Assessment of Indoor Allergen Exposure

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Current Allergy and Asthma Reports 2005, **5:**394–401
Current Science Inc. ISSN 1529-7322

Of the four modes of treating human allergic disease, avoidance or separation of the allergic patient from the allergen source is most effective and least expensive. The clinical immunology laboratory has established efficient and inexpensive "reservoir" dust sampling and processing procedures to obtain a surface dust specimen that reflects the allergen burden of the environment. Following extraction, allergens are quantified by reproducible, validated immunoenzymetric assays for the quantification of "indicator" aeroallergen levels in home, school, and work environments. In this paper, the strategies and methods for collecting and processing dust samples are discussed, and assays are reviewed for quantifying indoor aeroallergen exposure from dust mites (Der p 1 and 2, Der f 1 and 2), animals (cat: Fel d 1; dog: Can f 1; mouse: Mus m 1; rat: Rat n 1), and insects (cockroach: Bla g 1 and 2). Accurate quantification of the levels of allergen in indoor environments facilitates avoidance therapy by identifying environmental risk factors for asthma and allergy exacerbation and allowing the allergic patient to monitor the effectiveness of environmental remediation actions.

Introduction

The management of immunoglobulin E (IgE)-mediated human allergic diseases, such as extrinsic asthma and rhinoconjunctivitis, involve a combination of four potential approaches: allergen avoidance, symptom-directed pharmacotherapy, allergen-specific immunotherapy, or hyposensitization and omalizumab (Xolair, Novartis, New York, NY; anti-IgE therapy). Of these, avoidance or the separation of the allergic patient from the allergen source is potentially the most effective, best-tolerated, and least expensive approach for reducing asthma and allergy symptoms [1•,2]. To facilitate avoidance of allergen exposure and document remediation actions in the allergic individual's personal and work or school environments, it is useful to document the actual level and location of the relevant allergens that can trigger symptoms. This is particularly important for areas where allergic individuals spend considerable time. The US Institute of Medicine [3] has identified eight indoor agents that are causally related to or associated with asthma exacerbation in school-aged children: dust mites, cats, dogs, cockroach, fungi, rhinovirus, environmental tobacco smoke, and nitrogen oxides. Other allergens, including proteins from weed, grass, and tree pollens; animals; insects; and foods can also be detected in indoor environments, but they are considered less causally related to asthma exacerbations [4•,5]. Table 1 lists the indicator allergens that are most commonly measured to assess indoor allergen contamination.

Primary Indoor Aeroallergens

Dust mites

House dust mites (Ascari order) are small (0.3 mm long) creatures related to spiders and ticks. The clinically important house dust mites are *Dermatophagoides pteronyssinus*, *D. farinae* in drier areas, and in subtropical and tropical regions, the glycyphagid mite *Blomia tropicalis* [6•]. Dust mites live on human and animal skin scales present in surface house dust, and they thrive in warm environments where the relative humidity is approximately 70%. Because humans shed large amounts of skin (5 g/wk), most homes, except those in arid climates or at high altitudes, are commonly infested with mites in beds (pillow, mattress, comforter), carpets, upholstered furniture, and stuffed toys. Mites reportedly produce up to 200 times their own weight in allergen-rich fecal pellets of a mean average diameter of 20 microns during their 2 to 3.5 month life span. Dust-mite allergen on the fecal particles can become airborne and inspired after a localized domestic disturbance during sleep or house cleaning. Acarologists use microscopic techniques to count the mite levels, with fewer than 20 mites per gram of dust being considered low and more than 500 mites per gram being a heavy infestation. Group 1 (25 kD) and 2 (14 kD) allergenic proteins from *Dermatophagoides* mites are considered the major allergenic specificities [7•], and, therefore, their presence and level in surface dust can serve as useful environmental indicators of the presence and relative quantity of house dust mites in an indoor environment. Dust-mite allergen measurements have also aided feather pillow and mattress manufacturers to optimize the processing of feathers used in bedding to minimize allergen content [8].

