What can we learn from multiplex allergen testing?

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Allergens and their Health Effects

In our practice, clients increasingly approach us with complaints related to asthma and allergy triggers. These need to be addressed separately from many of the other exposure-related risks we are used to dealing with on a daily basis. There is more to IAQ than monitoring radon, heavy metals, VOCs, biologic contaminants and molds. In the interest of your clients, you may want to include another class of biological compounds into your testing arsenal: Indoor Allergens.

Over the last few decades, there has been a progressive increase in the prevalence and morbidity of asthma in many parts of the world. This has arisen in parallel with changes in lifestyle towards sedentary living in warm houses with extensive furnishing and low ventilation. We now spend about 90% of our time indoors, and levels of many pollutants are 2-5 times higher indoors than outside. Current data suggest that approximately 10% of US children have asthma. This increase has been in perennial rather than seasonal asthma, and a large proportion of the patients are sensitized to one or more of the allergens found predominantly inside houses, that is, indoor allergens.[1] A large number of epidemiologic studies have established that allergen exposure in the home is a major risk factor for the development of allergic disease such as allergic rhinitis and asthma. In addition to allergen exposure in the home, occupational allergen exposure (i.e. laboratory animal handlers) is a common cause of occupational asthma.

A large proportion of the world's population is affected by allergies. If a person is allergic to, let's say cat, this person's immune system recognizes one or more certain proteins that the cat produces, and it mounts an IgE antibody-mediated allergic reaction to these proteins. These molecules are called allergens and tend to be small to medium-sized proteins or glycoproteins. What exactly it is that makes an allergen an allergen still remains to be defined. There appears to be no common biologic function or protein structure that characterizes a protein as an allergen. They have diverse functions and may be active or inactive enzymes, enzyme inhibitors, lipocalins, pheromone binding proteins or regulatory or structural proteins. A common feature, however, is that allergens are readily soluble and are therefore able to penetrate the nasal and respiratory mucosae, where they get in contact with our immune system and can cause an allergic reaction.

A systematic allergen nomenclature has been developed by the International Union of Immunological Societies' (IUIS) Allergen Nomenclature Subcommittee: the first three letters for the source genus followed by a single letter for the species and a number denoting the chronologic order of allergen identification. Thus, the abbreviated nomenclature for the dust mite *Dermatophagoides pteronyssinus* allergen 1 is Der p 1

and for the major cat allergen (*Felis domesticus*) is Fel d 1.[2] The most important indoor allergens are produced by the dust mites *Dermatophagoides pteronyssinus* (Der p 1, Der p 2) and *D. farinae* (Der f 1, Der f 2), cat (Fel d 1), dog (Can f 1), German cockroach (Bla g 1, Bla g 2), mouse (Mus m 1) and rat (Rat n 1). Sensitization and exposure to indoor allergens, particularly dust mites, animal dander, cockroach and fungi, are among the most important risk factors for asthma.[3]

The relationship between exposure, allergen sensitization and asthma has been most thoroughly explored for dust mite allergens. Epidemiologic studies in many parts of the world have established that exposure to 2 µg of mite group 1 allergen per gram of dust is likely to result in allergic sensitization. There is a dose-response relationship between exposure to the allergen and the number of individuals who become sensitized. There is further evidence that severity of asthma symptoms is related to the dose of mite allergen exposure. Childhood asthma is also strongly associated with sensitization to animal allergens, cockroach and, to a lesser extent, mold allergens. Cat allergen (Fel d 1) has a ubiquitous distribution in the environment and can be found at clinically significant levels even in houses that do not contain cats. This is also true for dog allergen (Can f 1). Rodent urinary proteins have long been associated with occupational asthma among laboratory animal handlers. High prevalence of sensitization and exposure to mouse allergen was reported among inner-city children with asthma.[4] Inner-city children are also at the greatest risk of developing cockroach allergy. Cockroach infestation of housing results in the accumulation of potent allergens that are associated with increased asthma mortality and morbidity among U.S. children, particularly African-American and Hispanic children, living in the inner-cities.[5] Cockroach allergens appear to be particularly potent, and cause allergic individuals to develop IgE antibody responses after exposure to ten- to hundred-fold lower levels of cockroach allergens than to dust mite or cat allergens.

