

Assay for the major dog allergen, *Can f I*: Investigation of house dust samples and commercial dog extracts

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Monospecific rabbit antibodies were used to develop a sensitive two-site enzyme immunoassay to measure a major dog hair and dander allergen, Can f I. This Can f I assay demonstrated no reaction with 17 heterologous allergen sources, including dog albumin, cat, guinea pig, and horse. Analysis of serial dilutions of purified Can f I and the international standard for dog was parallel. The assay was considered specific for Can f I with a lower limit of detection at 0.03 µg/ml. Total imprecision was from 2% to 6%. Commercial dog extracts for specific immunotherapy contained from 0.7 to 290 µg of Can f I per milliliter. The assay was used to measure Can f I in 136 house dust samples collected from 103 homes across the United States. Concentration of the dog allergen was expressed as micrograms of Can f I per gram of dust. Prevalence of Can f I in the dust samples ranged from <0.3 to 10,000 µg/gm. Serial dilutions of samples containing Can f I were parallel to the standard. The median Can f I value for homes with a dog in residence was 120 µg/gm, and for homes with no dog, 3 µg/gm. With few exceptions, homes with no dog in residence had <10 µg/gm. This Can f I assay will provide useful information for assessing commercial extracts as well as monitoring dog-allergen exposure and allergen-control methods. (J ALLERGY CLIN IMMUNOL 1991;88:847-53.)

Key words: Environmental allergen, aeroallergen, dog hair and dander, ELISA, rabbit antibody

Dogs are among the most popular companion animals in the world. However, dogs are an important source of allergen in the environment. Dog antigen has been observed in more than half the homes sampled in Baltimore, Md.¹ The clinical importance of dog allergy has been demonstrated by several investigators; positive SPT has been found in 14% of unselected adolescents in Finland,² and 40% of children with asthma demonstrated a positive SPT to dog.³

Recently, we purified the major allergen in DHD, *Can f I*, and verified its importance as a sensitizing agent.⁴ It has, however, hitherto not been possible to determine the relationship between dog-allergen exposure and development of allergic disease. Clinically relevant threshold limits for environmental house dust mite-allergen exposure have been suggested.⁵ The *Can f I*-specific ELISA assay presented in this article has primarily been developed as a tool for the

Abbreviations used

<i>Can f I</i> :	Major dog allergen
CIE:	Crossed immunoelectrophoresis
DHD:	Dog hair and dander extract
SPT:	Skin prick test
Ab:	Antibody
HRP:	Horseradish peroxidase
PBS:	Phosphate-buffered saline
CV _{total} :	Total imprecision
IT:	Immunotherapy
BSA:	Bovine serum albumin

measurement of exposure to dog allergen in the environment of allergic patients.

MATERIAL AND METHODS

Antigen preparations and allergen extracts

The following preparations were used in the present study: dog albumin (a kind gift from Dr. J. Ohman, Boston, Mass.); extract of cat hair and dander (raw material from Hässle AB, Göteborg, Sweden); extracts of horse, guinea pig, storage mite (*Acarus siro*), short ragweed (*Ambrosia artemisiifolia*), Bermuda grass (*Cynodon dactylon*), and fungi (*Aspergillus fumigatus* and *Mucor racemosus*) were from ALK, Hørsholm, Denmark; extracts of American

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Received for publication April 16, 1991.

Revised June 20, 1991.

Accepted for publication June 26, 1991.

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cockroach (*Periplaneta americana*), German cockroach (*Blattella germanica*), cricket (*Gryllus domesticus*), and dry-wood termite (*Cryptotermes brevis*) were produced in our laboratory⁶; standardized extracts of DHD, house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), birch pollen (*Betula verrucosa*), and timothy grass (*Phleum pratense*) were Aquagen SQ from ALK. Six commercially available dog extracts were obtained from the manufacturers. The international standard for dog was obtained from National Institute of Biological Standards and Controls, London, England.⁷

Reference allergen

Purified major allergen, *Can f* I, was used as a reference allergen and was purified as previously described.⁴

House dust samples

One hundred thirty-six dust samples were collected between September 1989 and March 1990 from 103 homes across the United States. The allergist of the individual household recommended the site of sampling. Dust sampling was performed by the occupant after careful instructions. The specified site was vacuumed for 5 minutes. The dust samples were collected on a filter (38 cm²; pore size, 6 μ m) in a special hose-nozzle sampling device (ALK Laboratories, Inc., Milford, Conn.) that connects to the hose attachment of most vacuum cleaners. After the vacuuming, the filter disk was removed from the sampling device and covered, and then both the filter and device were returned to the laboratory by mail. A member of each household was asked to provide information on whether or not they owned a dog and whether the dog had access to the room from which the dust sample was collected. Completed questionnaires were received from 99 households.