In terms of risk ranges for asthma developed from home studies, Der p 1 and Der f 1 levels higher than 2000 ng/G of fine dust have been associated with increased risk

Table 1. Indicator allergens for common indoor aeroallergen sources *

Common name	Genus/species	Indicator allergen	MW
Dust mite	*Dermatophagoides pteronyssinus*	Der p 1	25kD
		Der p 2	14 kD
Dust mite	*Dermatophagoides farinae*	Der f 1	25 kD
		Der p 2	14 kD
Cockroach	*Blatella germanica*	Bla g 1	NA
		Bla g 2	20 kD
Cat	*Felis domesticus*	Fel d 1	38 kD
Dog	*Canis familaris*	Can f 1	25 kD
Mouse	*Mus musculus*	Mus m 1	19 kD
Rat	*Rattus norvegicus*	Rat n 1	17 kD
Mold	*Alternaria alternate/tenuis*	Alt a 1	28 kD
Mold	*Aspergillus fumigatus*	Asp f 1	18 kD

* Reagents are available for IEMA quantification of these indicator allergens
Other potential allergns found indoors but which cannot be currently assayed by IEMA methods include pollens (trees, grasses, weeds), foods (peanut), mold spores (*Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*), and natural rubber latex (*Hevea brasiliensis*).
IEMA—immunoenzymetric assay; MW—molecular weight.

for sensitization in atopic individuals, and levels higher than 10,000 ng/G have been associated with an increased risk for symptoms [3,9].

Fur-bearing animals

Fur-bearing animals, including cats, dogs, guinea pigs, hamsters, rabbits, rats, and mice produce dander, saliva, and urine that contain allergenic foreign proteins capable of inducing allergic symptoms in humans. Larger pets, such as horses, cows, ducks, chickens, geese, goats, and pigs, also produce allergens that are considered to be primarily outdoor allergens, but they can be tracked into the homes, schools, and workplaces. Of these, the domesticated cat and dog and the mouse and rat are of the greatest concern in terms of inducing symptoms of allergy and asthma, owing to their status as household pets or pests.

The sublingual mucous salivary glands and hair root sebaceous glands of the domesticated cat (*Felis domesticus*) produce a potent 38-kD allergen (Fel d 1) [10]. It adheres tenaciously to fibers in carpets and dust particles from 2 to 10 microns in size that can be inhaled. Twenty or more weeks may be required after cat removal for levels of Fel d 1 to decline to those comparable with homes with no cats [11]. Fel d 1 serves as a heat-stable indicator of the level of cat allergen exposure in a home, school, and work environment. Levels of Fel d 1 higher than 8000 ng/G have been associated with an increased risk for sensitization, and levels > 80,000 have been associated with an increased risk for asthma symptoms [3,9].

Can f 1 (25 kD) is a major cross-reactive dog allergen, and it is used as an environmental indicator in surface dust for assessing the burden of dog allergen. Domesticated dog allergen is more heterogeneous than cat allergen, according to an international collaborative study that demonstrated IgE reactivity to more than 20 distinct protein bands [12]. Can f 1 is used as an indicator allergen to assess the level of dog allergen in indoor environments. Levels of Can f 1 higher than 10,000 ng/G have been associated with an increased risk for sensitization, and levels higher than 80,000 have been associated with an increased risk for asthma symptoms [3,9,13].

Mouse and rat allergens have been detected in air and surface dust collected from urban homes as part of the National Cooperative Inner-City Asthma Study [14•,15]. Mus m 1 (19 kD) and Rat n 1 (17 kD) are used as indicators of the presence of mouse and rat allergens, respectively, in indoor environments. Detectable levels of mouse allergen have been identified in 82% of US homes based on a National Survey of Lead and Allergens in Housing cross-sectional survey of 831 US households [16]. In 22% of US homes, the kitchen floor concentrations exceeded the 1.6 micrograms per gram level that is associated with increased sensitization rates.

