

Immunoassays for Indoor Allergens

**Martin D. Chapman,^{*,1} Lisa D Vailes,¹
and Kunio Ichikawa^{1,2}**

¹*Asthma and Allergic Diseases Center, Department of Medicine, Box 225,
University of Virginia, Charlottesville, VA 22908; and ²Department of
Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan*

Introduction

Ten years ago, monoclonal antibody (MAb)-based two-site enzyme immunoassays (ELISAs) for Der p 1 and Der f 1 were first published, and these assays have since become a cornerstone for assessment of indoor allergen exposure (1,2). Subsequently, assays for several other mite allergens have been developed, as well as assays for cat, dog, cockroach, and fungal allergens (3–17; Table 1). Over 300 publications using allergen immunoassays are now on medline databases covering a broad spectrum of clinical, epidemiologic, and immunologic studies. Early studies focused on the sites of allergen accumulation within the home and seasonal variation in allergen levels. There followed a series of case control studies and population studies designed to establish risk levels for allergen exposure leading to sensitization and correlation of exposure assessments with clinical symptoms and the severity of asthma (reviewed in ref. 2 and articles 5 and 7). Analysis of airborne allergen levels and particle size distribution defined the aerodynamic properties of mite, cat, dog, and cockroach allergens (*see* Article 4), and the source of the allergen (e.g., mite feces) or its anatomical site of production (e.g., cat anal glands). Measurement of major allergens by ELISA also became incorporated into programs for standardization of allergenic products used for diagnosis and treatment. Finally, immunoassays have been essential in designing and monitoring allergen avoidance studies. These include environmental studies where patients are moved from allergen containing to allergen-free conditions (e.g., at high altitude);

*Author to whom all correspondence and reprint requests should be sent. E-mail: mdc8m@virginia.edu

Table 1
Immunoassays for Indoor Allergens

Allergen	Capture MAb ^a	Second antibody ^b	Allergen standards	Reference
Mite				
Der p 1	5H8	4C1 ^B	N1BSC 82/518, UVA 93/03	(3)
	10B9	5H8 ^B	NIBSC 82/518, UVA 93/03	(3)
	PIA03	P1A01 ^B	92-Dp	(4)
Der f 1	6A8	4C1 ^B	UVA 93/02	(3)
	F1B01	F1A05	92-Df	(4)
Group 2	1D8	7A1 ^B	UVA 97/01	(5)
Group 7	WH9	HD19 ^B	n.a.	(6)
Blo t 5	4G9	4D9 ^B	rBlo t 5	(7)
Mammalian				
Cat, Fel d 1	6F9	3E4 ^B	CBER Cat E3, UVA 94/01	(8)
Dog, Can f 1	6E9	RaαCan f 1	NIBSC 84/685, UVA 98/01	(9,10)
Rat, Rat n 1	RUP-6	RUP-1 ^B	Affinity purified Rat n 1	(11)
Cockroach				
Bla g 1	10A6	RaαBla g 1	UVA 97/02	(12)
Bla g 2	8F4	RaαBla g 2	UVA 97/02	(12)
Per a 3	A-2	E-4 ^{AP}	Purified Per a 3	(13)
Fungi				
Asp f 1	4A6	RaαAsp f 1	Affinity purified Asp f 1	(14,15)
Alt a 1	121G	121G ^B	rAlt a 1	(16)
	MAb-1	MAb-2	Affinity purified nAlt a 1	(17)

^aMAb used to coat ELISA plates.

^bMAb or polyclonal rabbit antibody used for allergen detection. B = biotinylated; AP = alkaline phosphatase conjugate; n.a. = not available.

studies where the source of the allergen (e.g., cat) is removed from the environment; and intervention studies designed to reduce allergen levels in the home (e.g., using encasings, acaricides, or protein denaturants to reduce mite allergen levels) (Articles 6 and 7). Increasingly, immunoassays are being used to assess the efficacy of allergen control products and devices, such as encasings, air filters, and vacuum cleaners. This provides the opportunity for manufacturers to design products that are of proven efficacy by using objective methods to demonstrate their effects on allergens.

The scope, breadth, and sheer weight of these studies would have been inconceivable using previous immunochemical assays, such as radioallergosorbent technique (RAST) (or ELISA) inhibition, which require large amounts of human sera with high titers of IgE antibodies. The advantages of ELISA are high throughput, accurate quantitation (ng/mL or µg/mL allergen protein), and defined specificity, using MAbs to allergens of known molecular structure and allergenic importance. The limitations of this approach are that dust extraction, sample

preparation, and the assays themselves are time-consuming, and their use is confined to well-equipped research or clinical laboratories. As information about the importance of indoor allergens as risk factors for allergic disease has become widely understood by the public at large, it has become apparent that simple, rapid, dust-sampling and allergen-test systems are required. The guanine assay was the first patient-based test for mite allergen and, over the past two years, several MAb-based technologies have been developed for office- or home-based testing. This article reviews progress in ELISA for indoor allergen assessment and describes new approaches to allergen detection.

