

Original article

A new method for collecting airborne allergens

xxBackground: Measurement of airborne allergens has hitherto been done with the use of fixed-location pumps or personal air samplers. Our objective was to find out whether ionizers could be good tools for collecting airborne allergens. As a model we have used cat allergen (Fel d 1). We have compared Fel d 1 levels collected by the ionizer at different time periods, as well as comparing Fel d 1 levels obtained with the ionizer with those of low- and high-volume pumps.

Methods: Dust samples from floors and air samples collected with ionizers and pumps, obtained in 31 homes with cat, 23 homes without cats, and 28 day-care centres, were analysed for cat allergen content (Fel d 1) by ELISA.

Results: Fel d 1 was present in the reservoir in all homes with cats, ranging from 660 to 375 000 ng/g (GM 75 000) and in the air collected by the ionizer from 2.0 to 204 ng/24 h (GM 19.3). The allergen in homes without cat varied from <55 to 1800 ng/g (GM 166). Corresponding levels in air were found in two of these homes (2.3 and 7.3 ng/24 h). There was a correlation between the number of cats and the amount of airborne cat allergen ($r: 0.47$; $P < 0.05$). The levels in day-care centres were <55 to 3070 ng/g in dust (GM 360) and <1.1 to 7.9 ng/24 h in the air (GM 1.6). We obtained a moderately strong correlation between air and dust samples in homes with cats ($r_s: 0.64$; $P < 0.001$) and in day-care centres ($r_s: 0.49$; $P < 0.05$). We found that a collection period of 24 h is preferable for the ionizer. The intrahome reliability coefficient was nearly two times higher for the ionizer ($r: 0.69$) than the pump ($r: 0.39$).

Conclusions: The ionizer seems to be a good tool for monitoring the environment. It is easy to use and silent and does not disturb the airflow in the room.

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Many studies have shown that exposure to indoor allergens is an important cause of sensitization and development of asthma (1–3), and a relationship between exposure to these allergens and asthma should be considered (4). Cat allergen is an important indoor allergen, especially in Swedish homes (5, 6). This allergen occurs not only in homes but also in environments where pets are not kept such as schools and day-care centres (7–9), presumably owing to the indirect transfer of pet allergens.

Measuring airborne allergens is necessary to assess the relationship between airborne allergen exposure, sensitization, and symptoms. Many studies have measured pet allergen concentration in dust samples from mattresses and floors, but other surfaces have also been investigated such as sofas, curtains, and tables and chairs in schools (10). Risk levels for allergen sensitization have been proposed (4), and they are based on the amount of allergen found in settled dust. However, few studies have focused on the concentration of airborne allergens. Allergens are present in low quantities in the air compared to settled dust, where levels can be 100–1000 times higher. The amount of airborne allergen

has been shown to increase after disturbance, and the allergen remains in the air for extended periods of time (11, 12). The major allergens of cat (Fel d 1) and dog (Can f 1) are primarily carried by particles with an aerodynamic diameter greater than 10 μm (13, 14). However, up to 40% of particles carrying these allergens are less than 5 μm , giving them a relatively long settling time (11, 14), and making it possible to measure these allergens even in the absence of disturbance. The fact that individuals allergic to cats develop symptoms rapidly on entering a house with a cat suggests that the allergen must be continuously airborne. Conversely, subjects allergic to house-dust mites rarely react rapidly in a mite-infested house unless the dust is disturbed.

Airborne allergens have hitherto been measured with either fixed-location pumps or personal air samplers (12, 15). A disadvantage of pump/samplers is that they are noisy, while personal air samplers are inconvenient to wear. Ionizers, which (according to the manufacturers) clean the air by removing particles, have so far not been used for collecting airborne allergens for diagnostic purposes. Ionizers function by means of electrical attraction: first they load particles in the air

with a negative charge, and then they attract the charged particles to a positively charged collector plate. They make no noise and are easy to handle.

In this study, we investigated the efficiency of a modified ionizer for collecting airborne allergens. We used collection of the cat allergen, Fel d 1, as a model and compared the results from the ionizer with those obtained with low- and high-volume stationary pumps. We studied the amount of allergen found in settled dust in homes with and without cat and in day-care centres. The aim was to find out whether ionizers are a good tool for collecting airborne allergens in order to monitor the environment.