Assessment of Allergen Exposure and Environmental Control are Part of Asthma Management

Exposure Assessment

Agencies such as the American Academy of Asthma, Allergy and Immunology (AAAAI) and National Heart, Lung and Blood Institute (NHLBI) stress the importance of indoor allergens and environmental control, and recommend for patients with persistent asthma, that clinicians should (1) identify allergen exposures; (2) use skin testing or blood testing to assess specific sensitivities to indoor allergens; and (3) implement environmental controls to reduce exposure to relevant allergens (Table 1) [6]. In collaboration with the client's physician recommendations, indoor air quality professionals can help fulfill two of the three recommended points by determining levels of indoor allergen exposure in the home, and also by providing qualified environmental controls to reduce the exposure, if required.

Table 1: Key Points: Control of Environmental Factors that Affect Asthma

Excerpted from: US National Heart, Lung and Blood Institute: Guidelines for the Diagnosis and Management of Asthma (EPR-3) [6]

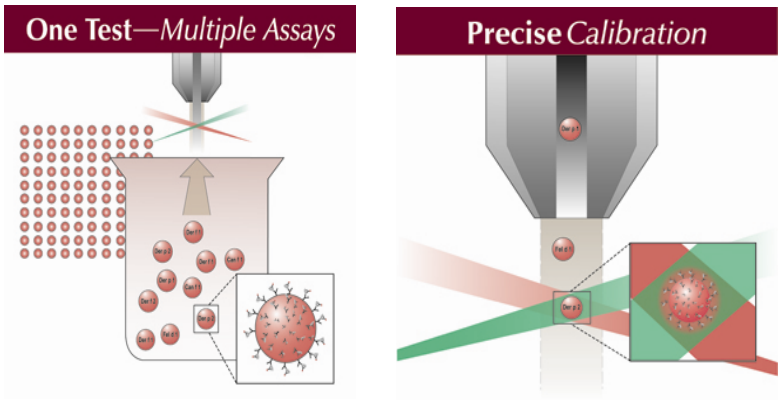
- **Exposure of patients who have asthma to allergens increases asthma symptoms and precipitates asthma exacerbations.**
- **Patients who have asthma should:**
  - **Reduce exposure to allergens to which the patient is sensitized and exposed.**
  - **Know that effective allergen avoidance requires a multifaceted, comprehensive approach**
  - **Consider allergen immunotherapy treatment**

How can we assess the level of allergens that patients are exposed to in their homes? In years past, biologists and pest management companies have previously counted dust mites in dust samples and trapped cockroaches to assess infestation and allergen exposure. While these methods are useful for certain purposes, they are unsuitable for routine measurement of allergen exposure. Allergen levels may remain high when mite or cockroach populations have been reduced; and simply counting mites or cockroaches is no reliable indicator of allergen exposure. Since the mid-1980s, quantitative assessments of allergen exposure have been made by measuring major allergens in reservoir dust samples. Samples are usually collected from three or four sites in the home, including mattresses, bedding, bedroom or living room carpet, soft furnishings or kitchen floors, by vacuuming each area of $1m^2$ for 2 minutes. It is also possible to collect airborne allergens using air filter cartridges. These dust samples or air filter cartridges are then processed in a qualified laboratory: Fine reservoir dust or filters are extracted in extraction buffer and the resulting extracts are analyzed for their allergen content using specific immuno-assays.

Quantitative allergen measurements of these extracts have been made for the past 20 years using monoclonal antibody-based enzyme-linked immunosorbent assays (ELISAs). These assays offer high sensitivity, high throughput, accurate quantification and defined specificity. Indoor allergen measurement by ELISA has been the gold standard for exposure assessment and a growing number of academic and commercial reference laboratories across the United States offer ELISA testing services. While the ELISA method provides reliable quantitative exposure assessment, it does require a separate test for each allergen. The associated time and cost involved is an impediment for large studies of exposure assessment and especially for studies involving multiple allergens.

Recently, a new state-of-the-art technology has been developed that allows the simultaneous detection of the most common indoor allergens in a single test. The Multiplex Array for Indoor Allergens (MARIA™) offers major advantages compared to ELISA such as the time savings achieved by analyzing multiple allergens at once, as well as improved sensitivity, accuracy and reproducibility. MARIA™ technology uses polystyrene microspheres that are internally dyed with distinct fluorophores to create as many as 100 distinctly coded bead sets. Capture antibodies can be covalently coupled to different beads and then used to develop quantitative immunoassays using biotinylated detector antibodies and a reporting fluorophore (Figure 1). For allergen exposure assessment, up to 11 common allergens of dust mite (Der p 1, Der f 1 and Mite Group 2), cat (Fel d 1), dog (Can f 1), mouse (Mus m 1), rat (Rat n 1), cockroach (Bla g 2), Alternaria (Alt a 1) and the pollen allergens of birch (Bet v 1) and timothy grass (Phl p 5) can currently be measured simultaneously using this technology. The detection panel is continuously being enlarged to include other allergens, such as food allergens and other molds.

