

An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: Studies with mite, roach, cat, mouse, and guinea pig antigens

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We describe a new high-volume air sampler for determining antigen concentrations in homes and illustrate its use for quantitating airborne house dust mite, cat, cockroach, mouse, and guinea pig antigens. The concentration of house dust-mite antigen was similar from houses in Rochester, Minn. and tenement apartments in Harlem, N. Y., but cockroach and mouse urinary proteins were present only in Harlem. The amount of cat or guinea pig antigen varied as expected with the number of pets in the home. In calm air the airborne concentration of mite and cat antigen was similar throughout the house but increased greatly in a bedroom when bedding was changed. In calm air most of the cat and mite antigens were associated with respirable particles $<5\ \mu\text{m}$ mean aerodynamic mass diameter, but in air sampled after the bedding was changed, more cat antigen was found in particles $>5\ \mu\text{m}$. The apparatus and technique described can provide objective data concerning the magnitude and the relative distribution and duration of suspended particles of defined sizes, which contain allergen activity. (J ALLERGY CLIN IMMUNOL 76:724-9, 1985.)

A variety of allergens are associated with airborne particles in home and work environments. Indeed, almost any organic dust or volatile chemical reactive with proteins may cause allergic rhinitis, asthma, or hypersensitivity pneumonitis. If sufficient levels of allergens are airborne at least sporadically during an extended period of time, individuals prone to allergy may become sensitized. Once sensitization has occurred, a brief exposure may suffice to elicit an allergic reaction, and if chronic exposure continues, there may be permanent disability. In order to study these issues quantitatively, it is important to develop means of measuring the concentration of antigen in the air. We have previously described an immunochemical method to quantitate for outdoor allergens,¹ allergens in industrial settings,² or a research laboratory,^{3,4} but the Accu-Vol (General Metal Works, Inc., Cleves, Ohio) sampler used in those studies

proved too noisy to operate in homes or offices. In this article we extend the immunochemical approach to indoor aeroallergen quantitation with the use of a new quieter volumetric air sampler. Because size of suspended particles containing allergenic activity determines the site of airway deposition, we also studied size distribution of indoor particles containing house dust-mite and cat allergen.

MATERIAL AND METHODS

Air sampling equipment

The Air-Sentinel (Fig. 1), a new volumetric air sampler, was used for aeroallergen quantitation. The device incorporates a multistage centrifugal blower (Kooltronics, Hopewell, N. J.) and a custom-built stainless steel filter head assembly (HiMec, Inc., Rochester, Minn.) requiring a 4 by 10 inch fiberglass filter (GMW 810, General Metal Works) that retains particles $0.3\ \mu\text{m}$ or larger. During operation, its noise level is 60 to 70 dB, about the same as an electric fan. Flow rates were established for each sampler by use of a calibrated No. 3 Fleisch pneumotachograph (Dynascope, Blue Bell, Pa.) in line with a DP 45 Validyne transducer (Validyne Engineering Corp., Northridge, Calif.) (± 1 inch H_2O). Flow rate variations among 10 samplers were within 5% of one another (average flow rate = 3 L/sec). Variation in flow rate for a single sampler was $<1\%$. After 72 hours of operation in a dusty environment, the drop in flow rate was $<10\%$.

The particle sizing experiments were performed by use

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of an Accu-Vol air sampler (General Metal Works) equipped with an Andersen cascade impaction head (Andersen Samplers, Inc., Atlanta, Ga.) as previously described.³

Filter extraction for aeroallergen quantitation

Air-filter extraction involves elution of allergen activity by descending chromatography.¹ The Air-Sentinel is designed with a triangular collection orifice. When compared to the procedure used with the Accu-Vol sampler for sample handling,¹ this feature expedites sample processing by minimizing sample manipulation (i.e., folding filter to a tapered tip and stapling) and permits elution of multiple filter samples in a minimum volume of 100 mmol/L NH_4HCO_3 buffer (Fig. 2). Saturation of the exposed triangular area requires approximately 5 ml of buffer in a humidified chamber. Optimal allergen activity is obtained when 10 ml of buffer have been eluted into a collection reservoir (data not shown). Therefore, the total volume of buffer for elution of aeroallergen proteins per sample was 15 ml. An eluate of 10 ml can be assayed directly if the specific allergen activity in an eluate approximates 1 $\mu\text{g}/\text{ml}$ (data not shown). For eluates with specific allergen activity equivalents of <1 $\mu\text{g}/\text{ml}$ lyophilization and reconstitution in a smaller volume are necessary to provide sufficient allergen concentrations for quantitation. After recording the volume, the eluate is sterilized by membrane filtration (Millipore Corp., Bedford, Mass.) into sterile evacuated bottles and stored at -20°C .

