

## Proficiency testing of allergen measurements in residential dust

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**Background:** Analyses of household dust for allergens are more common with increased efforts to reduce and control asthma. Currently, no laboratory accreditation or quality assurance program specific to household dust allergen analyses exists. Moreover, there is an absence of peer-reviewed data on within-laboratory and between-laboratory variability that is achievable for these analyses.

**Objectives:** The purpose of this study was to characterize the levels of intralaboratory and interlaboratory variability in analyses of allergen concentrations in residential dust and to investigate the utility of quality control samples for monitoring laboratory performance.

**Methods:** Aliquots from homogeneous batches of dust and dust extracts were provided to 8 commercial, academic, and municipal laboratories to be analyzed for as many as 6 allergens (Der p 1, Der f 1, Fel d 1, Can f 1, Bla g 1, Mus m 1) by using ELISA techniques.

**Results:** Coefficients of variation on the estimated geometric means from the analytical results ranged between 61% and 93%. In most cases, between-laboratory variability was the dominant component of total variability. In spite of this between-laboratory variability, reasonable agreement was observed between the means of allergen levels in the reference laboratory characterizations and the estimated geometric means from the model fitting of results across participating laboratories.

**Conclusion:** The results from this study indicate that, in most cases, participating laboratories could measure the

concentrations of allergens by using ELISA procedures with a level of accuracy and precision that may be acceptable in many situations. (*J Allergy Clin Immunol* 2005;116: 844-50.)

**Key words:** Allergens, ELISA, quality control samples, residential dust

Allergens from dust mites, pets, cockroaches, and rodents in residential dust have been identified as a potential risk factor for allergic sensitization and exacerbation of asthma.<sup>1</sup> Research on both the distribution of allergens in surface dust collected from bedding and floors and interventions to reduce allergen concentrations and surface loadings in homes and schools has increased substantially. This is caused in part by a rise in asthma over the last several decades<sup>2-4</sup> and identification of asthma as the most common chronic illness among children.<sup>1</sup> In addition to methodological studies that have been conducted to determine how and where dust samples should be taken and how they should be measured for allergen content,<sup>5-7</sup> multicenter research studies on asthma involving allergen measurements in residential dust have included the National Cooperative Inner-City Asthma Study,<sup>8</sup> the Childhood Asthma Management Program,<sup>9</sup> the National Survey of Lead and Allergens in Housing,<sup>10</sup> and smaller demonstration projects funded by the US National Institutes of Health and the US Department of Housing and Urban Development (HUD). Remediation and intervention efforts based on allergen detection in these studies have examined the utility of such practices as dust mitigation, moisture reduction, and carpet removal.<sup>11,12</sup>

Each of these studies has used the levels of allergens in surface dust to draw inferences about the degree of allergen exposure across residences and the efficacy of interventions to reduce exposure. Allergen content in dust has been quantified almost exclusively by using ELISAs. Currently, however, there is no laboratory accreditation or quality assurance program specific to these analyses. Moreover, there is a lack of peer-reviewed data on the within-laboratory and between-laboratory variability that can be achieved with these analyses. The objectives of this study were to (1) characterize the levels of intralaboratory and interlaboratory variability in ELISA analyses of allergen concentrations in residential dust, (2) study the

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#### Abbreviations used

|           |                                                                                                                |
|-----------|----------------------------------------------------------------------------------------------------------------|
| BDL:      | Below detection limit                                                                                          |
| CAP:      | College of American Pathologists                                                                               |
| CV:       | Coefficient of variation                                                                                       |
| HUD:      | US Department of Housing and Urban Development                                                                 |
| JHU-DACI: | Johns Hopkins University School of Medicine—Dermatology, Allergy, and Clinical Immunology Reference Laboratory |
| QC:       | Quality control                                                                                                |
| SOP:      | Standard operating procedure                                                                                   |

variation contributed by sample extraction by analyzing pre-extracted dust samples, and (3) examine the utility of quality control (QC) samples for monitoring laboratory performance.