Figure 1: Multiplex allergen detection using MARIA™



The major advantage of the multiplex array compared to ELISA is the time savings achieved by analyzing multiple allergens at once (Table 2a). The multiplex results have improved sensitivity, accuracy and reproducibility in comparison with ELISA [7] (Table 2b).

Tables 2 a and 2b: Comparison of Assay Performance between ELISA and MARIA™

2a: Comparison of required Assay Time
Example: 200 dust extracts to be analyzed for 8 allergens

	ELISA	MARIA™
Samples per plate	18	20
Number of plates required	12 x 8 = 96	10
Total technician time	**16 days**	**3 days**

2b: Lower Limit of Detection (LLOD):
MARIA™ is up to 40-fold more sensitive than ELISA

Allergen	**ELISA** (ng/ml)	**MARIA™** (ng/ml)
Der p 1	2	0.06
Der f 1	2	0.06
Mite Group 2	0.8	0.02
Fel d 1	0.8	0.02
Can f 1	2	0.06
Mus m 1	0.2	0.01
Rat n 1	0.8	0.02
Bla g 2	2	0.98
Alt a 1	0.8	0.02
Bet v 1	2	0.2
Phl p 5	4	0.2

As an extensive third-party validation of the technology, we conducted an NIH-funded multi-center ring trial of MARIA™ technology to establish reproducibility of data between as well as within laboratories. Nine laboratories within the US and Europe each analyzed the same set of 151 samples on three separate occasions. Data were compiled and then analyzed by an independent group of statisticians. Study results based on more than 32,000 individual allergen measurements showed that data obtained using MARIA™ technology are highly reproducible both within and between laboratories: Results within each study site correlated very closely ($r > 0.96$, $p < 0.001$). 53% of median coefficients of variance (CV%) within laboratories were within the 5% CV margin and 75% fell within 10% CV. Results between study sites were highly correlated for all allergens (~0.95, $p < 0.001$). Overall means of results were comparable between laboratories and inter-laboratory CV% ranged between 19% and 27%.

Simple qualitative or semi-quantitative tests are also available for certain allergens that can be used in allergy clinics, physicians' offices or by consumers. The aim of these "point of care" tests is to provide patients with tests that can be used to monitor allergen levels in their homes and to reinforce education about the role of allergens in causing asthma. Lateral flow technology has been used to develop rapid tests that can measure allergens within 10 minutes. These tests are analogous to pregnancy tests and are designed for use by patients and other consumers. The mite allergen test uses the same monoclonal antibodies as the mite group 2 ELISA and can detect both *D. pteronyssius* and *D. farinae*. The test includes a simple dust collection and extraction device (DUSTREAM Collector, Indoor Biotechnologies Inc. Charlottesville, VA, Figure 2) that allows dust to be collected and extracted within two minutes. The rapid test has indicator lines that provide patients with estimates of high, medium and low allergen levels. These lines have been shown to correlate with group 2 levels determined by ELISA (Figure 3).

Figure 2: *Dustream*™ dust collector

Figure 3: Semi-quantitative detection of mite allergen

Environmental Control

Expert guidelines recommend allergen avoidance as an important goal of asthma management. Targeted interventions in the homes of allergic individuals can significantly improve health and should be part of the management of children with asthma. Studies of inner city asthma demonstrated that reduction of indoor allergen exposure leads to improvement of asthma symptoms, associated with a reduced use of medication and also a reduction in lost work or school time due to asthma.[5] Allergen measurements have been used to validate the efficacy of a variety of physical and chemical control procedures and devices, including mattress encasings, vacuum cleaner filters, acaricides, protein denaturants, detergents and carpet cleaners, steam cleaning, humidity control and air filtration systems. It is important that products and devices be tested for their effects on specific allergens so that allergists can make objective recommendations based on scientific and technical data, and allergic patients and other consumers can verify claims made by the manufacturers.