Extraction of dust samples

Aqueous extracts were prepared by diluting a measured amount of fine dust (separated by mechanical sieving through 0.5 mm screens) from each sample, 1:10 wt/vol, with 0.125 mol/L of ammonium bicarbonate buffer. A minimum of 200 mg of dust was extracted by agitation on a wrist-action shaker for 2 hours at room temperature. The suspension was then centrifuged for 15 minutes at 1000 g. Dust extracts were stored at -20° C until *Can f* I analysis.

Can f I concentration

Content of allergen *Can f* I was expressed as micrograms of dry weight of *Can f* I. The protein content of purified *Can f* I was 87.5%, as determined according to the method of Lowry et al.⁸ with BSA as the reference protein.

Reagent Abs

Rabbit Ab to DHD was prepared by immunizing rabbits with DHD with an immunization schedule as described by Harboe and Ingild.⁹ Oligospecific rabbit Ab to *Can f* I was prepared by immunizing rabbits with partially purified *Can f* I. This Ab was used as a capture layer in the *Can f* I ELISA assay. For the detection layer in the *Can f* I ELISA assay, we used rabbit Ab monospecific for *Can f* I. This Ab was prepared by means of successive

immunoabsorption steps as described elsewhere.¹⁰ Yield of the procedure was 7 mg of dry weight of monospecific Ab from 15 ml of oligospecific rabbit serum to *Can f* I.

Aliquots of 1 mg of the monospecific Ab were conjugated with 1000 U of HRP, (260 U/mg, P8375, Sigma Chemical Co., St. Louis, Mo.), and possible remaining reactive groups were blocked by the addition of 0.2 mol/L of lysine. The HRP-Ab preparation was stored at -20° C with 15 mmol/L of sodium azide.

Assay procedure

Polystyrene microtiter trays (96-well Immunoplate, NUNC, Roskilde, Denmark) were coated overnight at 20° C with rabbit anti-*Can f* I diluted 1000 times in 0.1 mol/L of sodium carbonate buffer, pH 9.6 (100 μ l per well). After trays were coated, they were thoroughly washed in PBS, pH 7.4, 0.05% wt/vol of Tween 20, and duplicate dilution series of samples were incubated overnight at room temperature in PBS, 0.5% wt/vol of BSA. Incubation overnight was at 20° C. Each tray included dilution series of reference allergen. After trays were again thoroughly washed, the bound allergen was revealed by incubation for 1 hour at room temperature with HRP-conjugated monospecific anti-*Can f* I diluted 1000 times in PBS, 0.5% wt/vol of BSA. After a wash, substrate (3 mmol/L of hydrogen peroxide and 5 mmol/L of O-phenylenediamine in 0.1 mol/L of citrate-phosphate buffer, pH 5.0), prepared immediately before use, was added, and reaction occurred for 15 minutes at room temperature in darkness. After this procedure, 1 mol/L of sulfuric acid was added. The absorbance at 490 nm was read on a Teknunc Immunoreader, NJ-2000, Nippon InterMed, Tokyo, Japan.

Assay precision

CV_{total} was calculated according to the method described by Krouwer and Rabinowitz.¹¹ CV_{total} was calculated for samples run in duplicate in five individual experiments.

