

House Dust Mite (*Dermatophagoides farinae*) Allergen Levels in Three Different Sources from Homes of Patients with Allergy to House Dust Mite

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OVERVIEW

The purpose of this study was to develop a useful clinical tool for measuring house dust mite (*Dermatophagoides farinae*) antigen in homes of patients with allergy to *D. farinae*. Three sources of *D. farinae* antigen were assayed by radio-allergosorbent test inhibition. These were: (1) vacuum cleaner bags from patients' own vacuum cleaners, (2) stuffed animals brought in by patients, and (3) airborne particles collected on a filter sheet through which a constant air flow was maintained for 48h. The contents of the vacuum cleaner bags were extracted in phosphate buffered saline (PBS) to make a 2.5 g/40 mL w/v extract. The stuffed animal coats were extracted by washing with the PBS and combing with a fine tooth comb. The polyglass filter sheets were eluted with a bicarbonate buffer. The resultant extracts were then assayed by RAST inhibition for *D. farinae* antigen.

All three sources were obtained and assayed from six homes. An additional 14 vacuum cleaner bags and four stuffed animals were assayed. Detectable levels of *D. farinae* antigen were found in all of the extracts. Vacuum cleaner bag assays varied fivefold, air sampler eluates varied twofold and stuffed animal extracts varied tenfold.

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In conclusion, detectable levels of *D. farinae* antigen were found by RAST inhibition assay by all three methods of dust collection; these levels varied significantly from household to household. Patient acceptance and cooperation was best when requested to bring in a vacuum cleaner bag sample.

INTRODUCTION

Over the last 60 years the allergens of the house dust mite (*Dermatophagoides farinae* and *D. pteronyssinus*) had been shown to be the major allergens of house dust and the cause of a significant amount of morbidity among patients with allergy to these mites (1-9).

The recommendations concerning house dust mite and house dust avoidance given to patients can vary from a simple change of bed linen to extensive remodeling and the use of air filtration systems. In the past these recommendations were made without critical information, namely the level of house dust mite allergen in the patient's home.

The goal of our present study was to develop a clinically useful method for the detection of house dust mite (*D. farinae*) allergen in the homes of patients allergic to house dust mite. We believe that with this information the clinician can better advise the patient and family about house dust mite precautions.

In this study we compared the levels of *D. farinae* antigen obtained from three different sources: extracts from vacuum cleaner bag dust, extracts of the coats of stuffed animals, and air samples obtained from the same homes.

MATERIALS AND METHODS

Vacuum Cleaner Bag Dust Extraction

Patients were asked to bring in their vacuum cleaner bags. It was thought that the entire carpeted area of the house would be represented in this sample. Nineteen vacuum cleaner bags from 16 different homes were extracted and assayed. The extraction procedure for the vacuum cleaner bags entailed mixing the bag thoroughly and then taking 5-10, 2.5 g samples at different areas of the

bag. Each 2.5 g sample was then added to 40 mL of phosphate-buffered saline (PBS) in a 50 mL centrifuge tube, shaken, and extracted overnight in the cold. After centrifugation, the supernatant was collected and frozen until assayed. The vacuum cleaner bag results shown are the mean of these five to 10 individual determinations.

Stuffed Animal Extraction

Patients and selected staff from the Marshfield Medical Research Foundation were asked to bring in a stuffed animal. These stuffed animals were used in the extent that they had spent some time in the patients' rooms. A total of 10 stuffed animals were extracted with a wash and comb technique. The stuffed animal was suspended from a ring stand and sprayed with PBS in large enough quantity to dampen the coat. Then with a clean standard pocket comb, the outside covering was combed, collecting resultant extract in a large container. The extract was then passed through a micro-pore filter to remove large particles and frozen until assayed.

Air Sampling

The Air-Sentinel was used for air sampling. The device developed by Swanson et al. (10), incorporates a multistage centrifugal blower (Kooltronics, Hopewell, NJ) and a custom-built stainless steel filter head assembly requiring a 4 x 10 in fiber glass filter (GMW810, General Metal Works) that retains particles 0.3 μm or larger (10). Flow rate was determined to be 170 L/m with a calibrated pneumotachograph.