Material and methods

Ionizers

The ionizers used in this study were manufactured by Airpoint AB, Stockholm, Sweden. The device was modified in order to capture allergens by installing a metal cup with a conductive surface of 47 mm in diameter (GP plastindustri, Gislaved, Sweden) as the collector plate. The ionizer loads the particles in the air with a negative charge, making them adhere to the positively charged collector plate. The ionizer was placed about 1 m above the floor in the middle of each sampling site (living room or playroom). A sampling metal cup was fitted to the ionizer. After the end of the sampling period, the ionizer was turned off and the metal cup was covered with a lid and stored at -20°C until extraction.

Pumps

Two different stationary pumps were used. One pump sampled air with a constant flow rate of 2 l/min (mainly Alitea AB, Stockholm Sweden). The particles were collected with plastic IOM inhalable dust samplers (SKC Ltd, Blandford, UK), equipped with 25-mm polytetrafluoroethylene filters (pore size of $1.0\ \mu\text{m}$) (Millipore AB, Sundbyberg, Sweden). Henceforth this pump will be referred to as the "low-volume pump".

The other pump (Alitea AB, Stockholm, Sweden) sampled air with a constant flow rate of 17 l/min. The particles were collected onto 37-mm glass fibre filters (Whatman International Ltd, Maidstone, UK) placed in a clear polystyrene filter blank cassette (pore size of $0.3\ \mu\text{m}$) (SKC Ltd, Blandford, UK). This pump will henceforth be referred to as the "high-volume pump".

The pumps were placed about 1 m above the floor in the middle of each sampling site. After the end of the sampling period, the pump was turned off and the filters were placed into Minisorp[®] vials (Nunc A/S, Roskilde, Denmark) and stored at -20°C until extraction.

Experimental design

Our first aim was to determine whether the ionizer could collect airborne Fel d 1, and whether there was an association between the levels of Fel d 1 obtained in the air and in the reservoir. Fifty-four homes (31 homes with cat and 23 without) and 28 day-care centres were included in the study. The Fel d 1 levels in the air were sampled over a 24-h period. Dust samples were collected under normal conditions from the carpet/floor in the living room in the homes and from the floor in the playroom in the day-care centres while normal activities took place. These samples were obtained after the air sampling.

In the second part of the study, the amount of Fel d 1 sampled with the ionizer was compared during sampling periods of 2, 12, 24, and 48 h. Twelve homes with cat were included.

In the third part of the study, the efficiency of the ionizer at sampling airborne Fel d 1 levels was compared with those of a low- and a high-volume pump during a 2-h collection time. The ionizer and the low-volume pump were placed in 11 homes with cat, and in six of those 11 homes the high-volume pump was also placed. Each device was used on a separate day with one day of rest in between, and the sampling scheme started with the ionizer. The samples were collected from the living room. Normal domestic activities were taking place during the sampling periods, although dusting and vacuum cleaning were avoided. At the end of the whole sampling period, a sample of settled dust was collected from the living-room floor.

The reliability of the measurements was investigated by repeatedly collecting air samples on five separate occasions in 10 homes with cat and comparing the data obtained with the ionizer and with the low-volume pump. All the samples were collected during a 12-h period, starting with the ionizer and thereafter according to the same procedures as described above.

Dust sampling

Floor dust was obtained by instructing the subjects to use their own vacuum cleaner with a new dust bag fitted. A separate filter bag (Allergen Control plc, USA/Medeca AB, Uppsala, Sweden) used for the collection of dust samples was inserted into the inlet tube of the vacuum cleaner to collect the dust. In homes, an area of $1\ \text{m}^2$ and in day-care centres an area of $5\ \text{m}^2$ was vacuum-cleaned for 4 min. After collection, the filter bag was sealed in a plastic bag and stored at -20°C until extraction.

Extraction of dust and air samples

Dust samples were weighed, and a total of 100 mg dust was extracted (1/20 w/v) in phosphate-buffered saline containing 0.1% Tween 20 by end-over-end mixing

overnight at 4°C. The samples were then centrifuged at 3500 rpm for 15 min. The supernatants were transferred to new test tubes and centrifuged again at 10 000 rpm for 5 min. The supernatants were finally stored at -20°C until analysed for the content of allergen.

Before extraction of the air samples from the ionizer, the metal cup was first allowed to adjust to room temperature for about 5–10 min. The metal cup was then washed with 1 ml of phosphate-buffered saline containing 0.05% Tween 20 and 0.2% BSA (bovine serum albumin, Labora AB, Sollentuna, Sweden). The solution was transferred to a tube and stored at -20°C until analysed. The extraction of allergens from pump filters was done in Minisorp[®] vials with 2 ml PBS and end-over-end mixing overnight at +4°C. Extracts were then centrifuged (10 min at 10 000 rpm) and stored at -20°C.

