repeated at a two week interval and fold difference between them. ●, Bed samples. *, Floor samples.

variance table [38]. The single determination range was calculated as the anti-log of 1.96 multiplied by the single determination standard deviation. It represents the range, in fold units, above and below a single estimate within which the true value would be expected to lie in 95% of cases.

The standard deviation of the differences between repeated measures was estimated indirectly as $\sqrt{2}$ multiplied by the single determination standard deviation. This method yields an estimate which includes any consistent difference between the first and second measurements [38]. The coefficient of repeatability, representing the range, in fold units, within which 95% of the differences between two measurements would be expected to lie, was calculated as the anti-log of 1.96 multiplied by the standard deviation of the differences [36].

Sample size calculations were based on the two-tailed *t*-test with the assumption of a type I error rate of 5% ($2\alpha = 0.05$) and type II error rates of 10 and 20% ($\beta = 0.1$ and 0.2 , power 90% and 80%) [39: 181–4]. The sample size required for studies designed to detect the difference between groups in within-site change in HDM allergen level, that is parallel group, randomized controlled trials, was based upon the standard deviation of the difference between repeated measures (calculated as described above). The sample size requirement for studies designed to analyse cross-sectional differences between-houses was based upon the between-house standard deviation which was estimated in the population studies (C and D). For the bed site the pooled

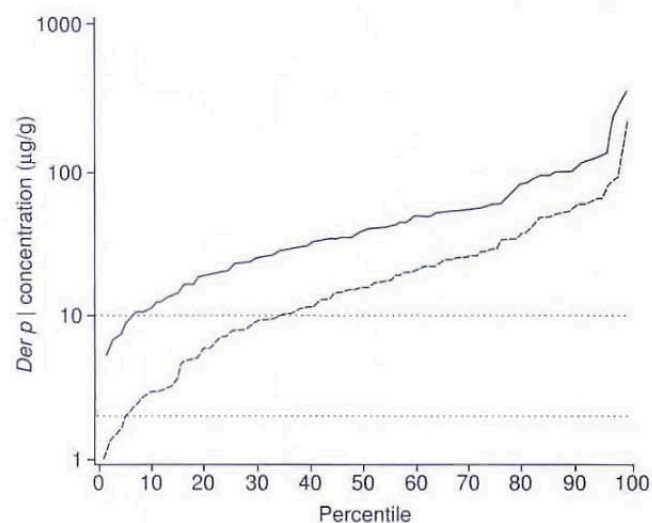


Fig. 2. Cumulative relative frequency distribution of *Der p* I concentrations in bed samples in Sydney (Study C, —) and Busselton (Study D, ---). The proportions of houses above and below the proposed threshold levels of 2 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ are shown.

Table 1. Repeatability of estimates of HDM allergen (*Der p I*). Data are from replicated samples collected at a 2 week interval

Site	<i>n</i> (replicates)	SD* of single determination	Range** of single determination	Coefficient** of repeatability
Bed	43	0.25	3.08	4.91
Floor	74	0.28	3.47	5.81
All sites	117	0.27	3.33	5.47

* standard deviation in log₁₀ units.

** in units of fold difference.

standard deviation using data from both studies was estimated using analysis of variance.

The 95% limits of agreement for the comparison between components of bedding were calculated as the range between two standard deviations (of the difference) above and below the mean difference. This represents the range within which 95% of pairs of observations would be expected to lie [37].

Results

Within-site repeatability

Using data from studies A and B, paired observations were available for 43 beds and 74 floor areas to assess repeatability over a 2 week interval. Figure 1 shows that, on the log-transformed scale, the difference between repeated measurements was not related to the mean of those measurements.

A single estimate of allergen concentration in the bed should be regarded as accurate to within approximately threefold above or below the true measurement (Table 1). Two measurements within fivefold of each other are within the range of variability of the measurement. The repeatability of allergen estimations from beds was slightly better than the repeatability of estimations from floors.

Between-house variability

The between-house distribution of allergen levels was examined using data from the population studies (C and D, Fig. 2). The mean values for both regions are shown in Table 2 along with the between-house standard deviation in log-units. Allergen levels in the bed were log-normally distributed in both studies ($P > 0.4$ for Shapiro-Wilk statistic) and were higher in Sydney (study C) than Busselton (Study D, $P < 0.0001$ for two

sample *t*-test). Allergen levels in the bedroom floor and the living room floor in Sydney were also log-normally distributed ($P > 0.1$) but the distribution was significantly non-normal for the living room samples in Busselton ($P < 0.001$).