Air sampling was accomplished in homes by running the sampler continuously for 36–48 h in or near the bedroom of the patient during same season of the year as vacuum bag collections were done.

Exposed filter sheets were eluted by a descending elution technique with 15 mL of 100 nmol/L NH_4HCO_3 buffer with recovery of 12 mL in a collecting container. The eluates were frozen in 1 mL aliquots until assayed. Eluates were lyophilized and reconstituted in a lesser amount of buffer if the original determination was below the detection range of our assay.

Mite (*D. farinae*) Antigen Quantitation

Vacuum cleaner bag extracts, stuffed animal extracts, and air sampler filter eluates were assayed by microtiter plate RAST inhibition assay. This procedure is similar to the RAST inhibition assay described by Gleich et al. (11), but microtiter plates were coated with Hollister-Stier *D. farinae* antigen (100 μ L of 1/5000 dilution) and used as the solid phase antigen. Serum from a person with perennial allergic rhinitis and a high level of specific IgE to house dust mite was used for these plate RAST inhibition assays. Optimal concentration of the patient's IgE was determined by checkerboard titration.

A standard curve of inhibition was determined by adding 50 μ L of Hollister-Stier house dust mite extract at various concentrations along with patient specific antisera. [The plates are incubated overnight at 2–8°C and then washed 10 times with PBS containing Tween 20 (PBS-T)]. Fifty microliters of ^{125} I antihuman IgE (Pharmacia) is then pipeted into each well and the plate covered with parafilm and incubated at room temperature for 4 h. The plate is then washed 10 times with PBS-T. The removable strips are broken apart, put in glass test tubes, and counted in a gamma counter. All assays are run in duplicate and the values shown are the mean of these two values.

Results were calculated by linear regression of percent inhibition versus antigen concentration.

$$\% \text{ inhibition} = (1 - \text{cpm well with antigen} / \text{cpm well without antigen}) \times 100$$

Results <90% inhibition and >10% inhibition are plotted vs the $\log_{10}$ [Ag] (Fig. 1). Unknown values were interpolated from this regression. Results are expressed as units of mite allergen/mL and defined as 100 U of mite allergen active equals the amount of mite allergen activity contained in 1/10,000 dilution of H/S mite antigen extract as detected by the RAST inhibition and standardized to 50 mg total protein/mL.

Assay specificity was determined by using the other allergens that our volunteer who donated the sera was sensitive to by standard prick test. These data are presented in Fig. 2 and show the assay to be specific up to a concentration of 100 protein/mL.

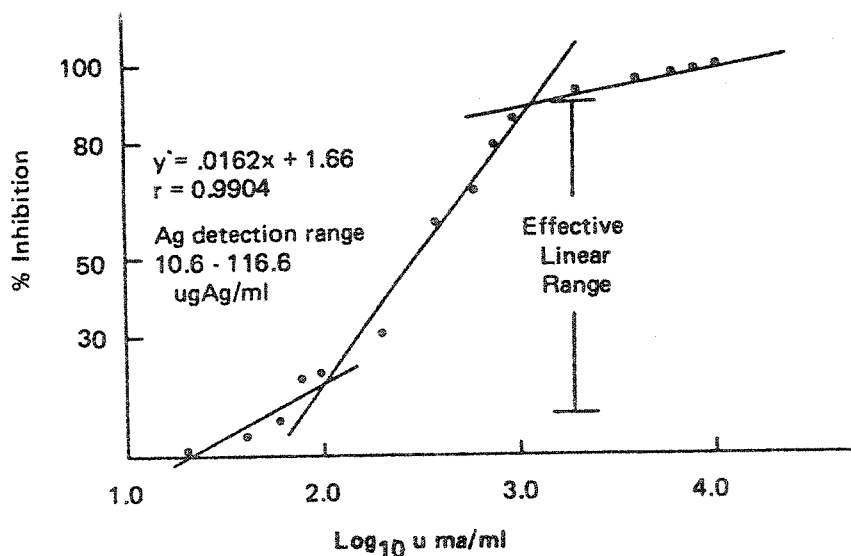


Fig. 1. Relationship of antigen dose to inhibition of antibody binding. The inhibition of antibody binding by free antigen concentration ($\log_{10}$ protein). The mid portion of the current (10–90% inhibition) was used as the standard curve.