Measurement of allergen concentration by ELISA

The level of Fel d 1 was determined by a (two-site) sandwich ELISA using monoclonal antibodies. The assay was performed according to the protocols from the manufacturer (INDOOR Biotechnologies Ltd, Charlottesville VA, USA; Professor Martin Chapman) with the exception that peroxidase-labelled conjugates were replaced with alkaline phosphatase-labelled (ALP) streptavidine (DAKO, Denmark). The dust samples were diluted in assay buffer at least 2.5 times, and the air samples were assayed undiluted. All samples were analysed in duplicates and reanalysed if they differed by more than 10%. Allergen concentrations in the reservoir were expressed as ng/g of dust. The allergen levels in the air were expressed as ng calculated as the total amount of allergen recovered during 2, 12, and 24 h. The aeroallergen levels collected with the pumps were also calculated as ng/m³ of air, taking into account the total amount of allergen recovered during the whole collection period, and the flow rate of the sampling device.

Dust samples with Fel d 1 levels below the detection limit (55 ng/g) were assigned a value of 54 ng/g, and air samples below the detection limit (1.1 ng/ml) were assigned 1.0 ng/ml for statistical analysis.

Statistical methods

Allergen levels are expressed as geometric mean (GM) and range. The Spearman rank correlation (r_s) was used to calculate the correlation between concentrations in dust and air samples, and between concentrations in air samples and number of cats. To determine the levels of significance between groups, the Mann-Whitney U test was used. P values of <0.05 were considered statistically significant.

ANOVA repeated measurement was used in order to test for differences over time within households. Intra-

home reliability coefficient (intra-class correlation coefficient [ICC]) was used to estimate the agreement between repeated measurements over time. The relevant interval for reliability coefficient is (r) $0 \leq r \leq 1$. The value 0 is to be interpreted as no reliability and 1 as perfect reliability.

The Wilcoxon matched pairs test was used to test whether the capability to detect allergen was equal for the two instruments.

Results

Reservoir and airborne Fel d 1 levels in homes and day-care centres

Fel d 1 was present in the reservoir in all homes with cat ($n=31$) ranging from 660 to 375 000 ng Fel d 1/g of dust with a GM of 78 000 ng/g. The corresponding airborne levels collected with the ionizer were 2.0–204 ng/24 h (GM 17 ng). The Fel d 1 levels obtained in the reservoir in homes without cat ($n=23$) ranged from <55 to 1800 ng/g of dust with a GM of 170 ng/g (Fig. 1). Measurable levels in the air were found in only two of these homes, and there the values were 2.3 and 7.3 ng/24 h, respectively. The Fel d 1 levels were significantly higher in dust in homes with cat than homes without cat ($P<0.001$). There was a moderate correlation between the number of cats kept in the household and the amount of Fel d 1 collected in the air by the ionizer (r_s : 0.47; $P<0.05$) (Fig. 2). However, we could not find any association between the number of cats in the household and Fel d 1 levels in settled dust.

The levels detected in the 28 day-care centres were <55 to 3070 ng Fel d 1/g of dust (GM 360 ng/g) and <1.1 to 7.9 ng/24 h in the air (GM 1.6 ng) (Fig. 1). The cat allergen levels were significantly higher in day-care centres than homes without cats ($P<0.05$).

Association between Fel d 1 levels sampled in the reservoir and in the air

The levels of Fel d 1 found in dust samples were much higher, as expected, than those collected in the air by the ionizers. In order to investigate the relationship between levels of Fel d 1 measured in air during 24 h and in settled dust samples, we calculated the correlation between these samples in homes with cat and in day-care centres. We obtained a moderately strong correlation in homes with cat ($n=28$, r_s : 0.64, $P<0.001$) and between samples collected in day-care centres ($n=28$, r_s : 0.49, $P<0.05$).