Electrophoresis

CIE was performed according to the method described by Weeke et al.¹²

RESULTS

Assay specificity

The specificity of the assay was tested by analyzing high concentrations of extracts from allergen sources likely to occur in the indoor dust samples. No significant reaction was found with any of the other extracts, even though they were tested at concentrations up to 1000 μ g/ml. The concentrations of allergen from cat and house dust mite likely to occur in dust samples will, only in extreme cases, reach this level.^{10, 13}

Measurements on samples containing *Can f* I

Serial dilutions of purified *Can f* I and DHD and the international standard for dog were run with duplicate determinations in five individual experiments (Fig. 1). The three curves were parallel ($r = 0.990$,

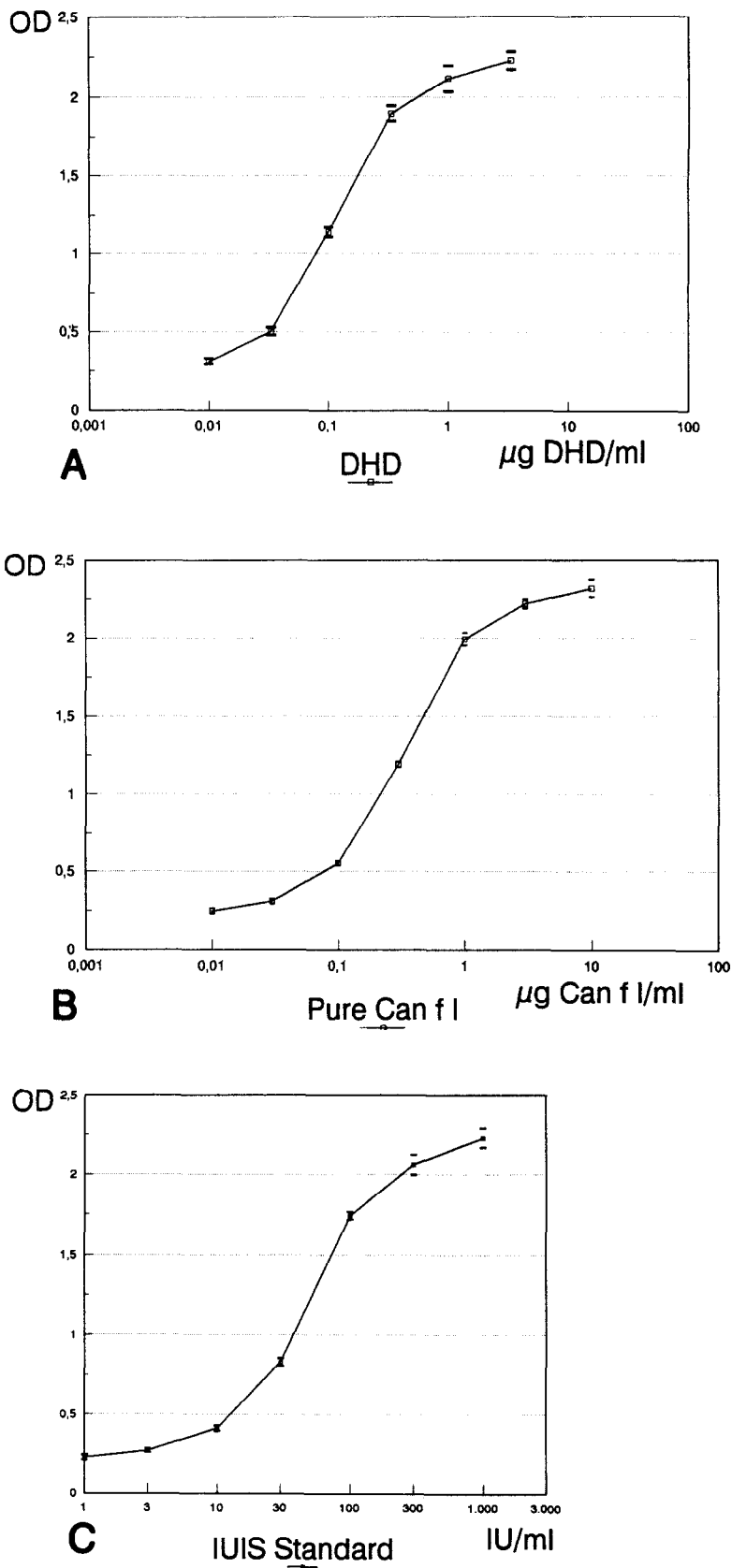


FIG. 1. A, Dilution curves of DHD. B, Purified *Can f I*. C, International standard for dog. Curves represent duplicate determinations of five individual experiments $\pm$ standard deviation; x axis for DHD is micrograms of DHD per milliliter; *Can f I* is micrograms of *Can f I* per milliliter; x axis for the international standard is international units per milliliter; approximately 30% of the DHD illustrated is *Can f I*.