## METHODS

The study was conducted in 2 phases, each with a different reference laboratory. The IBT Reference Laboratory (Lenexa, Kan) prepared dust specimens for a study conducted in 2001, whereas the Johns Hopkins University School of Medicine—Dermatology, Allergy, and Clinical Immunology Reference Laboratory (JHU-DACI, Baltimore, Md) prepared analogous specimens for 2004. These laboratories prepared homogenized batches of residential dust, characterized the levels of allergens in each batch, and shipped aliquots to participating laboratories. Each participating laboratory analyzed aliquots for 4 to 6 allergens by using their routine assay procedures and reported their results directly to the coordinating laboratory (Battelle, Columbus, Ohio).

Participating laboratories were required to be current or potential providers of ELISA analyses to grantees in HUD's Healthy Homes Initiative program. Seven commercial, academic, and/or municipal laboratories were selected for the 2001 study, and 5 laboratories participated in the 2004 study.

### Challenge dust specimen preparation

Three batches of dust were obtained for this study: 1 batch prepared by IBT Reference Laboratory for the 2001 study and 2 batches prepared by JHU-DACI for the 2004 study. Similar methods were used to prepare each dust batch. The 2001 dust was collected from several different houses in Lenexa by using a variety of vacuum cleaners. It was combined, rotated for 30 minutes at room temperature, and manually sieved through a 300- $\mu$ m screen for 30 minutes. Eleven aliquots of the mixed dust were taken and analyzed in ELISAs for dust mite (Der p 1, Der f 1), cat (Fel d 1), and cockroach allergens (Bla g 1, Bla g 2). To achieve the desired allergen levels, the dust was spiked with other dust containing elevated levels of dust mite and cockroach allergens. After a repeat mixing, the analysis process was repeated to obtain the final characterization of the allergen levels in the dust. The 2001 batch was prepared and analyzed in a single day by 1 technician by using ELISA reagent kits (INDOOR Biotechnologies Inc, Charlottesville, Va) from 1 lot.

Two complementary dust batches were prepared for the 2004 study by JHU-DACI. One contained higher levels of dust mite (Der p 1, Der f 1), cat (Fel d 1), and dog (Can f 1) allergens, whereas the other contained higher levels of cockroach (Bla g 1, Bla g 2) and mouse (Mus m 1) allergens. JHU-DACI's processing differed in that the initial dust for each batch was obtained from 200 to 300 homes in the eastern United States, the rotation of the combined dust was

conducted for 120 minutes, and the rotated dust was manually sieved twice through the 300- $\mu$ m screen for 30 minutes. Three distinct batch characterizations, each separated by approximately a 2-month period, were performed. Each time, a single technician analyzed 3 aliquots from each dust batch for Der p 1, Der f 1, Fel d 1, Can f 1, Bla g 1, Bla g 2, and Mus m 1. Finally, JHU-DACI produced a quantity of dust extract from each dust that was sufficient to ship to the participating laboratories. The allergen levels in this dust extract were quantified by analyzing each specimen in 10 runs over a 2-month period.

### Dust aliquots

In the 2001 study, triplicate 500-mg aliquots of fine sieved dust were shipped at ambient temperature by courier to each participating laboratory. All samples were treated in identical manner with regard to storage before shipment and packing. In the 2004 study, triplicate 250-mg aliquots of dust and triplicate 2-mL volumes of dust extracts were shipped by overnight courier to each laboratory. The participating laboratories were aware that the samples were part of a laboratory variability study but were masked to the expected allergen levels in the dust. Laboratories were reimbursed for their analyses per their standard fees.

### Analytical methods

All analyses were conducted by using ELISA methodology, and all laboratories acquired their reagent kits from INDOOR Biotechnologies, Inc. Each laboratory was asked to use its own standard operating procedure (SOP) for the analyses so that a characterization of the variability assessed in the study would result from different laboratories, each following their routine procedures. Tables E1 and E2 (see the Online Repository in the online version of this article at [www.jacionline.org](http://www.jacionline.org)) document the operating conditions under which the dust was extracted and analyzed in the 2001 and 2004 studies.