Cockroaches

There are approximately 50 varieties of cockroaches, only eight of which are considered important sources of indoor allergens. Of these, the German (*Blatella germanica*) and American (*Periplaneta americana*) cockroaches have been the most extensively studied. Cockroach allergen exposure is prevalent in inner-city homes and more common than previously thought in suburban middle class homes with asthmatic children [17]. Because cockroaches breed in kitchens, bathrooms, or in the basement near central heating systems, these are important sites for reservoir dust sampling. Bla g 1 (25–35 kD) and Bla g 2 (36 kD) are used as indicators of the level of cockroach infestation [18]. Cockroach allergen has also been detected in nebulizers, causing asthmatic children to experience life-threatening exacerbations of their asthma after use of their nebulizer [19]. Levels of Bla g 1 or Bla g 2 higher than 1 U/g have been identified as a level of concern that may lead to sensitization [3,9,20]. Alternatively, because dust in walls and hidden areas around furniture where cockroaches reside is

so difficult to collect, any detectable cockroach allergen in a dust specimen may identify an environment in which a predisposed, atopic individual is at risk for sensitization or increased allergic symptoms.

Mold

Fungi are plant-like organisms with no chlorophyll, definite nuclei, and rigid cell walls. Their bodies are composed of long chains of cells called hyphae that are aggregated into mats called mycelia. Fungi use both asexual and sexual reproduction to produce spores that vary in length (1 to 100 mm), shape (spherical, cylindrical, oval, or elliptical), and surface characteristics (smooth, warty, spiny, or wrinkled). Most spores are of a respirable size range of 7 to 12 mm in diameter. Allergic fungal species have been identified virtually everywhere that sampling devices have been placed [21]. Universally dominant outdoor fungal species that are detected in indoor reservoir dust include *Cladosporium* (*Hormodendrum*), *Alternaria*, *Fusarium*, *Helminthosporium*. Two universally dominant and clinically important indoor fungi are *Penicillium* and *Aspergillus*.

There is a general lack of standardized protocols for assessing the genus/species and quantity of fungi in indoor environments [22]. Currently, immunoenzymetric assays (IEMAs) permit the quantification of select allergens released from *Alternaria alternate/tenuis* (Alt a 1, 28 kD) and *Aspergillus fumigatus* (Asp f 1, 18 kD). Despite the availability of these assays, Alt a 1 and Asp f 1 are rarely measured in indoor surface dust because these allergens are not always released into the environment when their respective fungi are present. This is probably due to the lack of ideal growth conditions in many environments. Alternative surrogate markers of fungal contamination, such as extracellular polysaccharides specific for *Penicillium* and *Aspergillus*, such as ergosterol, and beta (1→3) glucan, have been studied; they are discussed elsewhere [23].

Environmental Aeroallergen Analysis

The decision to initiate an indoor aeroallergen analysis begins with the identification of allergic respiratory symptoms (asthma, rhinoconjunctivitis) in a patient who has a documented allergen-specific IgE antibody response to one or more of the indoor aeroallergen groups [24]. Sensitization to dust mite, animal epidermal, insect, and/or mold allergens can be documented by in vivo (skin testing) or in vitro (serology) testing. Sometimes, allergic symptoms begin after a definable event, such as moving into a new home, work, or school environment, or extensive remodeling that exposes areas of the environment that had been previously sequestered (eg, moist inner-wall surfaces). As indoor allergens accumulate, the allergy symptoms may gradually intensify, stimulating an increase in the individual's sensitivity. Assessing whether an individual's allergic symptoms stem from indoor allergen exposure can sometimes be difficult because symptoms vary with the level of allergen in the environment. Allergen levels can be influenced by the building structure, humidity, and temperature; pets in and around the environment; the furniture and carpeting; heating and cooling systems; season; and number of residents. Some individuals perform a personal avoidance study to investigate whether changing their living space by going on an extended vacation or visiting relatives diminishes their allergy symptoms. However, for individuals who suspect but are not sure that they are allergic to one or more of the common indoor allergens, the first step in the process is to be evaluated by an allergist for confirmation of an indoor allergy. Once sensitization has been confirmed by the detection of allergen-specific IgE antibody to allergens from dust mite, cat, dog, cockroach, mouse, rat, or molds, an indoor environmental survey becomes a cost-effective and useful procedure.