ELISA: The Gold Standard for Indoor Allergen Analysis

The key to successful immunoassays is the production of high-affinity MAb with defined specificity directed against nonoverlapping antigenic determinants (1). Pairs of MAb suitable for use in ELISA have been produced for a growing number of indoor allergens (Table 1). In most cases, one MAb is used for allergen capture and a second biotinylated or enzyme labelled MAb is used for allergen detection. When only a single MAb is available, polyclonal rabbit antibodies can be used for detection, (e.g., in assays for Can f 1, Bla g 1, Bla g 2, and Asp f 1). Recent developments include ELISA for *Dermatophagoides* spp. Group 5 and Group 7 allergens, *Blomia tropicalis* (Blo t 5), rat and mouse urine allergens (Rat n 1 and Mus m 1), and fungal allergens (Asp f 1 and Alt a 1). Exposure to Blo t 5 or Der p 5 is significantly lower than to the Group 1 and Group 2 *Dermatophagoides* allergens, which presumably explains the lower prevalence of sensitization to the Group 5 allergens (18). Assays for Group 7 allergens have been used to compare levels in commercial extracts and show 10–60-fold variation between Der p 7 levels in standardized *Dermatophagoides pteronyssinus* extracts (K. Ichikawa, unpublished data). Assays for mouse and rat urinary proteins have been used to compare airborne allergen levels in animal facilities, as part of occupational health studies aimed at reducing exposure of laboratory personnel to these potent allergens (11,19,20).

The last 10 years have also seen the cloning of major fungal allergens from *Aspergillus*, *Alternaria*, *Cladosporium*, and *Trichophyton* species. The role of fungi in causing allergic and other respiratory diseases is poorly understood and most exposure assessments rely on spore counts or culture techniques. The problem with fungi is whether ELISA assays will detect the growth form of the fungus found in dust. Assays for Asp f 1, which is produced when *Aspergillus* germinates, are usually negative on dust samples, which predominantly contain fungal spores (15). Alt a 1 can be eluted from *Alternaria* spores, however, the spore count has to be very high (>100,000 spores/mL) for

allergen to be detectable, and it seems unlikely that such high spore concentrations will routinely be found in domestic environments (16). Commercial *Alternaria* extracts have previously been thought to show great variability in allergen content, yet ELISA results show that these extracts usually contain ~1–2 µg/mL Alt a 1. In contrast, Asp f 1 levels in *Alternaria fumigatus* extracts were highly variable, ranging from undetectable to 10–60 µg/mL Asp f 1 (16).

Allergen Standards

Producing and maintaining allergen standards is critical to the reproducibility and accuracy of allergen assays. In the past, standards have been established by comparing the allergen content of a reference preparation (e.g., mite or cat extract) relative to a purified sample of a given allergen (Der p 1 or Fel d 1). It is important to realize that until recently all allergen standards were simply extracts that had been calibrated to a known concentration; they were not purified allergens. Two International Reference Preparations were established in the 1980s that fulfill World Health Organization (WHO) criteria for standards: mite, *D. pteronyssinus* (code NIBSC 82/518); and dog, *Canis familiaris* (NIBSC 84/685) (21,22). At the time these standards were prepared, they were estimated to contain 12.5 µg/mL Der p 1 or 100,000 ng/mL Can f 1, respectively. Subsequent studies suggest that the value for Der p 1 may have been overestimated and that 5 µg Der p 1/ampule for the WHO/International Union of Immunological Societies (IUIS) *D. pteronyssinus* reference may be more accurate (Table 2; 4,21–23). Other standards, listed in Table 2, have been established by researchers, regulatory agencies or manufacturers and biotechnology companies for “in house” or commercial use. Although these standards have been very useful for calibrating assays, in most cases, their stability has not been established and differences have emerged between estimates of the actual concentrations of allergen protein in the standards. In addition to Der p 1, estimates of the Fel d 1 content in cat standards also vary over a twofold range (Table 2).

Apparent differences in allergen content can in most cases be related to variability in protein measurements and the purity of the allergen used as the primary reference or standard. In the 1980s, allergen purification was limited to immunochemical techniques, the allergen sequences were not known, and relatively small amounts of protein were available for research purposes. With the advent of allergen cloning and the development of high-level expression systems, these problems have now largely been solved and there is great interest in using recombinant allergens for allergen standardization. Currently, bacterially expressed rBlo t 5 and rAlt a 1 are being used as allergen standards in ELISA, and most of the allergens listed in Table 1 have

Table 2
Estimates of the Allergen Content of Standards Used for ELISA

Standard	Allergen content	Reference
<i>Mite</i>		
<i>D. pteronyssinus</i>		
WHO/IUIS (NIBSC 82/518)	4.4–12.5 µg Der p 1/ampoule	(2,4,21)
CBER E5-Dp	24 µg Der p 1/mL	(23)
UVA 93/03 <i>D. pteronyssinus</i>	2500 ng/mL ^a	(3)
UVA 97/01	5000 ng/mL Der p 2	(5)
<i>D. farinae</i>		
CBER E5-Df	9.5 µg/mL Der f 1	(4)
UVA 93/03 <i>D. farinae</i>	2500 ng/mL ^a Der f 1	(3)
<i>Blomia tropicalis</i>	2500 ng/mL rBlo t 5	
<i>Cat</i>		
CBER Cat E5	9.7 U/mL	—
UVA 94/01	1.6 U/mL ^b	(24)
<i>Dog</i>		
WHO/IUIS (NIBSC 84/685)	100.000 IU/mL	(22)
UVA 98/02	5,000 IU/mL ^c	—
<i>Cockroach</i>		
UVA 97/02	100 U/mL Bla g 1	(24)
	2000 ng/mL Bla g 2	(25)

^aRelative to WHO/IUIS *D. pteronyssinus*, assuming 12.5 µg Der p 1/ampoule.