### Statistical methods

For each dust batch and study phase, allergen levels reported by participating laboratories were analyzed by using a balanced 1-way random effects ANOVA log-linear model (ie, a linear model fitted to logarithmically-transformed allergen measurements). The model included an intercept term, a random laboratory effect term representing between-laboratory variability (ie, variability in the mean log-transformed measurements of the laboratories) and a random error term representing within-laboratory variability (ie, variability in log-transformed measurements among aliquots analyzed within a laboratory). The model accounted for correlation in the measurements reported by a single laboratory. Once the data were exponentiated, the model's intercept term was interpreted as (1) the geometric mean measurement associated with the population of laboratories represented by those participating in this study, and (2) the predicted measurement that a laboratory from this population may report for a future aliquot from the given dust batch or extract. The analysis expressed between-laboratory and within-laboratory variability in the log-transformed measurements as a percentage of total variability (ie, the sum of these 2 variance components). The analysis also generated a 95% CI on the geometric mean measurement and a 95% prediction interval on the measurement of a future aliquot taken from the dust batch or extract.

By using a restricted maximum likelihood estimation approach, the MIXED procedure in version 8 (release 8.2) of the SAS System (SAS Institute, Cary, NC) was applied to the log-transformed study data to estimate the intercept term and the 2 variance components (between-laboratory and within-laboratory variability) associated with the random effects ANOVA model. On exponentiation, the

95% CI on the geometric mean laboratory measurement corresponded to the CI on the model's intercept term. The squared SE of the log-transformed geometric mean laboratory measurement was added to the estimated total variability in the log-transformed measurements, and the square root of this sum equaled the SE of the predicted future log-transformed measurement. This SE was used to calculate the 95% prediction interval associated with a future measurement. A Student *t* approach was used to calculate the bounds of the confidence and prediction intervals, and these bounds were exponentiated so that they could be expressed in proper units.

Separate, independent fits of the ANOVA model were made to data for each dust batch within each study phase, for each allergen type, and for dust aliquot analysis versus dust extract analysis (2004 study only). Measurements falling below a laboratory's detection limit were set to 1/2 of the detection limit and treated as measured values in the analysis. When all of a laboratory's measurements were below the detection limit (BDL) for a given dust batch and allergen type, then that laboratory's data were excluded from the model fitting for that batch and allergen type.

The 2001 Bl a g 1 results from laboratory A were reported 2 orders of magnitude higher than the other 2001 Bl a g 1 results. The laboratory director indicated that problems were encountered during these ELISA analyses. For these reasons, the 2001 Bl a g 1 results from laboratory A were excluded from all analyses.

## RESULTS

### Reference laboratory characterization of dust batches

The geometric and arithmetic means and coefficients of variation (CVs) of measured levels are presented by allergen type in Table I for each measurement of fine dust or pre-extracted dust performed by the reference laboratories. These data provided the coordinating laboratory with target ranges for all participating laboratories in the 2001 and 2004 studies. For the 2004 study, statistics are provided for each of the 3 characterizations performed on the pre-extracted dust aliquots, as well as pooled across the characterizations (labeled as *Pooled*). Because results of a 1-way ANOVA indicated that significant differences existed at the .05 level between these three characterizations for dust mite and dog allergens within batch 2, and for cat allergen within batches 2 and 3, caution should be taken when using these pooled results to characterize the dust batch for these allergens.

### Analytical results across laboratories

Allergen measurements reported by the participating laboratories are displayed by laboratory, dust batch, and allergen type within the *scatterplots* shown in Figs E1 and E2 (see the Online Repository in the online version of this article at [www.jacionline.org](http://www.jacionline.org)).

### Characterizing variance components

Table II presents the results of the random effects ANOVA modeling for each dust/extract batch and allergen. The 2nd and 3rd columns provide the estimated percentages of total variability in the log-transformed allergen measurements that are associated with between-laboratory and within-laboratory variability, respectively. The estimated geometric mean measurement across the

population of laboratories represented by those participating in this study is noted in the 4th column. This estimate also represents the predicted measurement of a future analysis from this dust or extract batch that a laboratory from this population may generate. The CVs (5th column) ranged from 61% to 93%.