Environmental allergen control protocols are dependent on the allergen [8]. The dust mites *Dermatophagoides pteronyssinus* and *D. farinae* live in the dust that accumulates in most homes, particularly within fabrics. Favorite habitats include carpets, upholstered furniture, mattresses, pillows and bedding materials. Their major food source is shed human skin scales, which are present in high numbers in most of these items, and the allergens are contained in the fecal particles that accumulate in their habitats. Dust mites prefer warm and humid conditions and grow very poorly when the humidity remains below 40%. They are susceptible to the effects of low as well as high temperatures. Freezing for 24 hours will kill most mites, as will exposure of carpets to direct sunlight for several hours, due to the high temperatures and/or low humidity.

Killing of the mites present in the home, however, will not necessarily reduce the mite allergen concentration, as the fecal particles containing the allergen will not be removed. Cost effective measures to reduce mite allergen in the home include encasing mattresses and pillows with tightly-woven materials that prevent penetration by mites as well as their fecal particles. Bed linens should be washed in hot water every 1-2 weeks. Stuffed toys should also either be washed in hot water or be removed from the bedroom. Carpets and hard floors should be vacuum cleaned and wiped regularly. It may also be helpful to remove carpets and soft furnishings, especially from the bedroom, in order to reduce potential mite habitats. An effective means to make the cleaned home less hospitable for new dust mite colonization is to reduce the relative indoor humidity to below 40%.

Control measures for animal allergens such as cat and dog allergens are more complicated. These allergens can be carried on particles of less than 5 micrometers in diameter that become airborne easily. Due to this characteristic as well as the fact that particles carrying cat and dog allergens appear to be very sticky, animal allergens can be found at significant levels even in homes, office buildings and schools that have never actually housed the respective animal. Environmental control of animal allergens should include removal of the source (i.e. finding a new home for the pet). Clinical benefits can, however, not be expected for weeks or months after removal of the pet, as particles are airborne and distributed over the entire home. Aggressive cleaning may help to reduce reservoirs of allergen. If the pet is not removed, HEPA or electrostatic air cleaners should be installed, especially in the bedroom, carpeting removed, and mattress and pillow covers should be replaced. If possible, animals may be washed at least twice a week.

Cockroach allergen in the home can be reduced by regular and thorough extermination of the infestation, followed by thorough cleaning. Neighboring dwellings should be included in the extermination program. In order to reduce the likelihood of further infestations, leaky pipes and faucets, holes in walls and other entry points should be repaired. Behavioral changes should be encouraged to reduce the availability of food sources that attract cockroaches, such as cleaning up immediately after cooking, avoiding open food containers and uncovered trash cans.

A wide variety of mold species can be present in both indoor and outdoor environments. *Aspergillus* and *Penicillium* species are generally regarded as the most numerous indoor molds, whereas *Alternaria* is important in both indoor and outdoor environments. Several mold allergens, including Alt a 1 and Asp f 1 have been characterized. Molds tend to grow best in warm and moist environments and mold exposure is therefore correlated with these conditions. Basements, window sills, shower stalls and bathroom carpets are common sites for mold infestations. Air conditioners and humidifiers have also been shown to be sources of significant mold exposure. The control of molds requires a concerted approach combining fungicides, measures to reduce humidity and removal of mold-infested items. A variety of fungicides are commercially available that are highly effective, as long as the sites of mold growth are carefully investigated. Any measures that can reduce humidity should be recommended, including dehumidification, air-conditioning, increased ventilation, and a ban on the use of humidifiers and vaporizers.

Conclusions

Indoor allergens are of tremendous importance to allergic disease. Exposure is a risk factor for the development of asthma as well as other allergic disease. There are measures that can help to reduce exposure to most allergens, which can reduce symptoms and medication requirements. The guidelines for the management of asthma made a very clear statement regarding the importance of indoor allergens and environmental control, stating that for any patient with persistent asthma, the clinician should (1) identify allergen exposures; (2) use skin testing or *in vitro* testing to assess specific sensitivities to indoor allergens; and (3) implement environmental controls to reduce exposure to relevant allergens [6]. Currently, measurement of allergen in reservoir dust samples provides the best index of allergen exposure in the home. Allergens can be accurately measured by ELISA for major allergens, which have been reported in more than 300 peer-reviewed publications. The next generation of tests uses fluorescent multiplex array technology (MARIA™) and enables multiple allergens to be tested simultaneously in dust or air samples. The combination of improved allergen-monitoring techniques and validated allergen-avoidance procedures should enable low allergen conditions to be established in homes. This strategy should improve asthma management and reduce the public health problems associated with sensitization to indoor allergens.

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