Allergen extraction from particle-sizing impaction discs

The procedure for extraction of allergen activity from the impaction discs of the Andersen sampler (Andersen Samplers Inc.) is similar to that described for aeroallergen quantitation with minor modifications. The circular paper impaction discs are folded into quarters and stapled in a triangular pattern for elution with 50 ml of buffer. The extract is lyophilized and reconstituted in 1 ml of buffer.

Air sampling

Air-filter samples for aeroallergen quantitation were obtained from nine private homes in Rochester, Minn., and from five tenement apartments in Harlem, N. Y. Sampling time varied from 24 to 72 hours, which represents a range from 260 to 780 m^3 of air per sample. Samples were also obtained from basement, study, and bedroom areas within a single Rochester home heated with a force-air furnace. Air samples (48 hours; 3400 m^3) for airborne particle size separation were also obtained from this Rochester home.

We recognized the potential for particle fragmentation during the course of particle-sizing experiments, thus producing an overestimate of allergen carriage by smaller airborne particles. To evaluate this potential, impaction disc media were mounted on each stage of the Andersen head as usual (Fig. 3) along with an additional one half disc covering half of each stage. The sampler was then placed in a bedroom for 20 minutes while the bedding was changed and shaken vigorously. A cat often slept on this bed. The half disc was removed for analysis, and the sampler was



FIG. 1. The Air-Sentinel, a volumetric air filtration device expressly designed for collection and quantitation of indoor airborne antigens.

moved to the basement to collect a 24-hour sample of calm air. The allergen activity recovered from the half disc combined with that of the half portion below it should be equivalent to the total activity of half disc exposed for the entire duration. This would control for fragmentation during long-term sampling (i.e., 24 hours). Sampling turbulent air and calm air should demonstrate a difference in the distribution of allergen activity associated with particles of defined sizes. We hypothesized that recently suspended particles would be of all sizes but that larger particles would quickly settle out and be underrepresented in the 24-hour calm air sample.

Reference allergens

Fifty percent glycerinated extracts of cat hair/dander (1:50 w/v, Albay AP), containing 1.8 units of cat 1 antigen per milliliter, *Dermatophagoides farinae* (1:100 w/v), and cockroach mix (1:10 w/v) were purchased from Hollister-Stier Laboratories, Spokane, Wash. Male Dunkin-Hartley guinea pig urine was collected in metabolic cages, centrifuged, dialyzed, and lyophilized.³ Mouse urine was manually expressed from BALB/c male mice.⁴ In as much as these are not purified antigen preparations, the concentrations of allergen are not comparable between the various antigen sources.

Protein content

Protein determinations for each allergen extract were performed according to the method of Lowry et al.⁵ The protein

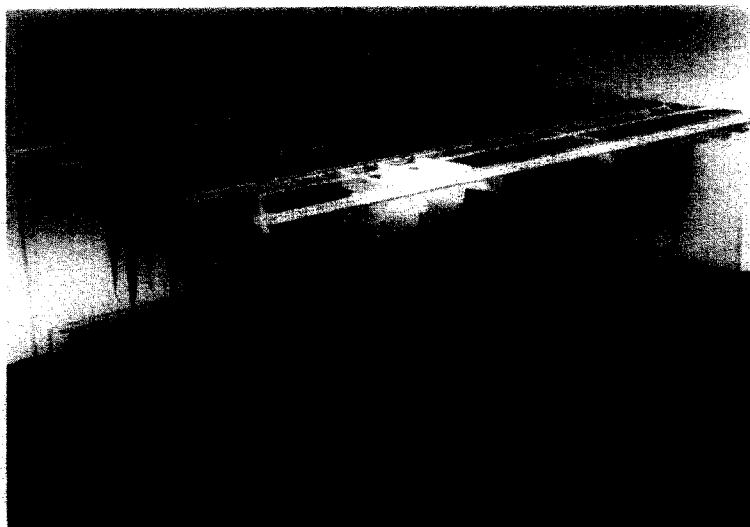


FIG. 2. Elution jig for processing up to 50 air-filter samples at a time.