In most cases, between-laboratory variability was the dominant component of total variability in the log-transformed measurements, especially for the 2004 extracts and the 2001 dust. Within-laboratory variability was dominant in dust mite, cockroach, and mouse allergen levels with batch 3 (dust), in cat and mouse allergen levels with batch 2 (dust), and in cat and dog allergen levels with batch 2 (extracts). However, when within-laboratory variability was estimated to represent virtually all of total variability (eg, Mus m 1, batch 2 dust), the contribution of within-laboratory variability could be reduced considerably after removing an extreme measurement that had highly influenced the size of the variability (eg, 1 measurement from laboratory D for Mus m 1 in batch 2). When extreme values were identified based upon the size of Studentized residuals from the ANOVA modeling, they were not removed from the analyses because no problems with the ELISA analyses could be identified for these measurements.

### Estimating geometric mean and predicted future aliquot measurements

Estimates of the geometric mean and predicted future aliquot measurements, reported in Table II for each dust batch and allergen type across the participating laboratories, were similar to the geometric means from the initial characterizations (Table I). The analysis also yielded a 95% CI on the geometric mean measurement across the participating laboratories. For laboratories that analyze and report results in the same manner as the laboratories participating in this study, this CI can be interpreted as 95% likely to contain the true geometric mean allergen level associated with the dust batch. The variability used to calculate this confidence interval takes into account both between-laboratory and within-laboratory variation.

The 95% prediction intervals on the outcome of a future ELISA analysis on an aliquot from a given dust batch also were calculated (last column of Table II). For a laboratory within the same population as those participating in this study (which are assumed to represent a random sample from the population, even though these laboratories were not selected randomly for the study), this interval would be expected to contain the future sample measurement 95% of the time.

As seen in Table II, the prediction intervals on a future aliquot measurement are wider than their corresponding CIs on the geometric mean. This occurs because a geometric mean is calculated from multiple sample measurements, and therefore, it has a lower degree of uncertainty than individual measurements. Furthermore, the predicted value of a future measurement has a higher degree of uncertainty than does a measurement that has already been taken, because of the additional uncertainty

**TABLE I.** Geometric\* and arithmetic means (and CV) of allergen levels resulting from analyses performed to characterize the dust aliquots and extracts prepared from each batch

|               |            |        |     | Dust mite allergens |                   | Cat allergen      | Dog allergen      | Cockroach allergen | Mouse allergen    |
|---------------|------------|--------|-----|---------------------|-------------------|-------------------|-------------------|--------------------|-------------------|
| Study         | Dust Batch | Char.  | N   | Der f 1<br>(μg/g)   | Der p 1<br>(μg/g) | Fel d 1<br>(μg/g) | Can f 1<br>(μg/g) | Bla g 1<br>(U/g)   | Mus m 1<br>(μg/g) |
| Dust aliquots |            |        |     |                     |                   |                   |                   |                    |                   |
| 2001          | 1          | —      | 11† | 0.53                | 1.28              | 45.7              | —                 | 0.52               | —                 |
|               |            |        |     | 0.53 (3%)           | 1.28 (3%)         | 45.8 (6%)         |                   | 0.53 (12%)         |                   |
| 2004          | 2          | 1      | 3   | 2.70                | 56.3              | 1480              | 70.8              | 0.4‡               | 0.035             |
|               |            |        |     | 2.73 (19%)          | 56.9 (20%)        | 1500 (20%)        | 71.6 (18%)        | 0.4 (0%)‡          | 0.037 (35%)       |
|               |            | 2      | 3   | 3.09                | 41.5              | 1460              | 107               | <0.4               | 0.023             |
|               |            |        |     | 3.10 (11%)          | 41.5 (4%)         | 1460 (4%)         | 107 (4%)          | <0.4               | 0.032 (99%)       |
|               |            | 3      | 3§  | 4.52                | 41.5              | 2130              | 89.2              | <0.4               | —                 |
|               |            |        |     | 4.53 (9%)           | 41.5 (3%)         | 2130 (5%)         | 89.3 (6%)         | <0.4               |                   |
|               |            | Pooled | 9§  | 3.35                | 45.9              | 1660              | 87.8              | <0.4               | 0.028             |
|               |            |        |     | 3.46 (26%)          | 46.7 (21%)        | 1700 (21%)        | 89.3 (19%)        | <0.4               | 0.034 (63%)       |
| 2004          | 3          | 1      | 3   | 0.330               | 0.159             | 35.3              | 0.317             | 11.8               | 2.24              |
|               |            |        |     | 0.333 (17%)         | 0.167 (35%)       | 35.5 (13%)        | 0.400 (87%)       | 13.3 (55%)         | 2.60 (68%)        |
|               |            | 2      | 3   | 0.520               | 0.184             | 48.1              | 0.378             | 29.0               | 1.83              |
|               |            |        |     | 0.530 (25%)         | 0.184 (1%)        | 48.1 (2%)         | 0.378 (6%)        | 32.3 (58%)         | 1.84 (14%)        |
|               |            | 3      | 3   | 0.510               | 0.171             | 53.9              | 0.203             | —                  | —                 |
|               |            |        |     | 0.520 (25%)         | 0.172 (10%)       | 54.0 (10%)        | 0.204 (13%)       |                    |                   |
|               |            | Pooled | 9   | 0.444               | 0.171             | 45.0              | 0.290             | 18.5               | 2.02              |
|               |            |        |     | 0.461 (30%)         | 0.174 (18%)       | 45.9 (19%)        | 0.327 (60%)       | 22.8 (73%)         | 2.22 (54%)        |
| Dust extracts |            |        |     |                     |                   |                   |                   |                    |                   |
| 2004          | 2          | —      | 10¶ | 3.82                | 47.3              | 1770              | 90.6              | 0.4#               | 0.024             |
|               |            |        |     | 3.84 (11%)          | 47.6 (12%)        | 1790 (16%)        | 91.4 (14%)        | 0.4 (0%)#          | 0.0193 (12%)      |
| 2004          | 3          | —      | 10¶ | 0.486               | 0.176             | 53.4              | 0.263             | 9.08               | 2.52              |
|               |            |        |     | 0.497 (7%)          | 0.177 (7%)        | 53.6 (11%)        | 0.265 (13%)       | 9.16 (14%)         | 2.02 (12%)        |