^bRelative to CBER E5 (Estimates of 1 U = 1.9–4 µg protein).

^cRelative to NIBSC 84/685 (1 U = ~ 1 ng protein).

been expressed as recombinants with immunoreactivity comparable to the natural allergen. As part of a new initiative in allergen standardization, the WHO/IUIS Allergen Standardization Committee is embarking on a program to produce purified natural and recombinant allergens for standardization. This program will involve collaborations between academic researchers, allergen manufacturers, and biotechnology companies to produce the purified allergens, which will serve as primary standards for immunoassays and be stored in WHO-approved repositories. Criteria will be established for allergenic purity and for acceptable protein measurements. One approach will be to use the allergen extinction coefficients for protein determinations, because these are now known from the cloning data, and are available on the World Wide Web. Optical density (OD) measurements are in general laboratory use and provide independently verifiable methods of allergen determination. An excellent correlation has been reported for two different ELISAs for Der p 1 and Der f 1 ($p > 0.95$, $p < 0.001$) and the authors reported that these assays were “identical” (4). Quantitative differences between allergen standards are an important source of assay variability, which will be resolved once the WHO/IUIS international standards are established. As an

example, mite allergen Der p 2 has been expressed at high level in *Escherichia coli*. Recombinant Der p 2 is highly immunoreactive and has been used in nuclear magnetic resonance (NMR) and X-ray crystallographic studies to determine the tertiary structure of the allergen (26,27). The allergen is available in sufficient quantities to initiate an international collaborative study to establish a primary standard using rDer p 2, and this standard would form the basis of future immunoassay standards.

Practical Considerations and Assay Performance

The widespread use of immunoassays for measuring allergen exposure spurred the need to examine the reproducibility of the assays and factors affecting assay performance. At the Third Indoor Allergen Workshop in 1995, a multicenter study of assay variability was designed to compare the results of assays for Der p 1 in house dust samples in 20 laboratories worldwide. This study, coordinated by Rob Siebers of the Wellington Asthma Research Group in New Zealand, has produced interesting data. Initial results showed that Der p 1 values from the different labs varied over a six- to sevenfold range and that temperature of extraction significantly affected the results. Dust samples extracted at 4°C (overnight) contained 50–60% less Der p 1 than samples extracted at room temperature (2 h) (28). Other predicted sources of variability were differences in allergen standards and plate-to-plate variation. Our results on samples analyzed as part of this study showed that temperature affected Der p 1 extraction, but that plate-to-plate variability was <10% and the results obtained using two standards were very similar (Table 3). Interestingly, the results on extraction temperature appear to apply only to Der p 1 because temperature does not affect extraction of Der p 2 or Fel d 1. Several published studies show that intraassay variation of indoor allergen ELISA is ~5% and that inter-assay variation ranges from 5–18% depending on the allergen (3,29–31). These results suggest that the assays behave consistently within a given laboratory. However, the inter-laboratory comparison leaves room for improvement. Siebers et al. (32) report sevenfold variation in Der p 1 results from 29 different labs (from 16–110 µg/g) in a follow up study. Close inspection of these results reveals that approx 60% of the labs gave results within a two- to threefold range, which would be acceptable under Food and Drug Administration (FDA) guidelines for an allergen standard (50–200% of the reference preparation). Clearly, further studies are required to reduce the variability and to bring the “outliers” into the range of the majority of the participating labs.

Table 3
Comparison of Variables in Der p 1 ELISA

Sample ^a	Extraction at room temp.				Extraction at 4°C			
	Plate 1		Plate 2		Plate 1		Plate 2	
	UVA 93/03	NIBSC 82/518	UVA 93/03	NIBSC 82/518	UVA 93/03	NIBSC 82/518	UVA 93/03	NIBSC 82/518
A	0.47	0.57	0.45	0.53	0.3 (36%) ^b	0.35 (39%)	0.3 (33%)	0.34 (36%)
B	26	29	24	27	10 (62%)	12 (59%)	10 (57%)	12 (56%)
C	29	33	27	32	9 (69%)	11 (67%)	8.8 (67%)	10 (69%)
X	95	113	92	108	40 (58%)	47 (58%)	38 (59%)	45 (58%)
Y	159	189	160	187	82 (48%)	96 (49%)	81 (50%)	95 (49%)
Z	18	21	17	20	5.3 (70%)	6.2 (70%)	5 (71%)	6 (70%)

^aDust samples A-Z were extracted for 2 h at room temperature (25°C) or overnight at 4°C, respectively. Results were compared using two different allergen standards (UVA 93/03 or NIBSC 82/518) and the assays were carried out in duplicate on two separate ELISA plates. Results are expressed as Der p 1 content (µg/g).