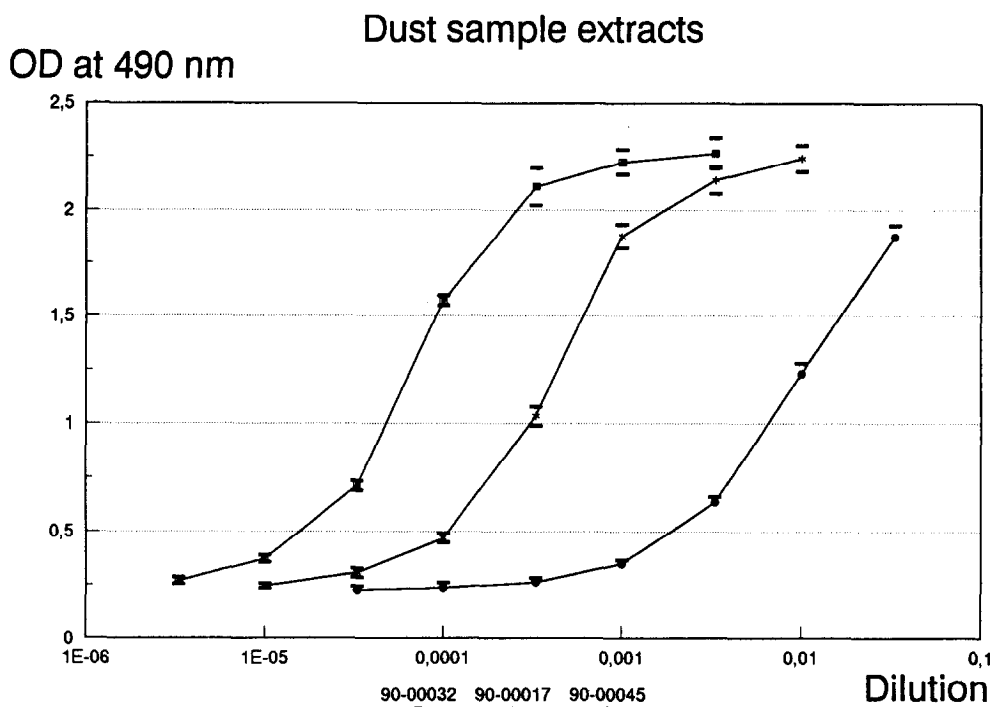


FIG. 2. Dilution curves of house dust-sample extracts. Curves represent duplicate determinations of five individual experiments $\pm$ standard deviation. Sample No. 90-00032 contained 10,000 μg of *Can f* I/gm; sample No. 90-00017 contained 1450 μg of *Can f* I/gm; sample No. 90-00045 contained 58 μg of *Can f* I/gm.

TABLE I. American dust samples

	Median	Geometric mean	Range
Homes			
With dog (N = 56)	120	158	10-10,000
Without dog (N = 43)	3	3	<0.3-23

Values are micrograms of *Can f* I per gram of dust.

0.996, and 0.965), and it was thus decided, for economical reasons, to use DHD as a standard curve for comparison with serial dilutions of dust-sample extracts. The lower limit of detection was set at the value of blanks plus 2 SD corresponding to 0.03 μg *Can f* I per milliliter. CV_{total} varied from 2% up to 6% at the lower detection limit. With the assay, the concentration of *Can f* I in the international standard was found to be 1.16 μg of dry weight of *Can f* I, corresponding to 1.02 μg Lowry protein per 100 IU.

One hundred thirty-six dust samples were obtained from 103 households scattered across the United States. The samples were extracted, and serial dilution

curves were compared to the standard curve. Curves produced by serial dilution of dust samples, differing approximately four orders of magnitude with respect to concentration of *Can f* I, are illustrated in Fig. 2; the curves ($r = 0.967$, 0.992 , and 0.977) were parallel to the standard curve ($r = 0.996$). In the house dust samples collected for this study, the concentration of *Can f* I varied from below detection, that is, <0.3 $\mu\text{g}/\text{gm}$ of dust to 10,000 $\mu\text{g}/\text{gm}$ of dust. The results are illustrated in Fig. 3. Ninety-nine of the 103 households examined completed a questionnaire about the presence of dog in the home. The median value of *Can f* I concentration in 56 homes with dog was 120 $\mu\text{g}/\text{gm}$ of dust and 3 $\mu\text{g}/\text{gm}$ of dust in 43 homes without dog (Table I). In 37 of 56 households with dog, the animal had access to the room in which the dust sample was taken; the median concentration in these samples was 160 μg of *Can f* I per gm of dust (range, 15 to 10,000 $\mu\text{g}/\text{gm}$). In the remaining 19 homes, the dog did not have access to the room from which the dust sample was collected; the median concentration here was 46 $\mu\text{g}/\text{gm}$ of dust (range, 10 to 440 $\mu\text{g}/\text{gm}$).