Char., Characterization number.

\*Geometric means are italicized in the table.

†N = 10 for Fel d 1.

‡One measurement was reported below the detection limit of 0.4 U/g, whereas the remaining 2 were equal to 0.4 U/g.

§No data were available from the 3rd characterization for Mus m 1.

||No data were available from the 3rd characterization for Bla g 1 or Mus m 1.

¶N = 8 for Mus m 1.

#Eight measurements were below the detection limit of 0.4 U/g, whereas the remaining 2 were equal to 0.4 U/g.

that is associated with estimating a measurement that will be conducted at a future time.

## DISCUSSION

In recent years, the measurement of indoor allergens derived from dust mites, pets, insects, and rodents in surface dust has moved from a research tool into use in clinical practice to assess the exposure risk from the environments of children and adults with asthma. As more laboratories perform these measurements with commercial reagents, external proficiency testing as a part of an overall quality assurance program becomes essential. This report describes the first systematic study of variability among laboratories that measure indoor allergens. The laboratories participating in this study were selected because they are actively engaged in providing allergen measurements to research studies funded by federal agencies such as HUD, but also because they represent the current level of practice with laboratories that have experience in allergen testing. As such, the observed variance of the analytical

methods used for allergen quantification should represent a more accurate assessment of the actual variance expected from assays used in clinical practice.

The predicted CIs shown in Table II are tight enough to imply that laboratory-reported allergen results have sufficient precision to allow distinguishing between residences with relatively low or high allergen levels. Thus, individuals wishing to identify residences that are significantly above or below target risk levels for sensitization and symptom induction (eg, 2 μg/g and 10 μg/g, respectively, for dust mite allergens<sup>13</sup>), which is generally the goal of these analyses when conducted in a clinical context, may be able to do this with reasonable confidence. Such decisions would have been possible using the dust mite results from this study—ie, the confidence intervals obtained for the dust mite results show that the Der f 1 and Der p 1 results for batches 1 and 3 were below 2 μg/g, the Der f 1 level for batch 2 was between 2 μg/g and 10 μg/g, and the Der p 1 level for batch 2 was above 10 μg/g, which are all consistent with the original characterizations of the dust. The relatively high analytical variability observed in this study could be more problematic, however, when