^bFigures in parenthesis indicate the percent reduction in Der p 1 levels seen in samples extracted at 4°C.

The sensitivity of most indoor allergen ELISAs is of the order 0.5–2 ng/mL, with a linear range of 5–50 ng/mL. For assays of reservoir dust samples, this level of sensitivity is quite adequate and these assays have been widely used in air sampling studies (8,33–37). Increased assay sensitivity can be obtained by increasing the concentration of biotinylated detector MAb or by the use of various amplification systems that are commercially available (e.g., AMDEX, Amersham Pharmacia Biotech; ELAST, NEN Life Science products; and streptavidin-peroxidase polymer, Sigma, St. Louis, MO) (11,38). These systems use either catalyzed reporter deposition or polymeric streptavidin to amplify the assay signal and have detection limits in the pg range. This level of sensitivity may improve airborne allergen and personal sampling (*see* Article 4).

Rapid Tests for Indoor Allergen Detection

Environmental allergen avoidance is recommended as the first line of treatment for asthma and is endorsed by recent National Heart, Lung and Blood Institute (NHLBI) guidelines, an American Academy of Allergy, Asthma and Immunology (AAAAI) position statement and a European Allergy White Paper (39–41). In some cases, the recommendations suggest that exposure assessment can be obtained by patient history and tests for allergen sensitization (39). However, patient history may not be a good guide to mite or cockroach sensitization, or to animal allergen exposure, if the patient does not keep furred pets. Even if sensitization is demonstrated (e.g., by skin testing or *in vitro* tests), patients may not be, or may not believe themselves to be, significantly exposed at home. These considerations, coupled with the need to educate patients about the role of indoor allergens in causing asthma, have spurred the development of qualitative or semiquantitative tests for assessing allergen exposure. These tests are simple enough to be carried out in doctors' offices, clinics, or other health-care facilities, and in patients' homes. The forerunner of these tests was the guanine assay (mites are the major producer of guanine in house dust), but this test showed a variable correlation with allergen levels and did not achieve widespread acceptance (42).

A new generation of allergen detection tests is now becoming available that use specific MAbs or polyclonal antibodies (PABs) bound to membranes in simple dipstick or cassette formats. Three tests that are currently available are DUSTSCREEN, the ACLOTEST, and gold-based lateral flow tests. DUSTSCREEN (CMG-HESKA, Fribourg, Switzerland) is a nitrocellulose-based strip test that detects multiple allergens in a single test using one dust sample. The nitrocellulose strip has six "spots" that are precoated with capture MAb.

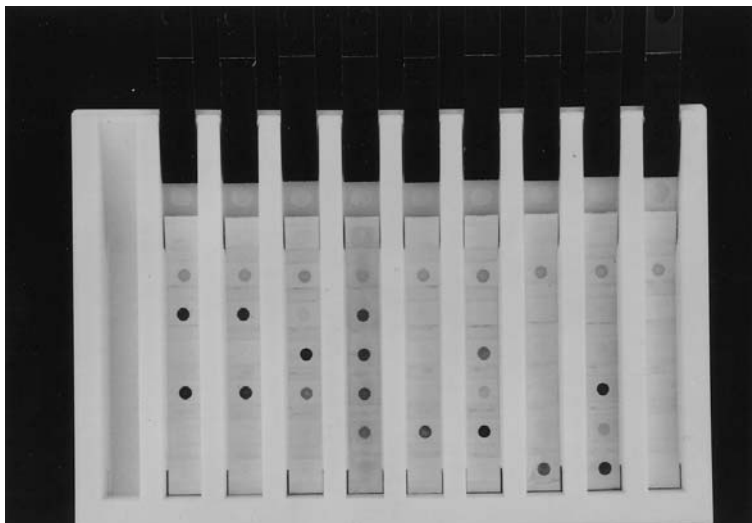


Fig. 1. DUSTSCREEN assay for multiple-test allergens in house dust. The strips are slotted into a plastic incubator and washing tray. Each strip contains controls and five dot areas for allergen detection: negative control, positive control, Der p 1, Der f 1, mite Group 2, Fel d 1, and Bla g 2 (top to bottom). Samples (left to right) were: mite extracts UVA 97/01; 93/03; 93/02; house-dust extract containing mite allergen; Fel d 1 standard 94/01; dust extract from house containing cats; Bla g 2 standard; dust from cockroach-infested house; PBS control.