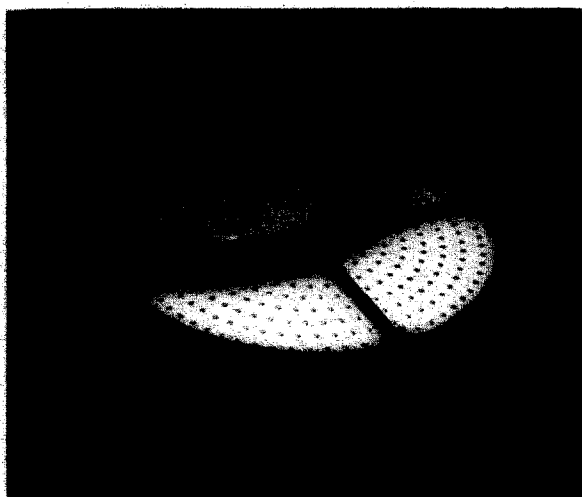


FIG. 3. Andersen cascade impaction head (Andersen Samplers, Inc.) illustrating the first impaction stage of five stages for airborne particle separation into defined sizes of >4.1 , <4.1 , >2.3 , <2.3 , >1.4 , <1.4 , >0.8 , and <0.8 μm .

concentrations were as follows: cat, 5 mg/ml; mite, 0.35 mg/ml; cockroach, 8 mg/ml; guinea pig urine, 0.9 mg/ml; and mouse urine, 0.9 mg/ml. Subsequent expression of aeroallergen levels per cubic meter of air are in units of protein with respect to these reference standards.

Solid-phase allergens

The various allergens (cat, mite, roach, mouse urine, and guinea pig urine) were coated onto the surface of Immulon-2 microtiter Removawell strips (Dynatech Laboratories, Inc., Alexandria, Va.). Each well received 1.0 μg of protein in 100 μl of 200 mmol/L of sodium bicarbonate buffer titrated with 200 mmol/L of sodium carbonate to pH 9.2.

The plates were incubated overnight at room temperature in a humid box and washed five times with RAST wash (0.1 mol/L of phosphate buffer, 1% Tween 20).

Measurement of allergenic activity

Quantitation of various allergens in air-filter eluates was done immunochemically in plate-RAST inhibition assays. Pooled IgE antibodies from persons highly sensitive to cat, mite, cockroach, mouse urine, or guinea pig urine allergens were used for plate-RAST inhibition assays.

Fifty microliter aliquots of appropriate reference allergens (cat, mite, roach, mouse urine, or guinea pig urine) at various concentrations were placed in the allergen-coated microtiter wells along with 50 μl of specific human antisera diluted 1:2. Similarly, air-filter eluates (50 μl) were also placed in wells along with the specific antisera. The plates were incubated at room temperature overnight in a humid box, washed five times, and 100 μl (20 ng) of ^{125}I -antihuman IgE was added to each well. The plate was incubated again at room temperature in a humid box for 18 hours. The plate was subsequently washed five times, and the Removawell (Dynatech Laboratories, Inc.) strips were broken apart into 16 by 125 mm glass test tubes and counted in a gamma scintillation counter. Plate-RAST inhibition results were evaluated by regression analysis with a Hewlett-Packard 9845B computer (Hewlett-Packard Co., Palo Alto, Calif.).

Because the air-filter eluates contain a mixture of many proteins, both allergenic and nonallergenic, the specificity of the assays is crucial. The cat, mite, cockroach, mouse, and guinea pig urinary antigens were each specific and did not cross-react with one another.