Environmental Specimen Collection

Although airborne allergen should ideally be collected to assess what an individual is inhaling, collection of valid reproducible air samples that can be repeatedly tested for allergen content is complicated, even for the experienced industrial hygienist. For this reason, the specimen that is routinely collected by an inexperienced lay individual is surface dust that is considered the reservoir from which allergen is redistributed into the individual's air space. The locations within an environment that serve as ideal indicator sites for the assessment of allergen in reservoir dust vary widely with the construction of the building and the living tendencies of the inhabitants. Areas where the allergic individual spends most of his or her time should be identified and sampled. Historically, indoor dust collection before 1970 was restricted to wipe-sampling techniques [25•]. Despite its simplicity and low expense, surface sample collection with a swab or wipe (either wet or dry) may be considered, at best, a qualitative procedure with limited quantitative capability due to the low dust-carrying capacity of the collection device and poor reproducibility of the method. Other samplers, such as the surface deposition plate, mats, microscope slide plates, sticky tapes and rollers, and attic dust samplers, have been largely dismissed for environmental allergen collection, owing to their limited ability to sample large surface areas. In contrast, a surface reservoir dust sample can be collected with a number of vacuum sampling devices and procedures [26–28]. The greatest advantage of the vacuum-collection method is its ability to collect a large quantity of crude dust mass from a large surface area that can then be processed into a uniform fine-dust specimen. Moreover, the vacuum can remove allergen deeply embedded in carpeted surfaces more effectively than other collection methods.

An initial evaluation of an indoor environment often begins with a "global" dust specimen that is collected from all the principal living and working areas. This composite

dust specimen allows the allergic individual to survey the whole space in question at minimal expense. This strategy of sample collection also partly addresses the heterogeneity of the house dust composition that varies throughout a home as well as between homes, across seasons, and among locations within a given country [29]. For instance, specimens collected from kitchens may contain a low fiber content, whereas environments with high fiber that adsorb allergen tend to be found where pets reside. Particles greater than 75 microns in diameter that are commonly found in dust specimens collected from different areas of an indoor environment include food crumbs, dander, hair, synthetic fibers, soil starch, plant and insect parts, and pollens.

Once a high level of allergen has been identified with the global specimen, dust may then be collected from individual areas, some where allergic symptoms are minimal and others where symptoms are severe. The dust specimens should contain all particulate present in the environment, including sand or dirt, human and animal hair, textile fibers and lint (carpet fuzz), metal items (paper clips, pins, coins), human dander, and insect parts. Most of the nonallergenic components are then removed from the dust specimen by further processing (see later). If possible, dust collection should precede the administration of cleaning solvents, freshener powders, and other substances that are used on carpets and upholstered furniture (eg, talcum or baby powder, baking soda). These may compromise the analytic measurement by altering the pH, ionic strength, or protein matrix of the extracted allergen. Unfortunately, they cannot be removed from the specimen during its processing and extraction [30].

Sercombe *et al*. [31] have recently identified the (MITEST) dust collector (Indoor Biotechnologies, Charlottesville, VA), electrostatic cloth sampler, and press tape sampler as comparable to a vacuum sampler for collecting dust mite allergen samples. Alternatively, the nozzle sock (Johns Hopkins DACI Laboratory, Baltimore, MD) is an inexpensive vacuum cleaner adapter that is inserted into the end of a vacuum hose attachment [28]. It is manufactured from a refined flash spun polyethylene fiber pulp media (Pulplus, Hysurf, DuPont, Wilmington, DE) that gives it strength, high surface area, and relatively high porosity. Its retention efficiency at 10 ft/min is more than 95% for 1 micron latex particles and 53% to 78% for 0.3 micron particles, and it possesses a high dirt-holding capacity. In contrast with other collection devices, the Hysurf nozzle sock is not affected by water. It is stronger than filter paper, porous, and has static potential.

