



# Validation of a novel sampling technology for airborne allergens in low-income urban homes

Allergens are distributed between the settled dust and airborne compartments. The airborne compartment represents inhalable allergen that penetrates deeply in the respiratory tract and may trigger asthma symptoms.<sup>1</sup> Larger airborne particles may also trigger allergic rhinitis. Because methods of air sampling have been technically demanding and limited by assay sensitivity, dust collection has been a standard surrogate. Raulf et al reviewed methods for measurement of airborne allergens.<sup>1</sup> More recent studies have described potentially improved methods. Barnes et al<sup>2,3</sup> used capture of allergens by air filters run over a period of months in people's homes. We<sup>4</sup> introduced a simple plug-in device (Inspirotec, Inc, Chicago, Illinois) for air sampling. It is silent, unobtrusive, and requires no technical skill to operate.

Due to the relatively low concentration of allergens in the airborne compartment, their detection, especially of dust mite, has been problematic. The Inspirotec sampler has been optimized for sample capture by ionic propulsion (US patent numbers 8,038,944, 9,216,421, 9,360,402, 9,481,904, and 9,618,431). The combination of Inspirotec's high air flow rate and highly sensitive Multiplex Array for Indoor Allergens (MARIA, Indoor Biotechnology, Charlottesville, Virginia) multiplex immunoassays facilitates the detection of a full spectrum of airborne allergens, including those previously undetectable. Hitherto, other investigators have been able to find significant correlations between allergen content of dust and air for mouse,<sup>5,6</sup> cat,<sup>7–10</sup> and dog.<sup>7–9</sup> Herein we evaluate the performance of the Inspirotec sampler by establishing correlations with reference methods of air sampling and dust collection for multiple allergens. This was done as part of a larger, ongoing, randomized clinical trial of home environmental intervention in which multiple air and dust samples were collected from low-income homes in Baltimore, Maryland. We found significant correlations for Inspirotec sampling compared both with standard particulate matter 10 (PM10; ie, particle sizes  $\leq 10 \mu\text{m}$ ) air filtration, and dust collection, for mouse, cat, and dog allergens (Fig 1).

Only the 3 allergens indicated in Figure 1 were observed in a sufficient number of positive samples to permit bivariate analysis. Of the 12 common household allergens tested, all except birch pollen, Bet v 1, showed relatively more positive samples (higher sensitivity) with the Inspirotec sampler than the PM10 (eTable 1). This was likely accounted for by the larger air volume sampled by the Inspirotec device during the time of running in the homes (100 L/min compared with 5 liters/min). eTable 1 also shows a higher proportion of samples positive for dust and negative for airborne samples for dust mite, dog, and cockroach. This is consistent with the preponderance of larger particles containing those allergens.

The higher sensitivity of the Inspirotec sampler also appears when the airborne allergen values are characterized by percentile ranges, as shown in eTable 2. Although allergens were more likely to be detected with Inspirotec sampling, the concentrations of allergens tended to be lower than those measured from PM10 sampling (eTable 2). These differences in allergen concentrations likely reflect the lower capture efficiency of the Inspirotec sampler. A model system of fluorescent latex particles estimated capture efficiency to be 23%. Although, theoretically, a correction factor could be applied to account for capture efficiency, the capture efficiency of allergens would likely differ from that for latex particles, which have different particle characteristics.

Although threshold concentrations considered clinically meaningful have limitations, physicians and other health-care providers and patients need such information to guide decisionmaking. As a result, thresholds of allergens expected to be of clinical significance have been proposed. Salo et al<sup>11</sup> proposed 10  $\mu\text{g/g}$  of dust for Can f 1 and 8  $\mu\text{g/g}$  for Fel d 1 as cutoff points for "elevated levels." Although there are limitations to extrapolating from a single study, corresponding airborne levels can be calculated from the generalized estimating regression equations used to estimate relationships between Inspirotec and settled dust allergen concentrations. This calculation would lead to equivalent levels of 0.04  $\text{pg/m}^3$  for both Can f 1 and Fel d 1.

In conclusion, the results obtained with the Inspirotec sampler show that it is more sensitive, but yields lower allergen concentrations than airborne allergen sampling with PM10. The Inspirotec sampler is compact, easily deployed, and unobtrusive, so larger scale studies can be done without the logistical problems that would be associated with more technically demanding and noisier alternative equipment. It is also a reasonable alternative to dust collection because the use of a vacuum cleaner in a controlled manner and vacuuming of defined areas is technically demanding, and airborne samples are a closer approximation to the air inhaled. The present results, combined with the ease of deployment and unobtrusiveness of the Inspirotec samplers, show that direct measurement of allergens in the airborne fraction, which is most relevant for identifying exposure in allergic asthma or rhinitis, can be used in large-scale studies involving multiple sites.