RESULTS

Aeroallergen quantitation

Airborne allergen levels from single family dwellings in Rochester, Minn. and tenements in Harlem,

TABLE I. Indoor aeroallergen concentrations (picograms of protein per cubic meter)

Dwelling	Mite	Cat	Roach	Mouse urine	Guinea pig urine
Rochester					
1	1610	92,000	<1,000	<3	<1,000
2	370	<1,000	ND	<3	<1,000
3	1600	17,000	ND	<3	<1,000
4	2600	51,000	ND	<3	<1,000
5	4090	ND	ND	<3	ND
6	5570	ND	ND	<3	ND
7	2790	ND	<1,000	<3	ND
8	ND	<1,000	ND	<3	495,000
9	ND	<1,000	ND	<3	118,000
Harlem					
1	2400	5,110	20,010	2960	ND
2	3700	6,290	16,240	430	ND
3	3140	55,900	25,100	230	ND
4	2160	13,150	42,480	1240	ND
5	3220	14,680	43,430	610	ND

ND = not determined.

TABLE II. Average mite and cat aeroallergen concentrations within a single Rochester home in November and December

Location	Allergen	
	Mite (pg protein/m ³)	Cat (pg protein/m ³)
Bedroom	1780 ± 340	87,000 ± 46,000
Study	1720 ± 1050	54,000 ± 28,000
Basement	1250 ± 390	129,000 ± 149,000
Bedroom	82,000*	9,262,000*

x ± SD; n = 6.

*Airborne allergen levels per cubic meter of air sampled for 10 minutes after making the bed.

N. Y. provide contrasting information about the prevalence and relative concentrations of various aeroallergens (Table I). In Rochester, dwelling 1 had three pet cats, dwelling 4 had two, and dwelling 3 had one pet cat. Dwelling 8 had three pet guinea pigs, and dwelling 9 had one. Thus, the amount of animal antigen in the air is proportional to the number of animals in the house.

Various areas within a Rochester home were studied to assess allergen distributions throughout a single dwelling heated with a forced-air furnace. Multiple experiments demonstrated that the distribution of cat and mite allergens were relatively consistent in bedrooms, study, and basement (Table II) except in turbulent air during the changing of bedding. This bed was chosen for the experiment because a cat often slept on it.

Particles of defined sizes were associated with allergen activity in each size range. These results were confirmed in three separate experiments. The bulk of the cat and mite allergen activities were associated with particles capable of penetrating the lower respiratory tract (Table III). Mite allergen activity was primarily in stages 2, 4, and 5 (<4.1 to <0.8 µg) both in quiet and disrupted air. Cat allergen activity was predominant in stages 1 and 5 (>4.1 and <0.8 µg) in turbulent air but was more evenly distributed throughout all stages in calm air (Table III). The cat and mite allergen activity recovered at 20 minutes and 24 hours was calculated from samples in series, whereas the 5-minute mite allergen recovery was an observation from a separate sample. The questions of particle fragmentation could not be unequivocally answered from these experiments.

TABLE III. Duration of suspended antigens associated with particles of various sizes in the air after making a bed

Particle size	Antigen concentration (pg protein/m ³)				
	Mite			Cat	
	5 Minutes	20 Minutes	24 Hours	20 Minutes	24 Hours
Larger than 4.1 μm	83,920	17,520	240	14,460	6,550
<4.1 μm >2.3 μm	162,790	63,280	520	9,110	6,430
<2.3 μm >1.4 μm	176,420	24,170	220	3,170	4,460
<1.4 μm >0.8 μm	218,490	44,510	470	7,720	5,510
Smaller than 0.8 μm	94,470	40,430	290	15,090	14,380

DISCUSSION

Immunochemical aeroallergen quantitation provides a practical method for the study of all airborne antigens, amorphous as well as morphologically defined particles such as pollens or viable particles.

Socioeconomic, geographic, and climatic factors are important in determining the kind of allergens present in an indoor environment. Although these factors are intuitively and empirically accepted, the variables involved have not been separated and studied to document and correlate clinical manifestations of allergic respiratory disease or asthma caused by aeroallergen levels. Furthermore, the "indoor biome" may have seasonal allergen much like the outdoors. In some instances the prevalence of a particular indoor allergen can be of great concern because the airborne concentrations may accumulate in a limited air volume. These aspects are crucial to the management of individuals already sensitized, as well as helping to define conditions that allow sensitization to occur initially.