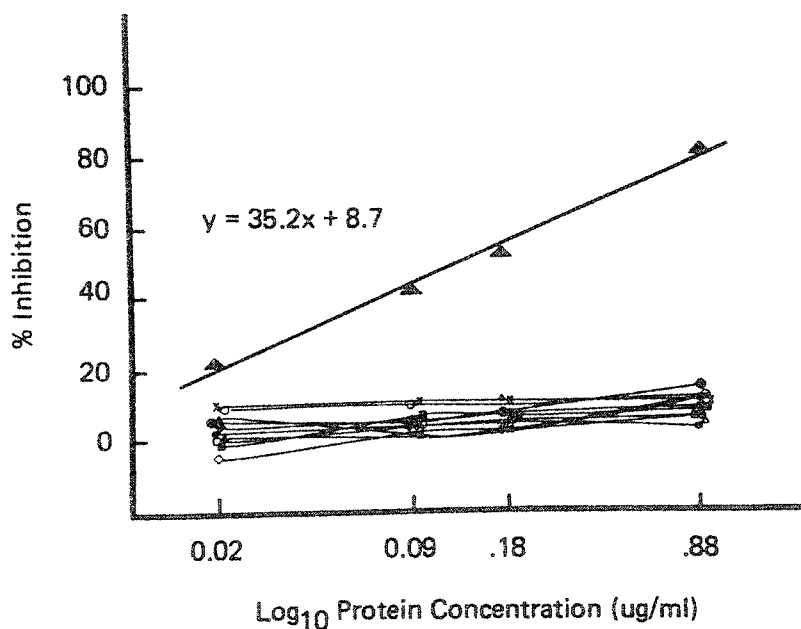


Fig. 2. Crossreactivity of unrelated aeroallergens with *D. farinae*. Dog (Δ), cat ($\times$), Ohio alternaria ($\circ$), early trees ($\diamond$), late trees ($\bullet$), grass mix ($\blacksquare$), ragweed ($\blacktriangle$), *A. fumigatus* ($\blacklozenge$), *P. notatum* ($\square$), and crude HD ($\bullet$) were each tested at various concentrations ($\log_{10}$ protein) and compared to the standard *D. farinae* ($\blacktriangle$). The equation of the standard curve only is presented.

RESULTS

A total of 19 vacuum cleaner bags from 16 different homes were assayed. The results are a mean of the five to 10 different extracts obtained from each bag. The range of values varied from a low of 136 U of mite allergen (U ma)/g of dust to a high of 942 U ma/g of dust. One home was sampled in May and had a level of 165 $\mu\text{g/g}$ of dust. (Two samples obtained in July and then September from the same home had values of 589 and 593 U ma/g of dust, respectively).

A total of 10 stuffed animals, 6 air samplings, and 19 vacuum cleaner bags were extracted and tested by RAST inhibition. The results of these determinations are depicted in Fig. 3. The vacuum cleaner bag and stuffed animal results are shown in U mite allergen/mL of extract, whereas the air samplings are shown as U mite allergen/mL of extract $\times 100$.

Air sampling was accomplished in six homes where we had also obtained vacuum cleaner bags and a stuffed animal. The air sampling was done during the months of May through September, the same season as the vacuum cleaner bags were obtained, though in most cases these were not obtained simultaneously. The results are shown as the amount of U ma/h of air sampled (Table 1). These data are combined in Table 1 with the results of the vacuum cleaner bag samples and stuffed animal extracts from the same homes. The results of the air sampling is depicted as $1000 \times \text{U ma/h of air sampled}$. When the levels of mite in the vacuum cleaner bag extract are compared by regression analysis to air sampler levels from the same homes, there was a correlation coefficient of 0.70. The same comparison between the stuffed animals and the air sampler revealed a correlation of 0.021.

Dust samples were collected by brushing the mattresses and livingroom upholstery from homes three and four in June of 1986 and examined for mite enumeration and speciation. No mites were detected from home three brushings, whereas *D. farinae* in small numbers were found in the brushings from four.