Following incubation with dust sample, bound allergen is detected using a cocktail of enzyme-conjugated secondary MAb to mite cat or cockroach allergens (Fig. 1). This test can be quantitated using a strip reader and shows a good correlation with ELISA (43). However, the test takes several hours to perform and the procedure is best suited for pharmacy, clinic, or office use where allergen levels on dust samples could be obtained the same day. The ACLOTEST (Lofarma, Milan, Italy) is a dipstick test for mite that uses polyclonal rabbit antibodies to mite extract for allergen capture and affinity purified PABs conjugated to colloidal dye (Samaron Brilliant Pink) for detection. This test correlates with ELISA for mite allergen over the range 0.5–10 $\mu\text{g/g}$ and takes 30–60 min to develop (44). Results are graded as “negative” (no spot); weakly positive (light pink spot, 0.5–2 $\mu\text{g/g}$); or strongly positive (pink spot, >2 $\mu\text{g/g}$). At present, the test is only available for mite allergen.

The most recent advance is the development of true rapid tests for mite allergen that use lateral flow technology and gold-labeled MAb for detection. Capture MAb is “striped” onto the membrane and gold-labeled secondary MAb is impregnated in a pad with an absorbent

wick at one end of the membrane. When dust extract is applied to the wick, allergen diffuses into the pad and binds to the gold-labeled MAb. This complex flows further along the membrane until it meets the line of capture MAb, where the complex binds and forms a visible red line (Fig. 2). The intensity of color development is proportional to the allergen concentration in the sample. The lateral flow test has been developed for mite Group 2 allergen and has the advantages that it detects both *D. pteronyssinus* and *D. farinae*, is sensitive (detection limit ~100 pg) and results are obtained within 10 min (45). The test uses the same technology as pregnancy tests and is ideally suited to home use. Prototypes have been developed for mite Group 1 allergen, and Fel d 1. In principle, lateral flow technology can be applied to other allergens, e.g., cockroach, rodent urinary allergens, foods, and latex, thus providing simple tests of exposure for a variety of environmental allergens.

A central issue for indoor allergen analysis that applies to all of the tests described above (including ELISA) is the collection, processing, and handling of dust or air samples. Unlike pregnancy testing and other clinical tests using body fluids, allergen sampling requires collection of dust and extraction in an appropriate buffer, prior to assay. This remains the most time-consuming and contentious area of exposure measurements. Typically, research labs and commercial reference labs sieve dust, weigh out 100 mg fine dust for extraction, and express the results as μg allergen/g dust. These procedures have been endorsed by three International Workshops on Indoor Allergens and Asthma (see ref. 2). Some researchers use vacuum cleaners with adaptors that separate fine from coarse dust and avoid the need for sieving. However, for office-based testing or for patient use, sieving is not a feasible option and it is advisable to reduce to a minimum the numbers of steps required to prepare the dust sample. The most promising device currently available is the MITEST dust collector (MITEST, Dublin, Ireland). This collector fits onto the suction end of a vacuum cleaner and has an inner tube with a permeable membrane, which permits air flow, but enables the dust sample to be collected. An area of 0.25 m² is vacuumed for 2 min, after which the collector is capped at the lower end and buffer (~10 mL) is added. The top end is capped, the solution shaken and extracted for 5 min, and then applied to the allergen test. Using this collector together with gold-based lateral flow tests, a dust sample can be collected, extracted, and tested within 15–20 min (45). Although the MITEST device shows promise, it cannot be used on vacuum cleaners that do not have tube wands (e.g., upright cleaners) and it is designed as a single-use plastic disposable device, which is not environmentally

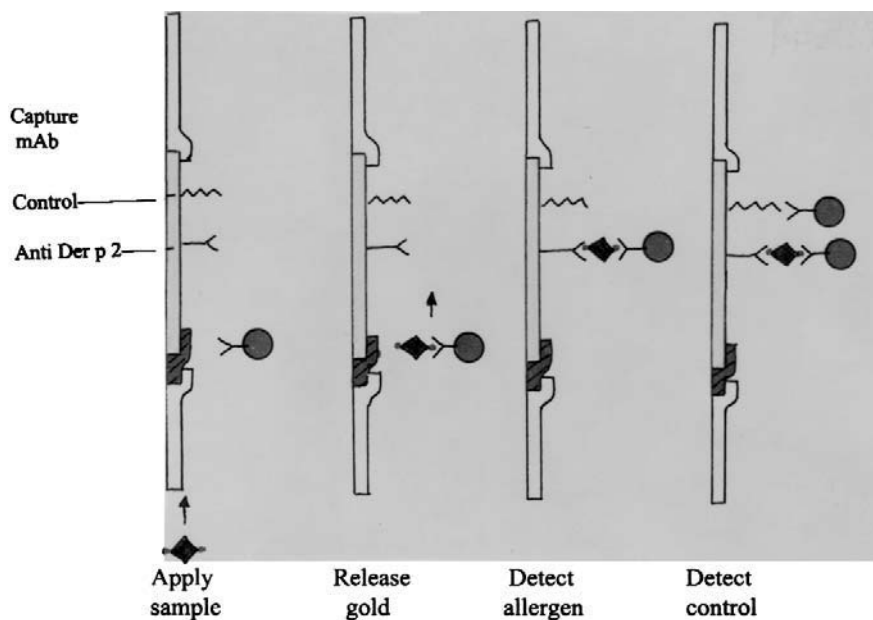


Fig. 2. Rapid lateral flow tests for allergen detection. Allergen sample diffuses along the wick, reacts with gold-labeled MAb and diffuses to the capture MAb zone. Here, the complex is bound and forms a red line. Unbound gold-labeled MAb diffuses further to a second capture MAb (anti-mouse IgG) and forms a positive control line.

friendly. Other possibilities include the use of paper collectors or filters that can be recycled.