The values presented here illustrate the possibility of the approach, but additional requirements should be met for future studies comparing antigen levels in different locations and at different times. Finite indoor air volumes and indoor air volumes with limited exchange rates require intermittent sampling to allow equilibration of resuspended aeroallergens. Continuous high volume sampling schedules may actually clean the air faster than particles are resuspended. Reference standard antigen preparations are needed so that the results can be expressed in terms of the standard. The antigen sources should be as well defined as possible. Variability among replicate assays may vary as much as 100% as a result of compounding errors at any or all of the handling steps. Typically, if replicate assays are performed by use of the same reagents within a reasonable time period, the vari-

ability should not exceed 50%. This is to say the absolute values may vary to this degree; however, the values relative to one another will not change. The possibility of losing allergen activity on storage is always present and, if it is possible, should be guarded against by eluting samples and processing them immediately. If they are to be stored for future analysis or reassay, they should be stored at -70°C in a carrier protein to minimize the loss of allergen activity.

In general the method is more sensitive for purified, or at least partially purified, allergens than for crude extracts. For example, the cockroach extract is a complex mixture of whole body proteins of three species, whereas the mouse urinary protein complex is a relatively simple mixture from an inbred strain with more relevant allergen per unit mass. For antigen extracts that require concentrating for analysis, it is necessary to determine whether they are stable during the concentration step, such as lyophilization. The IgE antibody pool also should be large enough for the entire study and stored at -70°C in aliquots to maximize stability. The introduction of plastic microtiter plates capable of adsorbing protein reproducibly to the surface of the well offers major advantages as well as the potential disadvantage of excluding the relevant allergen from the surface of the plate. If, for some allergen, this becomes evident, traditional sorbents that are chemically activated producing free radicals for protein linkage can be used such as discs or cellulose. The disadvantages in using these sorbent types are lower assay sensitivities and much slower assay turn around.

The size of allergen-associated airborne particles not only determines the site of airway deposition but also the length of time they remain suspended in the air. Platts-Mills et al.⁶ describe the settling rate for the fecal particles ($>10\ \mu\text{m}$) from *D. pteronyssinus* to be 95% complete within 5 minutes. The data in

Table III are consistent with this formulation. We, also, found the concentration of mite antigen, as well as cat antigen, was very much greater shortly after shaking bedding than it was in a 24-hour sample. We cannot exclude the possibility that some of the large particles become fragmented during the sampling procedure.

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Suppression of late-phase skin reactions by immunotherapy with ragweed extract

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*The cutaneous late-phase reaction (LPR) to ragweed was studied in untreated ragweed-allergic individuals and patients receiving 3 to 5 years of immunotherapy demonstrating clinical improvement. The magnitude of immediate skin reactions and the initial levels of the specific IgE and IgG antiragweed antibodies were similar in both groups. The LPR was elicited by administering a skin test with ragweed extract at 10 times the concentration required to elicit a 4+ immediate reaction and appeared as an erythematous-edematous lesion associated with pruritus. In the untreated group 94% developed an LPR (59 ± 32 mm at 4 hours and 67 ± 30 mm at 8 hours) at this dose. In the treated group only one third developed an LPR, one third had partial response measurable at one of these two times, and one third failed to develop any LPR (21 ± 20 mm at 4 hours, $p < 0.002$, and 20 ± 22 mm at 8 hours, $p < 0.001$). Therapy resulted in a twentyfold increase of IgG antiragweed level and in a decline of IgE antiragweed. The size of the LPR correlated inversely with the level of IgG antiragweed ($p < 0.01$; $r = -0.52$) but not with IgE antibody. Thus, in a retrospective analysis immunotherapy was associated with the suppression of the skin LPR, and the magnitude of the LPR was correlated with the level of IgG antiragweed. We suggest that the clinical efficacy of immunotherapy is related in part to effects on the LPR. (*J ALLERGY CLIN IMMUNOL* 76:729-34, 1985.)*

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