DISCUSSION

Using a RAST inhibition microtiter plate assay and three different types of samplings we were able to show that allergens of *D. farinae* could be detected in homes in central Wisconsin. The

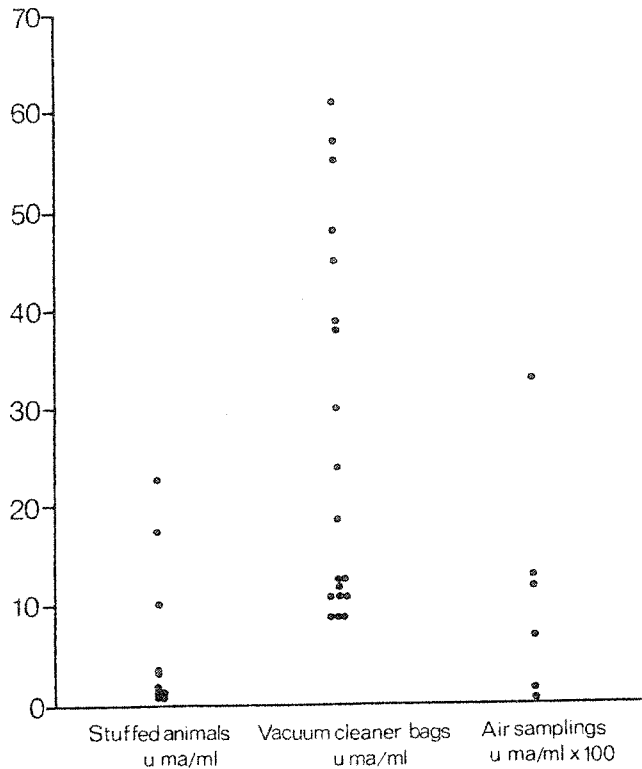


Fig. 3. Distribution of *D. farinae* aeroallergen by three sampling techniques. Samples were obtained from stuffed animals (U mite allergen/mL), vacuum cleaner bags (U mite allergen/mL), and air samplings (U mite allergen/mL x 100).

amount of mite allergen present in vacuum cleaner bag samples varied between homes up to sevenfold. The respirable allergen level provided by air sampler data also varied significantly. The mite allergen level of the stuffed animals also varied widely from 22.8 U ma/mL of extract down to 1.46 U ma/mL of extract. The allergen exposure that a patient has, particularly a pediatric patient, may not only be directly related to the respirable allergen in the air, but also upon the local allergens present in stuffed animals, upholstery and carpets. We do know that the levels of allergens may vary depending upon activity in the household. Swanson et al. (10) reported an increase in the level of aeroallergens of the house dust mite detected in a bedroom 46-fold resulting from simply making the bed. The data obtained from vacuum cleaner bags is interesting in that in the six homes where the air sampler and the vacuum cleaner bag assay was accomplished there was a correlation (0.70).

Table 1
Comparison of Sample Collection Methods
for *Dermatophagoides farinae* Allergen

House	Vacuum cleaner bag extract ^a	Air sampler ^b	Stuffed animals ^c
1	869	6.9	10.64
2	698	2.7	22.8
3	197	0.2	8.4
4	589	0.5	17.9
5	738	2.5	2.3
6	135	1.5	1.5
	$r = 0.700$	$r = 0.02$	

^aU mite allergen/g of dust.

^b1000 x unit of mite allergen/g of dust.

^cU mite allergen/mL of extract.

Because of small numbers, we did not achieve statistical significance between these two assays. We do feel that the correlation indicates that sampling vacuum cleaner bag dust extracts for the presence of house dust mite allergen does give one a good idea of the respirable allergen levels in those homes.

During this study it became apparent that the patients in our clinical practice were much more willing to bring in a vacuum cleaner bag sample than they were in taking an air sampler out to their home and running it over a 4- to 5-d period. If further data support our present correlation between the air sampling and the vacuum cleaner bag extracts, we feel confident that these vacuum cleaner bag extracts will provide us with useful information concerning the levels of house dust mite allergen in homes of our patients. Whether air sampling or vacuum cleaner bag dust extraction is the best method is not important, however. The importance of this work lies in the demonstration that it is feasible in a clinical practice to provide one's patients with knowledge of the actual levels of house dust mite allergen present in their homes, and to provide feedback to them concerning the actual effect of their house dust measures. With tools such as these we should be better able to deal with the allergens of the house dust mite and find more effective means for avoidance.

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