Locations where samples are ideally collected vary with the specific circumstances associated with the habits of the allergic individual. Carpeted and upholstered surfaces in bedrooms, living rooms, and recreation rooms, for instance, are key areas for sample collection if the question is the presence of dust mite contamination. In contrast, high humidity areas, such as the bath/laundry room, basement, and food-use sites, such as the kitchen, are areas where evidence of mold and cockroaches should be targeted. Because pet (cat/dog) and rodent epidermal allergens distribute themselves widely on small particles that remain airborne for long periods, sampling any environment where allergic patients spend their time should be satisfactory for assessing the presence of these allergens. Workplace and school reservoir dust specimen collection site selection should target areas where large numbers of allergic workers or students congregate, and where complaints have been reported. Paired dust samples should be collected from areas where symptoms are severe and compared with allergen levels in a "control" specimen that has been collected from areas where symptoms are absent.

Reservoir Dust Specimen Processing

Reservoir dust specimens commonly contain a myriad of particulates other than allergenic protein that need to be removed prior to extraction. This is especially important because environmental allergen results are reported in units or mass quantities of allergen per gram of dust instead of per unit area sampled. In a recent survey [32••], even experienced laboratories reported different methods for processing (sieving and extracting) reservoir dust specimens that contribute to interlaboratory variation. Most laboratories in North America sieve the crude dust through a 50-mesh metal sieve (50 wires/inch, each with a 0.009-inch diameter) onto waxed laboratory weighing paper to allow dust particles smaller than 250 microns to pass through. This process converts a nonreproducible dust specimen into a homogeneous specimen of fine dust particles that can be weighed out and extracted reproducibly for quantitative allergen measurements. Virtually all allergen is known to pass into the sieved fine dust fraction. Once sieved, the dust is weighed on an analytical balance. Typically 100 milligrams of fine reservoir dust are extracted in 2 mL of filtered phosphate-buffered saline (PBS) containing a carrier protein, such as 1% bovine serum albumin. The extraction process is rapid, with more than 99% of the allergen being extracted within 1 hour. However, for convenience, most laboratories rotate extraction tubes overnight (12–16 hrs). The next day, remaining solids are centrifuged into a pellet, and the supernatant containing the allergen is quantitatively removed and sterile filtered (0.22 micron filtration). The allergen extract is then frozen immediately at -20°C or lower. Exposure of the unprocessed fine dust specimen to the direct sunlight and repeated freeze thawing up to 20 times has not been shown to measurably alter the level of dust mite or cat allergen in the specimen [30].

Allergen Measurements

Two-site IEMAs are used to quantify house dust mite (Der p 1, Der f1, Der p 2, Der f 2), animal epidermal (Fel d 1, Can f 1, Rat n 1, Mus m 1), cockroach (Bla g 1, Bla g 2), and mold (Alt a 1, Asp f 1) allergens. The design of each of

these allergen immunoassays parallels the method initially reported for dust mite allergens [33]. To perform these assays, a capture antibody (typically monoclonal), calibrated allergen standard, and a biotin or enzyme-labeled detection antibody (typically monoclonal) are purchased from a commercial source (eg, Indoor Biotechnologies, Charlottesville, VA).

Each allergen IEMA is initiated by adsorbing the allergen-specific capture antibody on a plastic microtiter plate. Typical coating conditions involve 0.1 mL per well of chromatographically purified antibody at 3 to 10 micrograms per mL diluted in a non–protein-containing buffer (PBS or carbonate buffer). Following an incubation (1 hr at 23°C and overnight at 4°C), unreacted sites on the plate wells are blocked with an irrelevant protein (1% bovine serum albumin in PBS) for at least 1 hour, and then plates are washed four times with PBS 0.05% Tween 20 or another detergent-containing buffer. The calibrated allergen standard at eight serial dilutions, three control extracts (high, medium, and low) at 2 dilutions, and the test specimens, typically at three dilutions (undiluted, 1:20, 1:400) are pipetted in duplicate into the plate. Following a 1-hr incubation at 23°C or overnight at 4°C, the plate is rewashed, and allergen-specific detection antibody is added (1 μg/mL) to all wells. For some assays (Der p 1, Der f 1, Fel d 1), the detection antibody is biotinylated, and bound detection antibody is detected with an enzyme-conjugated streptavidin. For other assays, such as the Bla g 1, Bla g 2, and Can f 1 assays, the detection antibody is a rabbit IgG anti-allergen, and its binding is detected with an enzyme-conjugated anti-rabbit IgG reagent. Following the final conjugate incubation step, the plate is washed, and substrate is added. For horseradish peroxidase-based assays, 0.1 mL of 1 mM 2,2'-azino-di (3-ethyl benzthiazoline-6-sulphonic acid) (ABTS) is added to the plate in 70 mM citrate phosphate buffer, pH 4.3-containing hydrogen peroxide (1 μL of 30% hydrogen peroxide per mL of ABTS). The optical density (OD) is then read in each well, and the substrate reaction is stopped with sodium azide when the top point of the standard curve reaches an OD of 1.5. Unknown OD results are interpolated from the calibration curve in mass units per mL or units per mL for those assays that measure Bla g 1 and Bla g 2 and are not yet calibrated in mass units. The interpolated levels of allergen are then corrected for the mass of the dust extracted, and final results are reported as μg or U/g of fine dust.