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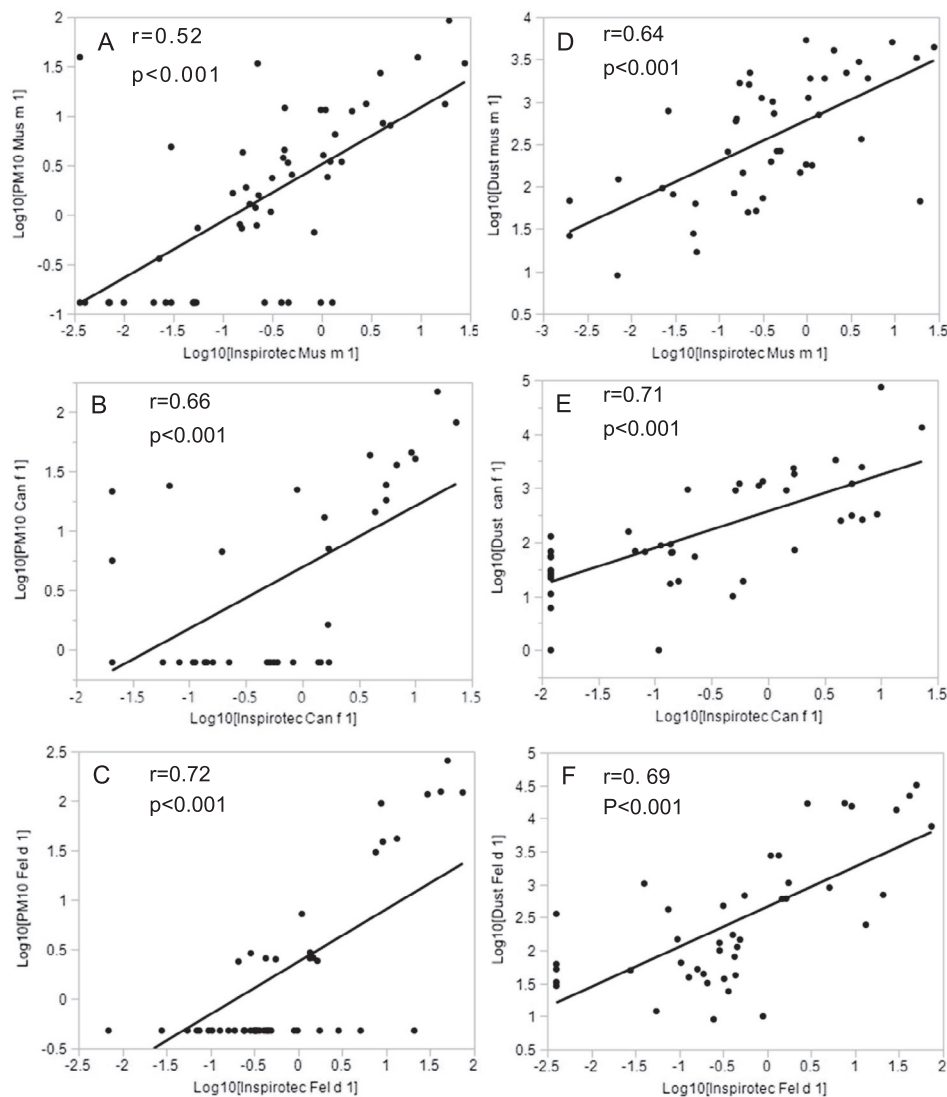


**Figure 1.** Bivariate analysis of Inspirotec airborne allergen capture compared with 2 reference methods. Reference methods were collection by air sampling and dust sampling. Samples were from 25 urban, low-income homes in Baltimore, MD, taken at baseline, 17 at 3 months, and 12 at 6 months. Study-blindedness was maintained regarding whether a home was in the active intervention group or the control group. Air sampling by PM10 (particles  $>10\ \mu\text{m}$  selectively removed with an impinger) and dust collection were obtained as described elsewhere.<sup>5</sup> In parallel, Inspirotec samplers were plugged into wall sockets and run for the same period of 5–7 days. Inspirotec samplers had precalibrated flow rates of  $100 \pm 20\ \text{L/min}$  and were stable within  $\pm 10\%$  over multiple uses. PM10 samplers were adjusted to  $4\ \text{L/min}$ . Samples from Inspirotec electrodes and PM10 filters were extracted in 1 mL and assayed using the Multiplex Array for Indoor Allergens (MARIA, Indoor Biotechnologies) for the 12 common household allergens: Der p 1; Der f 1; mite group 2; Fel d 1; Can f 1; Mus m 1; Rat n1; Bla g 1; Alt a 1; Bet v 1; Asp f 1; and Phl p 5. Dust samples were weighed, extracted by standard methods, and assayed by enzyme-linked immunoassay (Indoor Biotech) for Der f 1, Fel d 1, Can f 1, Mus m 1, and Bla g 2. Air sample results were expressed as picograms per cubic meter of air, and dust as micrograms per gram. JMP Pro version 13.0.0 (SAS Institute, Inc, Cary, North Carolina) and StataSE version 13.0 (StataCorp LLC, College Station, Texas) statistical software was used. Allergen variables were  $\log_{10}$ -transformed and values shown are Pearson's rho (r) from StataSE. Because there was repeated sampling within the same home, r and P values were established using generalized estimating equations, which account for the within-home correlation. The best straight line fit to the  $\log_{10}$  of the values is shown. Values below the LLOD were assigned a value of LLOD/2.

