

Brief communications

Measurement of cat allergen levels in the home by use of an amplified ELISA

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We previously reported that Fel d 1 was measurable in 10 of 40 air samples and 38 of 40 dust samples obtained from homes without cats.¹ We have reanalyzed the samples from 39 of these homes using a recently described amplified ELISA for Fel d 1.² This modification uses a signal amplification by catalyzed reporter deposition originally described by Bobrow et al.³ Because assay sensitivity is greatly increased, we reexamined the relationship of settled dust and airborne levels in samples that we had collected previously.

METHODS

Airborne and settled dust samples were collected from 1993 to 1994 and analyzed previously¹ for Fel d 1 with a two-site monoclonal ELISA.⁴ Airborne samples were collected on a glass fiber filter with a portable pump run at flow rates of 3 to 4 L/min.¹ Extracts were stored at -30°C until reanalysis with a monoclonal antibody-based ELISA⁴ and an amplified assay based on the catalyzed reporter deposition concept³ adapted for the monoclonal antibody-based ELISA.² Briefly, Immulon II microplates (Dynatech Lab Inc., Chantilly, Va.) were coated with anti-Fel d 1 monoclonal antibody 6F9 (Indoor Biotechnologies, Manchester, U.K.) and blocked with bovine serum albumin. Dilutions of cat allergen standard or unknown sample were incubated for 1 hour at 25°C . The plate was washed, then incubated for 1 hour at 25°C with anti-Fel d 1 monoclonal antibody 3E4, which had been coupled to horseradish peroxidase (Sigma Chemical Co., St. Louis, Mo.) with a periodate method.³ After a second wash, the plate was incubated with biotin-tyramine synthesized³ by mixing tyramine and N-biotin-4-hydroxybenzohydrazine (Sigma Chemical Co.). For amplification, 0.3 $\mu\text{L}/\text{mL}$ biotin-tyramine in 50 mmol/L Tris-HCl with 0.01% H_2O_2 (pH 8.0) was added, incubated for 15 minutes at 25°C , and washed. One hundred microliters per well of 0.25 mg/mL streptavidin-horseradish peroxidase 1:2000 was added and incubated for 15 minutes at 25°C . After washing the plate, 100 $\mu\text{L}/\text{well}$ 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-sulfonic acid) in 70 mmol/L citric acid-phosphate buffer

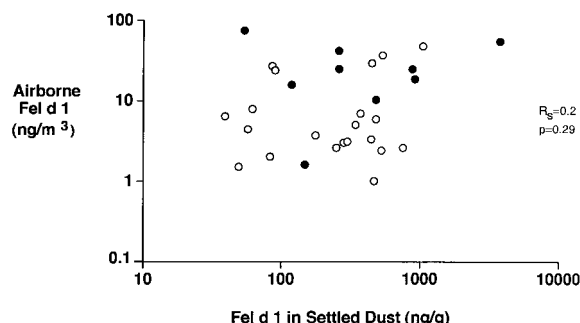


FIG. 1. Levels of Fel d 1 in paired air and dust samples collected from 30 homes without cats ($R_s = 0.2$, $p = 0.29$). Filled circles represent samples that had measurable Fel d 1 as determined by conventional ELISA. Of note is one value that was obtained on an unusual run in which the conventional assay was optimal and unusually sensitive. The majority of measurements in this range with previous ELISA had coefficients of variation that were too high ($>10\%$) or optical densities that were not twice blank value.

(pH 4.2) was added and developed for 8 to 10 minutes at 25°C . The reaction was stopped with 2 mmol/L NaN_3 and read at 405 to 490 nm. The detection limit was 156 pg of Fel d 1/mL.

Spearman's correlation coefficient was used to compare paired samples.

RESULTS

In our previous report, 30 of 40 homes without cats had undetectable levels of airborne Fel d 1. Nine of ten samples with initially measurable airborne Fel d 1 were available for reanalysis with the amplified assay. These results correlated well with original values ($R_s = 0.92$, $p = 0.00013$).

Air samples from all 30 homes with previously undetectable Fel d 1 were available for reanalysis. As seen in Fig. 1, by using the amplified assay, Fel d 1 was measurable in all 30 samples. Our previous report, which used the standard ELISA Fel d 1 assay, found that homes with detectable airborne Fel d 1 levels tended to have higher settled dust levels, although this correlation was not statistically significant ($R_s = 0.19$, $p = 0.15$). Even with all 30 airborne samples having measurable concentrations, the airborne levels still did not correlate significantly with settled dust levels ($R_s = 0.2$, $p = 0.29$).

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DISCUSSION

The amplified ELISA assay results correlate well with the standard ELISA results reported previously from our laboratory. Previously it has been necessary to use high flow samplers or long sampling times to measure airborne Fel d 1 concentrations in the range reported here.⁵ The amplified assay might allow measurements with very short sampling periods to better describe dose-response relationships, and may also be applied to measure airborne concentrations of allergens such as cockroach or dust mite that are airborne at very low concentrations.

Our finding that Fel d 1 allergen is measurable in all samples from homes without cats confirms the ubiquitous nature of cat allergen and may help to explain the high sensitization rate among people who do not live with cats.⁶ The poor correlation between air and dust samples supports the finding by others⁷ that settled dust levels may not provide an accurate estimate of airborne exposure. The practical importance of this more sensitive assay is that we can now measure airborne Fel d 1 concentrations with sampling techniques that involve lower flows, portable equipment, and shorter sampling times. Although the clinical relevance of low airborne Fel d 1 levels is yet to be determined, the amplified assay

provides a useful technique to examine the question experimentally.

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