Sample size requirements

Tables 3 and 4 show the sample sizes required for parallel group, randomized controlled trials and cross-sectional studies in which it is required to detect 1.5-fold, twofold and fivefold changes or differences in allergen levels. Randomized controlled trials involving HDM allergen measurements are more powerful for a given sample size than cross-sectional studies.

Variation between adjacent or similar sites

The reproducibility of concurrent sampling from two beds in different rooms, from the floor in the different

Table 2. Mean (95% range) *Der p I* concentrations ($\mu\text{g/g}$) in population studies

	Geometric mean	95% range	SD (in log units)
<i>Study C</i> (Sydney) (<i>n</i> = 74)			
Bed	38.9	7.1–213.4	0.38
Bedroom floor	22.4	4.1–123	0.38
Living room floor	13.7	1.7–109.6	0.44
<i>Study D</i> (Busselton) (<i>n</i> = 104)			
Bed	14.9	1.7–128.5	0.48
Living room	11.2	0.7–171.7	0.60*

* Non-normal distribution.

Table 3. Sample size requirements for each group in a parallel group, randomized controlled trial

Site	Power (1 - β)	Change required to detect		
		1.5-fold	twofold	fivefold
Bed	80%	63	22	4
	90%	85	29	6
Floor	80%	78	27	5
	90%	104	36	7

parts of the same bedroom and different parts of the same living room and from the floor in different bedrooms in the same house was examined using data from study A (Table 5). It can be seen that the variation between beds in the same house and between areas within the living room is greater than the variability of repeated measurements (Table 1). On the other hand the variation between bedroom floor samples, within the same room or different rooms, is no greater than would be expected from the repeatability of the measurement.

Relation between components of the bed

The concentration of *Der p* I in the pillow was 2.78-fold less than that in the whole bed ($P = 0.011$) (Table 6). Allergen concentrations in the mattress and upper bedding did not differ significantly from those in the whole bed ($P > 0.15$). The limits of agreement for the comparison of mattress and upper bedding with the

Table 4. Sample size requirements for cross-sectional studies. Numbers are houses required in each group to be compared

Site	Power (1 - β)	Difference required to detect		
		1.5-fold	twofold	fivefold
Bed*	80%	98	34	7
	90%	131	45	9
Bedroom floor**	80%	72	25	5
	90%	96	33	7
Living room floor**	80%	100	35	7
	90%	133	46	9

* pooled estimate based on data from Studies C and D.

** based on data from study C only.

Table 5. Reproducibility of estimates of HDM allergen (*Der p* I). Data from two samples collected concurrently at similar sites in the same house

Site	N (pairs)	SD* of single determination	Range** of single determination
Beds in different rooms	15	0.33	4.37
Floor areas in the same bedroom	18	0.14	1.91
Floor areas in the same living room	18	0.36	5.11
Floor areas in different bedrooms	17	0.27	3.32

* standard deviation in $\log_{10}$ units.

** in units of fold difference.

whole bed are not substantially different to the coefficient of repeatability for samples from the bed.

Discussion

All measurements contain a component of random error and the use of environmental exposure measurements in clinical or epidemiological practice requires quantification of this error or variability. The within-house and within-site variability in the measurement of HDM allergen which we have demonstrated should be considered in interpreting a single measurement relative to a threshold or a previous observation. Estimation of this variability has allowed us to make recommendations for the sample sizes required to conduct studies involving the measurement of HDM allergen.

Our findings about the repeatability of HDM allergen measurements should be regarded as a starting point for

Table 6. Difference in *Der p* I concentration between the whole bed and separate sites within the bed

Site	Mean proportion of main bed (P)*	95% Limits of agreement
Mattress	0.87 (0.51)	0.18 to 4.2
Upper bedding	0.72 (0.15)	0.13 to 4.1
Pillow	0.36 (0.011)	0.02 to 5.6

* significance of mean difference from the main bed.

investigators planning studies involving the measurement of HDM allergen. These findings will have been influenced by the setting in which the studies were conducted and also by our method of dust collection, extraction and analysis. The setting for the studies was a high allergen environment for studies A, B and C and a moderate allergen environment for study D. There is some evidence of heterogeneity in the between-subject variance estimates and it is possible that the variance will be different in other settings. In all settings $\log_{10}$ transformation of *Der p* I levels resulted in a normal distribution with stable within-subject variance, implying this is an appropriate transformation of the data for parametric analysis.