Conclusions

Over the past 10 years, indoor allergen avoidance has become recommended as an integral part of the management of patients with asthma, together with anti-asthma drugs and immunotherapy. This recommendation is in large part owing to the extensive epidemiologic studies on sensitization and exposure to indoor allergens that have been carried out worldwide and in which immunoassays for measuring exposure have played a major role. Currently, several prospective studies are underway in Manchester and Southampton, UK, and in Boston, MA, to investigate the effects of early childhood exposure on asthma prevalence, including controlled trials of avoidance in infancy to see if effective reductions of allergen exposure at home decrease the prevalence of the disease (*see Article 7*). The outcomes of

these studies could have an enormous impact on the significance of indoor allergens from a public health perspective. The recent AAAAI position statement on environmental allergens emphasizes the fact that patient adherence to allergen avoidance recommendations is often low, between 20 and 50% in clinical trials, and likely lower in clinical practice. This underscores the need for better patient education and care to ensure that avoidance procedures are properly implemented. Monitoring allergen exposure can play a role in demonstrating whether patients are exposed to significant concentrations of indoor allergens and in encouraging compliance with avoidance procedures. However, there are notes of caution. The rub with allergen avoidance has always been that even if avoidance reduces allergen exposure by 80–90%, reservoir levels may remain higher than the proposed threshold levels for sensitization, e.g., 2 $\mu\text{g/g}$ mite allergen, in homes with very high allergen levels ($>10 \mu\text{g/g}$). This is a potential problem for patient-based tests, such as ACLOTEST or lateral flow tests, which may give positive reactions even when substantive allergen reduction is achieved. At present, such tests should be used as screening tests to demonstrate exposure, whereas ELISA is the preferred technique for following avoidance procedures. It has been estimated that a twofold reduction in allergen exposure could reduce the risk of developing asthma and asthma severity (46–48). Although this effect for a relatively modest reduction in allergen exposure may be optimistic, it suggests that reducing allergen levels of key reservoirs (in bedrooms, living rooms, and basements) by more than 50% may reduce asthma morbidity.

Immunoassays have clearly had major impact on research and clinical studies involving indoor allergens, but their potential in other areas of allergy research is only just beginning to be realized. Detection of food, latex, fungal, and pollen allergens is in its infancy and these are all areas that could benefit from this technology. Contamination of foods with allergens, particularly peanut, is a potentially life-threatening problem for highly sensitive patients and the use of immunoassays could help manufacturers prevent cross-contamination in food-processing operations. Immunoassays could also be used to verify that food products are free of allergens, e.g., by measuring the peanut allergens, Ara h 1, Ara h 2, or Ara h 3. Assays for latex allergens are needed to assess the allergen content of latex products and medical devices, as well as to assess environmental exposure to latex among health care and other workers, e.g., in operating rooms and clinics. Tests for fungi need to be improved by developing assays for different species, including species that may not necessarily present an allergy problem, but may be associated with toxic products, e.g., *Stachybotrys*. Finally, there are few assays currently available for grass,

weed, or tree pollen allergens and these are needed for allergen standardization and possibly for evaluation in exposure assessment. Pollen counts have been used for over 100 years and are now widely used in Western countries to predict pollen exposure for hayfever patients, e.g., through such organizations as the AAAAI and the National Pollen Network in the United States. Pollen-counting stations are time-consuming and potentially could be replaced by immunoassays.

The rapid tests have great potential for empowering asthma patients with simple methods for determining exposure to indoor allergens and to other allergens. Once these tests become widely available, it will be interesting to see whether new approaches to avoidance or new devices will come directly from patients. Another application of lateral flow technology is to measure allergen specific IgE antibodies in finger-prick blood from patients using membranes coated with cocktails of recombinant allergens. Although this approach may seem futuristic, the use of patient based self-diagnostic tests is increasing. Given the high percentage of the population that is allergic, the allergy market is an attractive target for this type of testing, especially in countries where allergy testing is not a high priority (e.g., the United Kingdom, where allergy evaluation does not routinely form part of asthma management). Over the next decade, we can look forward to further applications and advances in immunoassay technology, which should provide better understanding of the role of allergen exposure in asthma and lead to the development of effective intervention strategies.

Acknowledgments

We are grateful to Wanda Harvey for secretarial support. This work was supported by National Institutes of Health Grants AI 32557 and AI 34607.

References

1. Chapman MD. (1988), Allergen specific monoclonal antibodies: new tools for the management of allergic disease. *Allergy* 43(suppl. 5):7–14.
2. Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. (1997), Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* 100:S1–S24.
3. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. (1989), A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der p* I and *Der f* I. *J Immunol Methods* 118:227–235.
4. Yasueda Y, Saito A, Akiyama K, Maeda Y, Shida T, Sakaguchi M, Inouye S. (1994), Estimation of *Der p* and *Der f* 1 quantities in the reference preparations of *Dermatophagoides* mite extracts. *Clin Exp Allergy* 24:1030–1035.

5. Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD. (1994), Monoclonal antibodies to Group II *Dermatophagoides* spp. allergens: Murine immune response, epitope analysis and development of a two-site ELISA. *J Allergy Clin Immunol* 94:537–546.
6. Shen HD, Lin WL, Tsai LC, Tam MF, Chua KY, Chen HL, et al. (1997), Characterization of the allergen Der f 7 from house dust mite extracts by species specific and cross-reactive monoclonal antibodies. *Clin Exp Allergy* 27:824–832.
7. Bavbek S, Vailes L, Wah LB, Arruda LK, Chapman MD. (1997), Development of an ELISA for recombinant *Blomia tropicalis* allergen, Blo t 5: use in monitoring allergen exposure. *J Allergy Clin Immunol* 99:S162.
8. DeBlay F, Chapman MD, Platts-Mills TAE. (1991), Airborne cat allergen (Fel d 1): environmental control with the cat *in situ*. *Amer Rev Respir Dis* 143:1334–1339.
9. Ingram JM, Sporik R, Rose G, Honsiger R, Chapman MD, Platts-Mills TAE. (1995), Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relationship to sensitization and asthma among children living in Los Alamos, New Mexico. *J Allergy Clin Immunol*, 96:449–456.
10. Custovic A, Green R, Fletcher A, Smith A, Pickering CAC, Chapman MD, Woodcock A. (1997), Aerodynamic properties of the major dog allergen Can f 1: distribution in homes, concentration, and particle size of allergen in the air. *Amer J Resp Crit Care Med* 155:94–98.
11. Renstrom A, Larsson PH, Malmberg P, Bayard C. (1997), A new amplified monoclonal rat allergen assay used for evaluation of ventilation improvements in animal rooms. *J Allergy Clin Immunol* 100:649–655.
12. Pollart SM, Smith TF, Morris E, Gelber LE, Platts-Mills TAE, Chapman MD. (1991), Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol* 87:505–510.
13. Wu C-H, Chiang BT, Fann MC, Lan JL. (1990), Production and characterization of monoclonal antibodies against major allergens of American cockroach. *Clin Exp Allergy* 20:675–681.
14. Arruda LK, Mann BJ and Chapman MD. (1992), Selective expression of a major allergen and cytotoxin, *Asp f I*, in *Aspergillus fumigatus*: implications for the pathogenesis of *Aspergillus* related diseases. *J Immunol* 149:3354–3359.
15. Sporik RS, Arruda LK, Woodfolk J, Chapman MD, Platts-Mills TAE. (1993), Environmental exposure to *Aspergillus fumigatus* allergen (*Asp f I*). *Clin Exp Allergy* 23:326–331.
16. Vailes LD, Sridhara S, Weber B, Cromwell O, Breitenbach M, Chapman MD. Quantitative differences between specific allergen levels in allergenic products derived from fungi (*Alternaria* and *Aspergillus*). (Submitted.)
17. Portnoy J, Brothers D, Pacheco F, Landuyt J, Barnes C. (1998), Monoclonal antibody-based assay for Alt a 1, a major *Alternaria* allergen. *Ann Allergy Asthma Immunol* 81:59–64.
18. Bavbek LK, Vailes LD, Wah LB, and Chapman MD. Exposure and sensitization to *Blomia tropicalis* Group 5 allergens. (In Preparation.)
19. Hollander A, Gordon S, Renstrom A, Thissen J, Doekes G, Larsson PH, et al. (1999), Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy* 54:142–149.
20. Renstrom A, Gordon S, Hollander A, Spithoven J, Larsson PH, Venables KM et al. (1999), Comparison of methods to assess airborne rat and mouse allergen levels. II. Factors influencing antigen detection. *Allergy* 54:150–157.
21. Ford AW, Rawle FC, Lind P, Spielsma FThM, Lowenstein H, Platts-Mills TAE. (1985), Standardization of *Dermatophagoides pteronyssinus*: assessment of potency and allergen content in ten coded extracts. *Int Archs Allergy Appl Immunol* 76:58–68.