Comparison between 2-, 12-, 24- and 48-h collection times

The levels of Fel d 1 collected by the ionizer during 2, 12, 24 and 48 h showed an increase with time (Fig. 3). The results demonstrated that a collection time of at

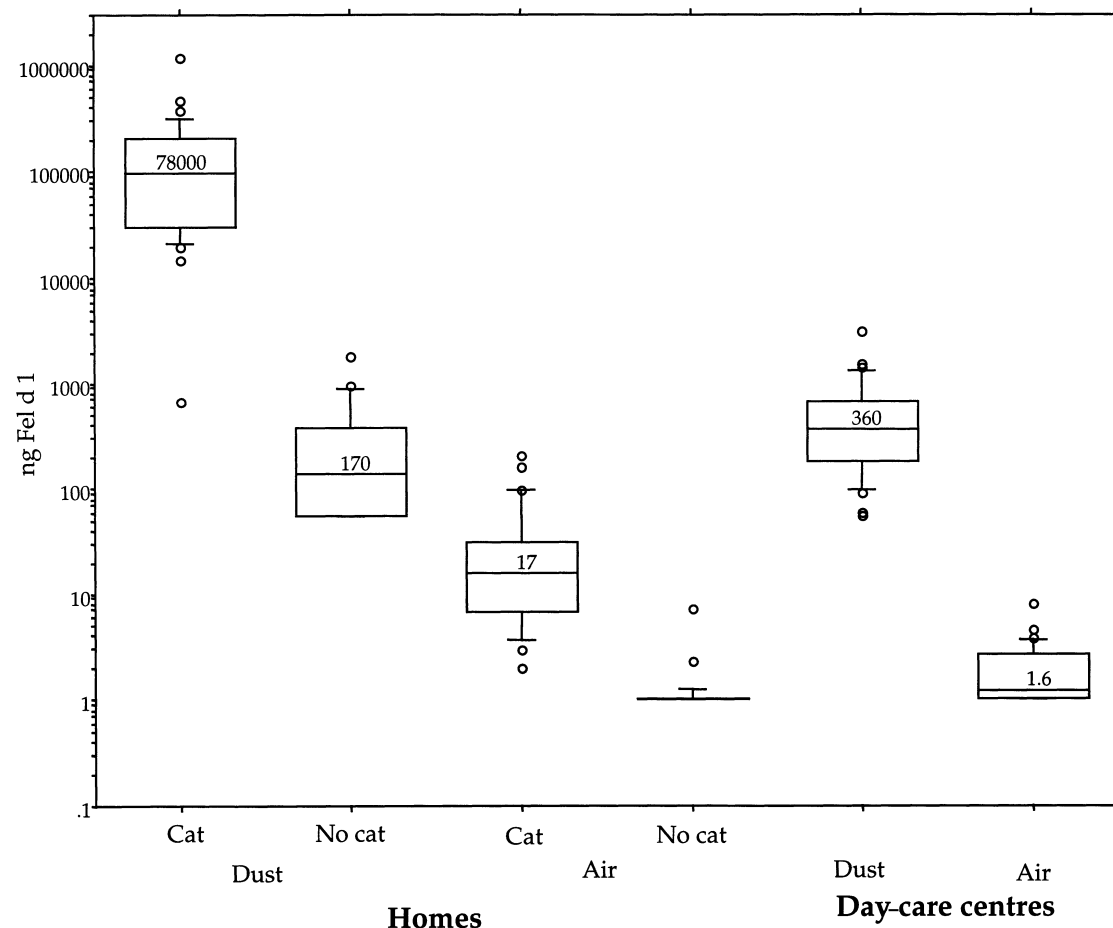


Figure 1. Concentrations of cat allergen (Fel d 1) in dust and air samples from homes with and without cat and day-care centres. Fifty per cent of each group is presented within boxes, and numbers given are GM.

least 24 h is preferable, even though there was a slight increase in allergen concentrations beyond 24 h (Fig. 3).

Comparison between the ionizer and the low- and high-volume pump

The efficiency of the ionizer at collecting airborne Fel d 1 was compared with that of the low- and high-volume pumps for 2 h. The ionizer and the low-volume pump were used in 11 homes with cat, and in six of these homes the high-volume pump was also included. Detectable levels of Fel d 1 (2.1–58.6 ng Fel d 1) could be found with the ionizer in all 11 homes, but only in one home with the low-volume pump (8.4 ng Fel d 1 or 35.3 ng/m³) (Table 1). However, when the high-volume pump was used, detectable levels of Fel d 1 were obtained in 4/6 homes, and the concentrations were in the same range as the levels collected by the ionizer. Fel d 1 levels in dust were found in all 11 homes with cats in the range 8–7300 ng/g of dust. There was a significant association between Fel d 1 levels obtained in dust and in air collected by the ionizer ($n=11$, r_s : 0.78, $P<0.05$).