The estimation of the repeatability of allergen measurements does not distinguish the sources of variability. Unpublished work from our laboratory has shown that, when two samples are collected simultaneously from the same bed or floor site using a checker-board pattern, the standard deviation of single determination is 0.15 log units ($n = 21$ pairs of samples). This is equivalent to a range of single determination of 2.0. This variability may be attributed to factors such as the assay and the extraction of allergen from dust. The remaining variability observed over 2 weeks includes that attributable to the dust collection procedure and real changes in the concentration of allergen present.

For the purpose of interpreting a single allergen result or for study design and analysis, it is the overall repeatability which is important. However, it is likely that repeatability will vary according to the methods used. For both these reasons, variation in study setting and methods, it is important that investigators include measures of repeatability in the design of all new studies of HDM allergen.

The degree of repeatability of HDM allergen measurements affects the classification of subjects relative to a threshold. *Der p* I concentrations of 2 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ have been proposed as thresholds for risk of sensitization and for risk of acute attacks of asthma [1]. The values for the range of single determination (Table 1) show that a single measurement of HDM allergen concentration can be regarded as accurate within a 3–3.5-fold range. Hence one could be confident that the true level for an individual was less than 10 $\mu\text{g/g}$ if a single measurement was less than 3 $\mu\text{g/g}$. As these measurements are much lower than the population mean, it is likely, due to regression to the mean, that subsequent estimations on the same individuals will be higher. The use of a single measurement to classify subjects relative to these thresholds will result in substantial misclassification bias [40].

Similar considerations apply to the interpretation of changes in allergen levels in a single house. The values for

the coefficient of repeatability show that, for individuals engaged in attempts at allergen avoidance, only changes greater than fivefold (for the bed) or sixfold (for floor areas) can be regarded as greater than expected due to the variability of the measurement.

There are two main factors which determine the sample size required for an investigation: the variance of the measurement and the minimal difference it is desired to be able to detect [39]. The minimum difference required to be able to detect should be selected in the context of the study hypothesis and clinical considerations. It is unlikely that differences or changes smaller than twofold would be clinically relevant. In a randomized controlled trial 30 subjects in each group (treatment and control) would yield 90% power to detect a twofold change in the beds and 80% power to detect such a change in floor areas. To detect a similar difference between groups in a cross-sectional study 35 subjects in each group would be required for a study with 80% power.

The assessment of HDM allergen exposure as a risk factor for disease often involves the estimation of regression coefficients in a linear or logistic model in which the dependent variable is the outcome of interest and the HDM allergen measurement is the predictor variable. Error in the measurement of HDM allergen exposure leads to attenuation of the estimate of the regression coefficient (that is bias towards the no-effect value) [41]. The adverse consequences of measurement error, both for attenuation of estimates and for misclassification, can be lessened if conclusions are based on replicated measures.

The variability in HDM allergen measures between floor areas in the same bedroom or different bedrooms was not greater than the repeatability of the measurement. Hence it is not relevant or necessary to specify the area of bedroom floor which is sampled. On the other hand, the variability between areas of the living room floor was greater than the repeatability of the measurement and it is relevant to focus on a specific site within the living room. This greater variation within the living floor may be due to the larger area covered by most living rooms and the greater variation in the use of the room. As might be anticipated there are important differences between beds within a house.

HDM allergen measurements collected separately from the mattress and the upper bedding were not significantly different from estimates of allergen concentrations obtained from the whole bed. The level of agreement was within the range of the repeatability of the measurement. The allergen concentration in dust collected from pillow was generally less than that in the whole bed and the limits of agreement were very wide.

These findings imply that allergen measurements from the mattress or upper bedding can be taken as representative of the whole bed.

In conclusion, we recommend that at least 35 houses per group be sampled in cross-sectional studies and 30 houses per group in parallel group, randomized controlled trials. The subject's own bed must be sampled and this may be done by collecting a sample from the layer of bedding below the bottom sheet. A single site within the bedroom floor may be taken as representative of this site but this is not true for the living room floor. The log transformation is appropriate for the analysis of allergen levels. In monitoring individuals' exposure to HDM allergen it is important to take cognisance of the variability inherent in the measurement. The reliability of estimates used for this purpose will be increased if they are based on replicated measurements.

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