**TABLE II.** Estimated percentage of total variability attributed to between-laboratory and within-laboratory variability, model-based estimates of the geometric mean and CV across laboratories, and 95% CIs and prediction intervals on these estimates\*

| Allergen                    | Percent of total variability<br>(on the log scale) attributed to |                                  | Model-based estimates<br>(across laboratories)                                      |               |                                    |                                                          |
|-----------------------------|------------------------------------------------------------------|----------------------------------|-------------------------------------------------------------------------------------|---------------|------------------------------------|----------------------------------------------------------|
|                             | Between-laboratory<br>variability                                | Within-laboratory<br>variability | Geometric mean/<br>predicted future<br>measurement<br>( $\mu\text{g/g}$ ) $\dagger$ | CV $\ddagger$ | 95% CI<br>on the geometric<br>mean | 95% Prediction<br>interval on a<br>future<br>measurement |
| 2001 Dust aliquots, batch 1 |                                                                  |                                  |                                                                                     |               |                                    |                                                          |
| Der f 1                     | 67.2%                                                            | 32.8%                            | 0.540                                                                               | 93%           | (0.254, 1.15)                      | (0.07, 4.38)                                             |
| Der p 1                     | 97.9%                                                            | 2.1%                             | 0.691                                                                               | 85%           | (0.324, 1.47)                      | (0.10, 4.57)                                             |
| Fel d 1                     | 87.6%                                                            | 12.4%                            | 68.1                                                                                | 66%           | (47.0, 98.8)                       | (26.2, 177)                                              |
| Bla g 1                     | 67.8%                                                            | 32.2%                            | 0.520                                                                               | 70%           | (0.164, 1.64)                      | (0.123, 2.19)                                            |
| 2004 Dust aliquots, batch 2 |                                                                  |                                  |                                                                                     |               |                                    |                                                          |
| Der f 1                     | 79.9%                                                            | 20.1%                            | 4.20                                                                                | 62%           | (3.40, 5.19)                       | (2.70, 6.54)                                             |
| Der p 1                     | 82.5%                                                            | 17.5%                            | 41.5                                                                                | 65%           | (26.9, 64.0)                       | (16.9, 102)                                              |
| Fel d 1                     | 28.6%                                                            | 71.4%                            | 1,500                                                                               | 61%           | (1,300, 1,740)                     | (1,030, 2,200)                                           |
| Can f 1                     | 72.9%                                                            | 27.1%                            | 67.5                                                                                | 64%           | (45.5, 100)                        | (29.1, 156)                                              |
| Mus m 1                     | 0.03%                                                            | 99.97%                           | 0.017                                                                               | 71%           | (0.0078, 0.0379)                   | (0.0042, 0.0713)                                         |
| 2004 Dust aliquots, batch 3 |                                                                  |                                  |                                                                                     |               |                                    |                                                          |
| Der f 1                     | 0.3%                                                             | 99.7%                            | 0.522                                                                               | 62%           | (0.439, 0.622)                     | (0.331, 0.824)                                           |
| Der p 1                     | 0.1%                                                             | 99.9%                            | 0.210                                                                               | 65%           | (0.125, 0.355)                     | (0.082, 0.538)                                           |
| Fel d 1                     | 92.1%                                                            | 7.9%                             | 59.8                                                                                | 65%           | (37.5, 95.4)                       | (23.4, 153)                                              |
| Can f 1                     | 89.5%                                                            | 10.5%                            | 0.252                                                                               | 84%           | (0.0733, 0.870)                    | (0.0320, 1.99)                                           |
| Bla g 1                     | 19.8%                                                            | 80.2%                            | 12.6                                                                                | 70%           | (8.02, 19.7)                       | (3.65, 43.3)                                             |
| Mus m 1                     | 28.5%                                                            | 71.5%                            | 2.06                                                                                | 64%           | (1.45, 2.92)                       | (0.977, 4.33)                                            |
| 2004 Dust extracts, batch 2 |                                                                  |                                  |                                                                                     |               |                                    |                                                          |
| Der f 1                     | 79.9%                                                            | 20.1%                            | 4.02                                                                                | 65%           | (2.61, 6.18)                       | (1.63, 9.88)                                             |
| Der p 1                     | 77.7%                                                            | 22.3%                            | 37.7                                                                                | 66%           | (23.5, 60.6)                       | (13.9, 102)                                              |
| Fel d 1                     | 35.7%                                                            | 64.3%                            | 1620                                                                                | 62%           | (1360, 1930)                       | (1050, 2510)                                             |
| Can f 1                     | 49.9%                                                            | 50.1%                            | 63.8                                                                                | 68%           | (38.8, 105)                        | (19.9, 204)                                              |
| Mus m 1                     | 56.8%                                                            | 43.2%                            | 0.0213                                                                              | 62%           | (0.0142, 0.0320)                   | (0.0126, 0.0361)                                         |
| 2004 Dust extracts, batch 3 |                                                                  |                                  |                                                                                     |               |                                    |                                                          |
| Der f 1                     | 54.6%                                                            | 45.4%                            | 0.611                                                                               | 63%           | (0.424, 0.882)                     | (0.307, 1.22)                                            |
| Der p 1                     | 99.1%                                                            | 0.912%                           | 0.193                                                                               | 63%           | (0.0957, 0.389)                    | (0.0868, 0.429)                                          |
| Fel d 1                     | 85.8%                                                            | 14.2%                            | 51.2                                                                                | 65%           | (33.4, 78.5)                       | (21.3, 123)                                              |
| Can f 1                     | 96.3%                                                            | 3.66%                            | 0.297                                                                               | 91%           | (0.0718, 1.23)                     | (0.0291, 3.02)                                           |
| Bla g 1                     | 62.4%                                                            | 37.6%                            | 6.91                                                                                | 64%           | (4.90, 9.74)                       | (3.22, 14.8)                                             |
| Mus m 1                     | 72.0%                                                            | 28.0%                            | 2.57                                                                                | 63%           | (1.75, 3.78)                       | (1.30, 5.07)                                             |