A few of the dust-sample extracts contained high enough concentrations of *Can f* I to be comparable in this respect with commercial DHDs. CIE of these dust

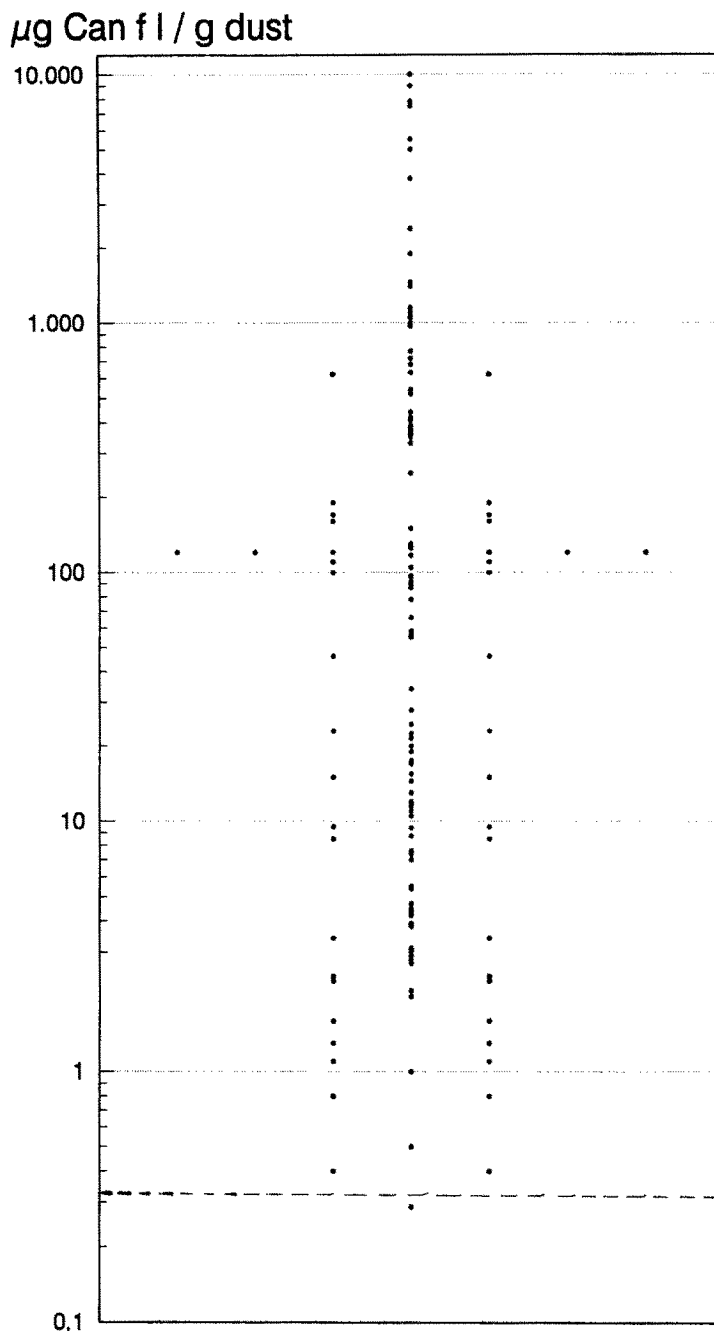


FIG. 3. Concentration of *Can f I* in extracts of 136 house dust samples from the United States. Each dot denotes a dust sample; dashed line denotes the lower limit of detection.

samples revealed that the composition of dog antigens was different from that in the international standard (Fig. 4). This finding indicates that some of the dog antigens are prone to degradation when they are exposed to environmental stress, whereas *Can f I* appears a relatively stable molecule.