Standards are calibrated against various reference preparations. For example, the Der p 1 standard has been substandardized against the World Health Organization and International Union of Immunological Societies *D. pteronyssinus* International Reference Preparation (NIBSC 82/518), assuming a Der p 1 content of 12.5 μg/ampoule. In contrast, the Fel d 1 standard is prepared from cat hair extract, and it is sub-standardized for Fel d 1 content from the US Food and Drug Administration Center for Biologics Evaluation and Research Cat E10 standard, which has a Fel d 1 content of 13.47 U/mL. Other standard preparations are calibrated as described by the supplier (Indoor Biotechnology, Charlottesville, VA)

Although the two-site IEMA is considered the most quantitative assay available, it can only be performed in a clinical immunology laboratory by technologists who have experience with highly complex immunoassays. An alternative semiquantitative lateral flow assay, the MITEST (referred to earlier), uses a colloidal gold-labeled antibody to detect dust mite group 2 allergen. A dust sample is collected with a vacuum using a special collector that permits extraction without sieving and centrifugation. The extracted sample is then applied to the assay device and results are provided as negative, low, medium, and high within 10 minutes. The semiquantitative levels from the MITEST correlated significantly with the levels of dust mite allergen detected by the quantitative IEMA [34].

Viable Mold Spore Assessment

Because Alt a 1 and Asp f 1 are not routinely measured in reservoir dust specimens by IEMA because of their limited utility as indicators for mold allergen contamination, a number of laboratories perform a conventional microbiological analysis. The number of viable mold spores in a sieved dust is determined by assuming that each viable fungal spore will produce a separate colony from which mycelia will be visually detected. A weighed sample of sieved but not extracted dust is aseptically layered onto a microbiological culture plate containing Sabouraud's dextrose agar. Selected antibiotics (penicillin, streptomycin, gentamycin) are added to the agar to prevent bacterial growth. All plates are incubated at 21°C in an inverted position, and the number of mold colonies is visually quantified at 24 and 36 hours. Once corrected for the starting weight of the sample, the number of colonies per gram of sieved dust observed at 36 hours is reported.

Dust samples with a high level of mold spores that overgrow their plate at 24 hours are automatically recultured using smaller dust samples. An aliquot of a quality control dust specimen containing a modest level of mold spores (10,000 to 13,000 colonies/g) is repeatedly analyzed in every run to ensure good inter-run consistency. Comments on the rate and density of mold growth are provided as supportive information related to the mold burden in the environment. The presence of high levels of bacterial growth (even in the presence of antibiotics) is noted when observed. Speciation of the fungi found in the specimen is rarely undertaken because its clinical value is unclear, except in cases in which the allergic individual inhabiting the environment is known to have a sensitivity to a restricted number of mold specificities.