22. Larsen JN, Ford A, Gjesing B, et al. (1988), The collaborative study of the international standard of dog, *Canis domesticus*, hair/dander extract. *J Allergy Clin Immunol* 82:318–330.
23. Mayer CH, Bond JF, Chen M-S, Kasaian MT. (1994), Comparison of the levels of the major allergens Der p I and Der p II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 24:1041–1048.
24. Chapman MD, Smith AM, Slunt JB, Vailes LD, Arruda LK. (1995), Immunochemical and molecular methods for defining and measuring indoor allergens: in dust and air. *Ped Allergy Immunol* 6 (7):8–12.
25. Arruda LK, Vailes LD, Pomes A, Chapman MD. Establishing standards for cockroach allergens, Bla g 1 and Bla g 2. (In Preparation.)
26. Mueller G, Smith AM, Williams DJ, Hakkart G, Aalberse RC, Chapman MD, et al. (1997), Expression and secondary structure determination by NMR methods of the major house dust mite allergen Der p 2. *J Biol Chem* 272:26,893–26,898.
27. Mueller GA, Benjamin DC, Rule GS. (1998), Tertiary structure of the major house dust mite allergen Der p 2: sequential and structural homologies. *Biochemistry* 37:12,707–12,714, 1998.
28. Siebers R, Luey B, Crane J, Fitzharris P. (1997), The effects of temperature and buffer on the extraction of Der p 1 from dust. *J Allergy Clin Immunol* 100:580.
29. Patchett K, Lewis S, Crane J, Fitzharris P. (1997), Cat allergen (Fel d 1) levels on school children's clothing and in primary school classrooms in Wellington, New Zealand. *Clin Exp Allergy* 100:755–759.
30. Zhang L, Chew FT, Soh SY, Yi FC, Law SY, Goh DYT, Lee BW. (1997), Prevalence and distribution of indoor allergens in Singapore. *Clin Exp Allergy* 27:876–885.
31. Sawyer G, Kemp T, Shaw R, Patchett K, Siebers R, Lewis S, et al. (1998), Biologic pollution in infant bedding in New Zealand: high allergen exposure during a vulnerable period. *J Allergy Clin Immunol* 102:765–770.
32. Siebers R, Rains N, Fitzharris P, Crane J. (1999), The worldwide external Der p 1 quality control program: round three. In: *Mites and Domestic Design III*. (Siebers R, Cunningham M, Fitzharris P, Crane J, eds.), Wellington Asthma Research Group, Wellington, 2000; pp. 21–25.
33. DeBlay F, Heymann PW, Chapman MD, Platts-Mills TAE. (1991), Airborne dust mite allergens: comparison of Group II with Group I mite allergen and cat allergen, Fel d 1. *J Allergy Clin Immunol* 88:919–926.
34. DeBlay F, Sanchez J, Hedelin G, Perez-Infante A, verot A, Chapman MD, Pauli G. (1993), Dust and airborne exposure to allergens derived from cockroach (*Blattella germanica*) in low cost public housing in Strasbourg (France). *J Allergy Clin Immunol* 99:107–112.
35. Wood R, Laheri A, Eggleston P. (1993), The aerodynamic characteristics of cat allergen. *Clin Exp Allergy* 23:733–739.
36. Custovic A, Green R, Fletcher A, Smith A, Pickering CAC, Chapman MD, Woodcock A. (1997), Aerodynamic properties of the major dog allergen Can f 1: distribution in homes, concentration, and particle size of allergen in the air. *Amer J Resp Crit Care Med* 155:94–98.
37. Custovic A, Simpson A, Pahdi H, Green RM, Chapman MD, Woodcock A. (1998), Distribution, aerodynamic characteristics, and removal of the major cat allergen Fel d 1 in British homes. *Thorax* 53:33–38.
38. Bollinger ME, Wood RA, Chen P, Eggleston PA. (1998), Measurement of cat allergen levels in the home by use of an amplified ELISA. *J Allergy Clin Immunol* 101:124, 125.
39. National Asthma Education and Prevention Program, Expert Panel Report 2. (1997), Guidelines for the diagnosis and management of asthma. National

- Institutes of Health, National Heart Lung and Blood Institute. NIH Publication No. 97-4051.
40. American Academy of Allergy, Asthma and Immunology Position Statement. (1999), Environmental allergen avoidance in allergic asthma. *J Allergy Clin Immunol* 103:203–205.
 41. European Allergy White Paper. Allergic diseases as a public health problem in Europe. Van Moerbeke D (ed.) The UCB Institute of Allergy, Braine-l'Alleud, Belgium.
 42. Chapman MD. (1993), Guanine: an adequate index of mite exposure? *Allergy* 48:301–302.
 43. DeWeck AL, Derer M, Morrison-Smith G, Stadler BM, Walliser M. (1998), Dust-screen, a new assay for simultaneous determination of multiple allergens in house dust. *ACI Intl* 10:133–140.
 44. Mistrello G, Gentili M, Antoniotto P, Ottoboni F, Falagiani P. (1998), Dot immunobinding assay for the detection of mite antigens in house dust samples. *J Med Entomol* 35:143–147.
 45. Tsay A, Williams L, Chandler J, Chapman MD. (1999), Rapid test for mite allergen detection in the home. *J Allergy Clin Immunol* 103:S235.
 46. Peat JK. (1997), Can asthma be prevented? Evidence from epidemiological studies of children in Australia and New Zealand in the last decade. *Clin Exp Allergy* 28:261–265.
 47. Custovic A, Taggart SCO, Francis HC, Chapman MD, Woodcock AA. (1995), Exposure to house dust mite allergens and the clinical activity of asthma. *J Allergy Clin Immunol* 98:64–72.
 48. Peat JK and Li J. (1999), Reversing the trend: reducing the prevalence of asthma. *J Allergy Clin Immunol* 103:1–10.