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## Supplementary Material

**eTable 1**

Percent Detectable and Undetectable With Air Sampling Methods

|                      | Inspirotec | Positive (%) | Negative (%) |
|----------------------|------------|--------------|--------------|
| <b>PM10</b>          |            |              |              |
| Total mite           | Positive   | 1.8          | 12.7         |
|                      | Negative   | 3.6          | 81.8         |
| Fel d 1              | Positive   | 30.9         | 52.7         |
|                      | Negative   | 0            | 16.4         |
| Can f 1              | Positive   | 29.8         | 35.1         |
|                      | Negative   | 3.5          | 31.6         |
| Mus m 1              | Positive   | 67.3         | 27.3         |
|                      | Negative   | 1.8          | 3.6          |
| Rat n 1              | Positive   | 1.8          | 9.1          |
|                      | Negative   | 5.5          | 83.6         |
| Bla g 2              | Positive   | 1.8          | 23.6         |
|                      | Negative   | 3.6          | 70.9         |
| Alt a 1              | Positive   | 0            | 5.5          |
|                      | Negative   | 0            | 94.5         |
| Bet v 1              | Positive   | 7.3          | 9.1          |
|                      | Negative   | 21.8         | 61.8         |
| Asp f 1              | Positive   | 1.8          | 18.2         |
|                      | Negative   | 0            | 80           |
| Phl p 5              | Positive   | 0            | 16.4         |
|                      | Negative   | 12.7         | 70.9         |
| <b>Dust</b>          |            |              |              |
| Total mite v Der f 1 | Positive   | 9.1          | 6.8          |
|                      | Negative   | 40.9         | 43.2         |
| Feld 1               | Positive   | 95.3         | 0            |
|                      | Negative   | 4.7          | 0            |
| Can f 1              | Positive   | 68.2         | 2.3          |
|                      | Negative   | 27.3         | 2.3          |
| Mus m 1              | Positive   | 95.5         | 0            |
|                      | Negative   | 4.5          | 0            |
| Bla g 2 v Bla g 1    | Positive   | 18.2         | 9.1          |
|                      | Negative   | 29.5         | 43.2         |

Abbreviations: LLOD, lower limit of detection; PM10, particulate matter  $\leq 10 \mu\text{m}$ . Data indicate percent of samples above (positive) or below (negative) field LLODs. Assays are described in legend to [Figure 1](#) Total mite was the sum of Der f 1, Der f 2, and mite group 2 values.

**eTable 2**

Airborne Allergen Concentration by Percentile

| Allergen   | Sampler         | Percentile (pg/m <sup>3</sup> ) |      |      |        |
|------------|-----------------|---------------------------------|------|------|--------|
|            |                 | 25th                            | 50th | 75th | 95th   |
| Total mite | PM10            |                                 |      |      | 4.00   |
|            | Inspirotec, Inc |                                 |      |      | 0.29   |
| Fel d 1    | PM10            |                                 |      | 2.51 | 118.96 |
|            | Inspirotec, Inc |                                 | 0.33 | 1.36 | 34.37  |
| Can f 1    | PM10            |                                 |      | 7.06 | 42.39  |
|            | Inspirotec, Inc |                                 | 0.14 | 1.57 | 9.54   |
| Mus m 1    | PM10            | 0.13                            | 1.78 | 7.99 | 36.00  |
|            | Inspirotec, Inc | 0.05                            | 0.31 | 1.22 | 12.67  |
| Rat n 1    | PM10            |                                 |      |      | 1.74   |
|            | Inspirotec, Inc |                                 |      |      | 0.04   |
| Bla g 2    | PM10            |                                 |      |      | 4.65   |
|            | Inspirotec, Inc |                                 |      | 0.06 | 0.55   |
| Alt a 1    | PM10            |                                 |      |      |        |
|            | Inspirotec, Inc |                                 |      |      | 0.01   |
| Bet v 1    | PM10            |                                 |      | 3.43 | 13.47  |
|            | Inspirotec, Inc |                                 |      |      | 0.42   |
| Asp f 1    | PM10            |                                 |      |      |        |
|            | Inspirotec, Inc |                                 |      |      | 0.34   |
| Phl p 5    | PM10            |                                 |      |      | 10.78  |
|            | Inspirotec, Inc |                                 |      |      | 0.32   |

Abbreviations: LLOD, lower limit of detection; PM10, particulate matter  $\leq 10 \mu\text{m}$ . Values below LLOD were assigned a value of LLOD/2 for purposes of this calculation of percentiles. For clarity, the blanks are where the values were below the LLOD. Total mite was the sum of Der f 1, Der f 2, and mite group 2 values. Note that, when the majority of samples are negative, there are only significant values in the highest percentile range. There were no positives for Bla g 2 and Bet v 1 by PM10 sampling.