Commercially available dog-allergen extracts were

obtained from six suppliers (Table II) and investigated for the concentration of *Can f I*. Four of the extracts were specific IT maintenance-dose extracts (one of which, C, was tyrosine absorbed), one was for oral IT, and one extract was for SPTs. The measurable concentration of *Can f I* varied approximately four orders of magnitude, as presented in Table II, with

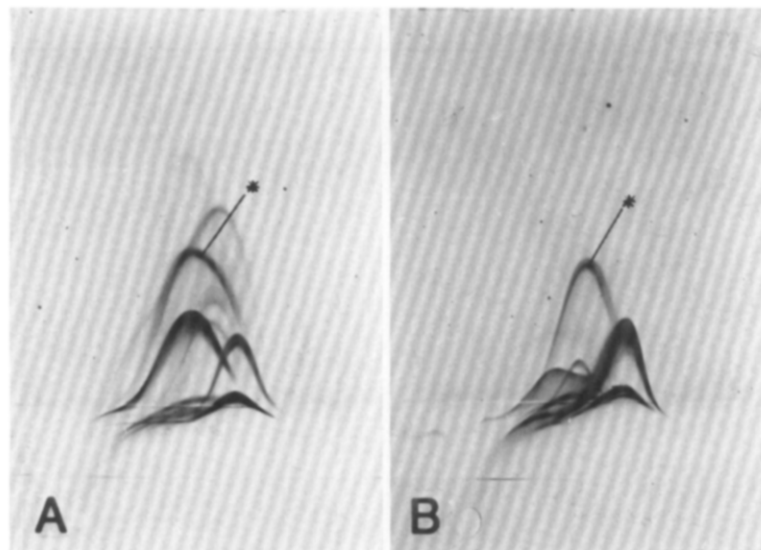


FIG. 4. A, CIE of the international standard for dog. B, House dust sample No. 90-00032 containing 10,000 µg of *Can f* per gram. First dimension separated 830 IU (A) and 10 µl of dust-sample extract (B). Second dimension gel contained 125 µl of rabbit Ab to DHD. Gels were stained for protein content; **Can f* I.

TABLE II. Concentration of *Can f* I in commercial dog-allergen extracts

Manufacturer	Concentrations (µg/ml)
A	290
B	45
C	0.1
D	60
E	0.7
F	1050

Extracts A through D were maintenance doses for specific IT (C was tyrosine adsorbed), extract E was for oral IT, and extract F was for SPT.

the lowest concentration observed in the tyrosine-absorbed extract and the highest in the skin test extract.

DISCUSSION

The *Can f* I assay presented here was developed to assess dog-allergen exposure.

The molecule selected as a marker allergen, *Can f* I, was previously demonstrated to be an important sensitizing agent^{4, 14, 15} and therefore has direct relevance for the allergic disease.

The specificity of the *Can f* I assay was judged sufficient, since we detected no significant binding to extracts of 17 different allergen sources likely to co-occur with dog allergen in house dust samples. The

allergen sources were tested at concentrations up to 1000 µg/ml, and none gave rise to a signal significantly above background.

The international standard was included in the study, and dilution curves were compared to curves of the purified *Can f* I molecule. The curves were parallel and allowed a conversion of absolute amount into international units. From five individual runs we found that 100 IU corresponded to approximately 1 µg of *Can f* I Lowry protein. This conversion factor will make the expression of dog-allergen exposure in international units possible.

Concentration levels of *Can f* I in house dust samples were a priori expected to be within the same orders of magnitude as observed for house dust mite, cockroach, and cat,^{6, 10, 13, 16} and we thus aimed for a sensitivity of approximately 0.01 µg/ml corresponding to 0.1 µg/gm of dust at extraction in 1:10 wt/vol.

It was demonstrated that *Can f* I is a relatively stable molecule and stays in the dust for extended periods. This phenomenon may account for its importance as a sensitizing agent.

The commercial dog extracts assayed varied about four orders of magnitude with respect to concentration of *Can f* I, and even within the four extracts for specific IT, there was a variation in measurable *Can f* I from 0.7 to 290 µg/ml. We have not tested the batch-to-batch variation of the extracts, but we believe that the assay presented here could be a valuable tool in standardization of commercial extracts of DHD.

The *Can f*1 assay presented here will make possible more detailed studies of the relationship between environmental exposure to dog allergen and the development of allergic disease.

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