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Validation of a Novel Sampling Technology for Airborne Allergens in Low Income Urban Homes.

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Keywords

Aerobiome; ELISA; MARIA[®]; airborne; aerosol; dustmite; cat; dog; mouse; mold; pollen

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 (1). 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. Methods for measurement of airborne allergens were reviewed (1). More recent publications described potentially improved methods. Barnes et al (2, 3) used capture of allergens by air filters in homes that run over a period of months. We (4) introduced a simple plug-in device (Inspirotec Inc, Chicago, IL) 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 MARIA[®] (Indoor Biotechnology, Charlottesville, VA) multiplex immunoassays facilitates the detection of a full spectrum of airborne allergens, including those previously non detectable. Hitherto, others have been able to find some significant correlations between allergen content of dust and air for mouse (5, 6), cat (7–10) and dog (7–9). Here, we wish to 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 on-going randomized clinical trial of home

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Conflicts of interest: JG, RR and PG are employees of Inspirotec Inc and JG and PG are co-founders and stockholders.

environmental intervention where multiple air and dust samples were collected in low income Baltimore homes. We showed significant correlations for Inspirotec sampling compared both with standard PM10 (particle sizes $\geq 10 \mu\text{m}$) air filtration, and dust collection, for mouse, cat as well as dog allergens (Fig 1).

Only the three allergens in Fig 1 were in a sufficient number of positive samples to permit the 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 (e-Table 1). This is likely accounted for by the larger air volume sampled by the Inspirotec device during the time of running in the homes (100 liters/min compared with 5 liters/min). Results in e-Table 1 also show a higher proportion of samples positive for dust and negative for airborne samples for dustmite, dog and cockroach. This is consistent with 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 e-Table 2. Although allergens were more likely to be detectable with Inspirotec sampling, the concentrations of the allergens tended to be lower than those measured from PM10 sampling (e-Table 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 decision-making. As a result, thresholds of allergens that are expected to be of clinical significance have been proposed. Salo et al (11) proposed $10 \mu\text{g}$ of dust for Can f 1 and $8 \mu\text{g/g}$ for Fel d 1 as cut-off points for “elevated levels”. Although there are limitations to extrapolating from one 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 pg/m^3 for both Can f 1 and for Fel d 1.

In summary, 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 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 closer approximation to the air inhaled. The results here, 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 assessing exposure in allergic asthma or rhinitis, can be used in large scale studies involving multiple sites.

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Abbreviations:

ELISA	Enzyme Linked Immunoassay
MARIA®	Multiplex Array for Indoor Allergens
LLOD	Lower Limit of Detection

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Fig 1. Bivariate analysis of Inspirotec airborne allergen capture compared with two reference methods.

Reference methods were collection by air sampling and dust sampling. Samples were from twenty-five urban, low income Baltimore homes at baseline, 17 at 3 months, and 12 at 6 months. Whether a home is in the active intervention group or the control group is still blinded. Air sampling by PM10 (particles $> 10 \mu\text{m}$ selectively removed with an impinger) and dust collection were as described elsewhere (5). In parallel, Inspirotec samplers were plugged into wall sockets and run for the same period of 5–7 days. Inspirotec samplers had pre-calibrated flow rates of 100 ± 20 liters/min and were stable within $\pm 10\%$ over multiple usages. PM10 samplers were adjusted to 4 liters/min. Samples from Inspirotec electrodes and PM10 filters were extracted in 1 mL and assayed by Indoor Biotechnologies MARIA™ assays 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, Phl p 5. Dust samples were weighed, extracted by standard methods and assay by Indoor Biotech ELISA for Der f 1, Fel d 1, Can f 1, Mus m 1 and Bla g 2. Air sample results were expressed as pg/m^3 and dust as $\mu\text{g}/\text{g}$. JMP® Pro 13.0.0 (SAS Institute Inc.Cary, NC) and StataSE 13.0 (StataCorp LLC, College Station, Texas) statistics packages were used. Allergen variables were log₁₀-transformed and

r values shown are Pearson's rho (r) from StataSE. Because there was repeated sampling within the same home, r and p-values were generated using Generalized Estimating equations, which account for the within-home correlation. The best straight line fit to the $\log_{10}$ of the values are shown. Values below the LLOD were assigned a value of LLOD/2.

e-Table 1.

Percent detectable and undetectable with air sampling methods.

	Inspirotec	Positive	Negative
		PM10	
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
Dust			
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

Numbers are percent of samples that were above (positive) or below (negative) field LLODs. Assays were as described in legend to Fig 1. Total mite was the sum of Der f 1, Der f 2 and Mite Group 2 values.

e-Table 2.

Airborne Allergen Concentration by Percentile

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

Values are in pg/m³ of air. Values below LLOD were assigned a value of LLOD/2 for the purposes of this calculation of the 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.