Reliability of air samples

In 10 homes with cat, the ionizer and the low-volume pump were used for 12 h on five separate occasions. In all homes, detectable levels of Fel d 1 were obtained with the ionizer and in five homes with the low-volume pump. Fel d 1 levels obtained with the ionizer were significantly higher than the levels obtained with the low-volume pump ($P<0.05$) (Fig. 4). We could not detect any systematic difference over the time period within the households. The reliability coefficient within homes was calculated to be nearly two times higher for the ionizer than the pump, r : 0.69 and r : 0.39, respectively.

Discussion

The purpose of this study was to test an ionizer as a new tool for quantifying airborne allergens. Since allergens are present in the air in small amounts, it is important to develop a sensitive sampling method together with a suitable quantification method. According to its manufacturer (Airpoint AB), the ionizer can collect particles over a large size range even down to $<0.5 \mu\text{m}$.

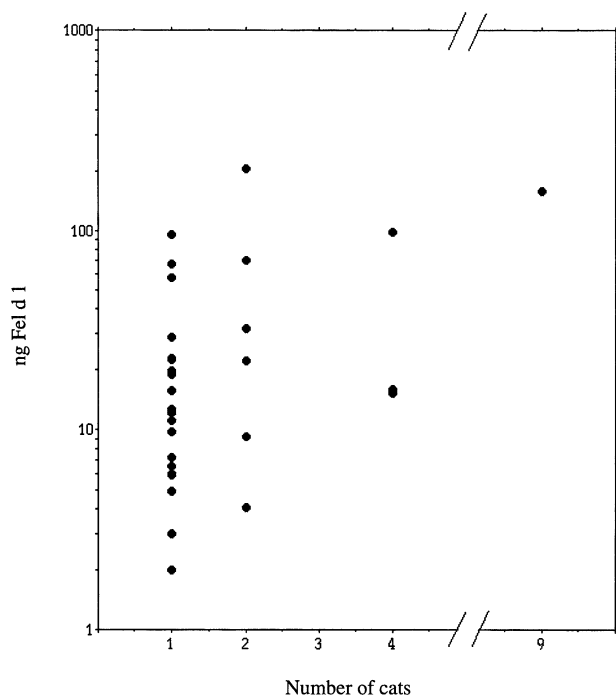


Figure 2. Cat allergen (Fel d 1) levels in relation to number of cats in home.

Warner et al. have recently used ionizers in an attempt to rid the air of particles in order to reduce clinical symptoms in the patients (15), but not for collection and quantification of allergens. They studied homes with mite-allergic children and reported that the airborne mite allergen concentrations in homes were reduced significantly by the use of ionizers, although they did not find a corresponding improvement in symptoms. They concluded that the ionizers may be useful as part of an allergen-avoidance regimen.

With the ionizer, we could detect airborne Fel d 1 in all homes with cats. Airborne cat allergen was also observed in 14 out of 28 day-care centres, although the levels were lower than those in homes with cat. Furthermore, detectable airborne allergen levels were also found in a few homes without cat. This is in

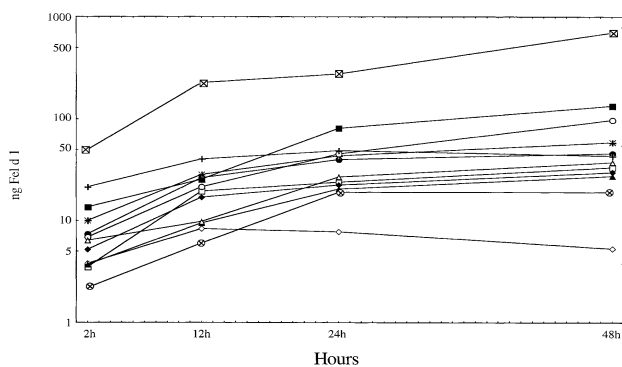


Figure 3. Cat allergen (Fel d 1) levels collected for 2, 12, 24, and 48 h in 12 cat homes.