\*Based on fitting the random effects ANOVA model to the log-transformed study data. Seven laboratories (A through G) participated in 2001 and 5 (A, B, D, E, H) in 2004. Data for the following laboratories were not included in the ANOVAs for the specified batches and allergens as a result of all data being BDL: 2001, Bla g 1, laboratories B, E, G; 2004, batch 3 (both dust aliquots and extracts), Der f 1, laboratory E; 2004, batch 3 (both dust aliquots and extracts), Der p 1, laboratories A and E; 2004, batch 3 (both dust aliquots and extracts), Can f 1, laboratory E. No analysis was performed on data for Bla g 1 for 2004 Batch 2 as a result of approximately 87% of data being BDL across all laboratories. In addition, laboratory A did not offer Mus m 1 analyses; thus, it is excluded from Mus m 1 ANOVAs.

$\dagger\mu\text{g/g}$  for Bla g 1.

$\ddagger$ Estimated as  $(\exp[\sigma_{\text{total}}^2] - 1)^{1/2}$ , where  $\sigma_{\text{total}}^2$  represents total variability of the log-transformed measurements (ie, the sum of between-laboratory and within-laboratory variability) as estimated from the ANOVA. This approach is used to calculate the CV of data that follow a lognormal distribution.

ELISA analyses are conducted in a research context (eg, as an exposure measure or to assess intervention effectiveness), in which case the variability would decrease the strength of statistical associations.

Between-laboratory variability was found to be the dominant component of total variability in all analyses for both the dust and pre-extracted dust. Possible causes of this variability include technician error, limited shelf life of reagents, differences in calibration of pipets and

balances, differences in extraction procedures, and differences in operating conditions. Between-laboratory variability was dominant for all allergens within at least 1 dust batch, although those batches with elevated levels of Fel d 1 and Bla g 1 tended to have higher within-laboratory variability compared with between-laboratory variability for these allergens.

As a result of the between-laboratory variability, individuals basing decisions solely on the results of

1 laboratory must be judicious in drawing inferences. For instance, it may be reasonable to use allergen levels measured in 1 laboratory to compare allergen levels pre-intervention and postintervention, because if the results from a laboratory were biased in 1 direction, then the bias would affect both the preintervention and postintervention results. However, attempting to compare allergen levels found in a home with standards or national averages may be misleading if analytical results are obtained from only 1 laboratory, although this is a common practice.

In spite of the between-laboratory variability observed in this study, relatively good agreement was observed between the means of the reference laboratory characterizations and the estimated geometric means from the model fitting of results across laboratories. In all but 2 cases (Bla g 1 in batch 3 dust and Fel d 1 in batch 2 extract), the means from the dust characterizations (Table I) fell within the 95% CI of the estimated geometric means (Table II), and the characterized means fell within the 95% prediction intervals on a future measurement in every case. Interestingly, the within-laboratory and between-laboratory variability observed with the pre-extracted dust was comparable with that observed with dust aliquots (extracted by the individual laboratories) for most allergens, indicating that the extraction step is not a significant source of variability. However, the seemingly clear reduction in the within-laboratory variability from analysis of the Bla g 1 extract for batch 3 (see Figs E1 and E2 in the Online Repository in the online version of this article at [www.jacionline.org](http://www.jacionline.org)) suggests that it might be more difficult to create QC dust with a homogeneous distribution of this allergen. This would suggest that rigorous SOPs for performance of the assay may promote less variable results among laboratories using the same reagents.

Although the 95% prediction intervals shown in Table II could be used in a proficiency testing program (assuming that sufficient information were available to allow selected laboratories to be rated as highly proficient in measuring allergen levels), various issues still must be addressed before a proficiency program can be established in which researchers obtain QC samples containing known amounts of allergens for submission to their laboratories to evaluate overall performance. First, it is not possible to obtain surface house dust specimens containing known allergen levels from official standards organizations such as the National Institute of Standards and Technology. As a result, private laboratories must collect sufficient quantities of dust so there is enough for characterization and use as QC samples. Results of this study indicate that the extent of variability in these QC results will depend on the laboratory that performs the characterization. Thus, multiple laboratories should participate in the dust characterization phase of the QC specimen development. Data obtained from the Seattle/King County Healthy Homes/ HUD Projects support this finding (Beaudet N, Pilot study of intra- and inter-laboratory variation of analyses of common allergens measured in residential house dust,

2004, abstract submitted to HUD in conjunction with quarterly report). Over a 5-month period in 2003, different aliquots of a single dust lot were sent masked in 6 batches of dust specimens collected under its research program. When analyzed by a contract laboratory for Fel d 1, the average of the 6 analyses was 209  $\mu\text{g/g}$  (CV = 61%), compared with pre-established target value of 273  $\mu\text{g/g}$ . Seven QC samples each were then sent to a different laboratory and the first contract laboratory for analysis. Both laboratories reported an average of 150  $\mu\text{g/g}$ , with CVs of 13% and 29%, respectively. These sets of analyses illustrate the dependency of both the mean results and the estimated variability on the laboratory. Other factors such as the heterogeneity and extent of mixing of the challenge dust specimen, as well as the residual humidity of specimen and relative humidity of the laboratory weighing out the specimen, can contribute to allergen result variability. These factors will need to be studied further before optimal QC dust specimens can be made available.

Results from 3 years of a study by the College of American Pathologists (CAP) indicate that allergens in surface dust are remarkably stable over years when stored frozen (CAP, Indoor Aeroallergen Year-End Critique, 2003). In its Indoor Aeroallergen Survey, CAP supplied aliquots from 2 different dust batches to 5 participating laboratories in biannual cycles. By using their normal ELISA techniques, the laboratories analyzed the aliquots for Der p 1, Der f 1, Fel d 1, Can f 1, and Bla g 1. The 2 laboratories that participated throughout all 3 years of the survey obtained within-laboratory CVs between 10% and 20%, and the mean results from the 2 laboratories were consistent with each other. Despite their apparent constraints associated with variability, QC dust samples can serve as a useful verification on whether observed laboratory variance is within expected boundaries or is excessive and deserves investigation.

The results from this study indicate that, in most cases, participating laboratories could measure the concentrations of allergens by using ELISA procedures with a level of accuracy and precision that may be acceptable in many situations. Variability between laboratories was generally found to be greater than within-laboratory variability. The variability associated with the analysis of dust extracts was similar to that for the unextracted dust for most allergens. The results support the need for the development and use of standard QC dust samples by laboratories conducting ELISA analyses for common residential allergens. The findings further suggest that the development of standard SOPs for sample preparation and the performance of assays could reduce variability among laboratories using the same reagents.

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