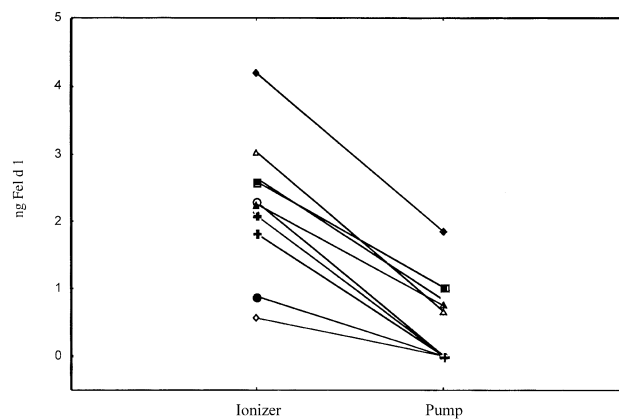


Figure 4. Difference in GM values of cat allergen levels measured with ionizers and pumps in 10 homes with cat.

agreement with other studies suggesting that the allergen is passively transported to these sites by occasional visits by cat owners, children, and staff (4, 16, 17). Thus, cat allergen is widespread in the community.

Furthermore, we were also able to demonstrate a moderate relationship between the number of cats and the Fel d 1 levels in air, but not in settled dust samples. We also observed an association between the levels of Fel d 1 detected in the reservoir with those measured by the ionizer.

When we compared the efficiency of the ionizer to collect Fel d 1 over periods of 2, 12, 24, and 48 h, we observed an increase in amount of allergen over time. The results showed that the collection period of 24 h is preferable to give an accurate estimation of the allergen level at a site. The advantage of sampling for 48 h compared to 24 h was not so great, since the greatest increase in allergen concentration took place between 0 and 24 h. This, together with the fact that a 24-h period is easier to handle, speaks in favour of a 24-h sampling period.

Comparison between the efficiency of the ionizer and low- and high-volume stationary pumps at collecting airborne Fel d 1 was done in homes with cat. We were able to measure airborne Fel d 1 levels in all 11 homes with the ionizer, but only in one home with the low-volume pump. This home in fact had the highest levels of Fel d 1 in its reservoir. When the high-volume pump was used, detectable Fel d 1 levels were found only in four out of six homes. These results indicate that the ionizer is as good as the high-volume pump for sampling aeroallergens. In comparison, the pumps are harder to handle and also too noisy to operate for a longer time period, especially the high-volume pump. Furthermore, the high-volume pump also disturbs the air in the room, contributing to the activity in the room while sampling (18). This could give rise to increased

Table 1. Fel d 1 levels in air (ng/2 h) and dust samples in 11 homes with cats

		Homes										
		I	II	III	IV	V	VI	VII	VII	IX	X	XI
Air	Ionizer	19.8	10.0	9.4	7.0	3.9	58.6	5.5	6.8	2.1	2.2	7.8
	Low-volume pump	8.4	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	High-volume pump	35.3*										
Dust		25	7.4	<1.0	13.2	<1.0	42	ND	ND	ND	ND	ND
	Carpet/floor†	12.5*	3.7*		6.6*		21*					
		7300	180	71	80	135	744	97	69	8	50	197

ND: not done.

*ng/m³.

†µg/g.

levels in the air samples collected by the air pumps compared to what can be expected with an ionizer. In a recent study by Custovic et al. (19), the levels of mite, cat, and dog allergens were studied in both the reservoir and in the ambient air in 127 homes. The airborne allergens were collected with a high-volume pump with a flow rate of 60 l/min for 1 h, with a total volume collection of 3.6 m³. In our study, we used a pump with a flow rate of 17 l/min for 2 h with a total volume collection of 2.04 m³. The Fel d 1 levels detected in the air by Custovic et al. ranged from 0.4 to 22.3 ng/m³ in homes with cat, a finding which was similar to the 3.7–21 ng/m³ obtained in this study.

Levels of allergens collected by pumps are often below the detection limit of the conventional ELISA. Several studies have therefore used an amplified ELISA to measure the amount of the allergen in the air (20, 21). The increased sensitivity makes these methods more difficult to use. However, collection with the ionizer resulted in a more concentrated sample, which was possible to quantify with an ordinary ELISA.

A drawback of the ionizer compared to the pumps is that the amount of allergen collected cannot be

calculated as ng/m³ of air, but must be given as a total amount per sampling period. However, when we compared the total amount of allergen collected by the ionizer and the high-volume pump during the same collection time (Table 1), the results were of the same magnitude, showing that the ionizer is as good as a high-volume pump in sampling aeroallergens.

In summary, the ionizer seems to be an efficient tool for collecting airborne allergens. It is silent, relatively small, and easy to use, and it does not disturb the airflow in the room. This new method of collecting airborne allergenic particles will be further evaluated in clinical studies to investigate whether airborne allergen concentrations correlate better with symptoms of allergy than levels of allergen in the reservoir.

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