Currently, there are no accepted risk ranges for any measure of environmental mold contamination, owing to the absence of definitive clinical studies. Because fungi are found in all outdoor and indoor environments, the level of

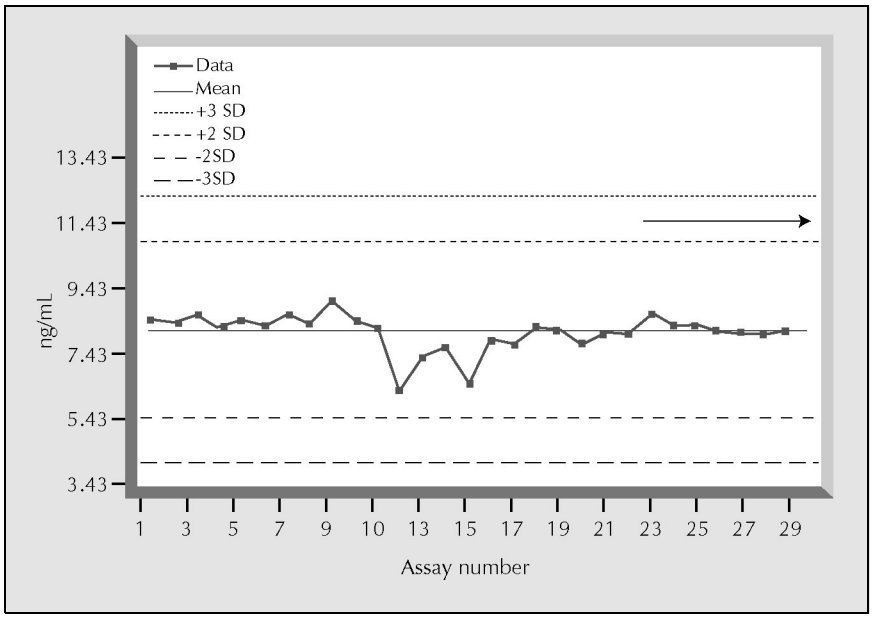

Figure 1. Representative Levy-Jennings quality control plot, *Dermatophagoides farinae*, of the middle quality control specimen for the Der f 1 immunoenzymetric assay. Der f 1 levels that were measured in 30 different assays are reported in nanograms per milliliter (ng/mL, Y axis) for each of the assay numbers (X axis) to examine trends and shifts. The mean and (+ or –) 2 and 3 standard deviations of expected values based on prior analyses of the same specimen are presented. SD—standard deviation.

mold spores in the outdoor environment directly influences the resultant level of spores detected in indoor reservoir dust. In one research study [21], *Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*, and *Rhodoturula* were identified in 65% of Baltimore homes, with total mold counts ranging from 133 to 25,367 colonies per gram of dust. The mold content was higher in homes where mildew had been reported for more than 3 months out of the year and in homes older then 30 years. In another survey of fine dust specimens collected from 358 homes throughout the United States, 10% contained very low levels from 200 to 1000 colonies per gram. Dust specimens containing mold colony counts in the top 50% of the group fell above a tentative action level of more than 10,000 colonies per gram of dust. Currently, colony counts more than 10,000 per gram may be considered sufficiently high to identify an environment where environmental intervention such as mildew removal, control of indoor humidity, and high-efficiency particulate air (HEPA) filtration of air may be appropriate. The correlation between an allergic symptom and the measured colony count is a subject that is under continuing investigation.

Quality Control and Proficiency Testing

There is inherent variability in every indoor aeroallergen measurement [35]. As household dust analyses become more common, there is an increased need for quality control procedures, to ensure minimal intralaboratory variability, and proficiency testing programs to assess interlaboratory variance with different methods of dust sample collection, processing, and analysis. Each laboratory should establish its own interassay quality control program that consists of analyzing extracts of house dust containing low, medium, and high levels of each allergen. Levels of allergen detected in nanograms per gram of fine dust following interpolation from a reference curve are plotted in a Levy-Jennings plot and are accepted at the discretion of the laboratory supervisor if they are in the three standard deviation control range, as defined by 10 previous measurements. Typical working ranges of the allergen IEMAs are Der p 1 (1 to 61 ng/mL), Der f 1 (1 to 45 ng/mL), Fel d 1 (1 to 80 U/mL), Bla g 1 (0.02 to 1.5 U/mL), Bla g 2 (0.02 to 1.7 U/mL), Mus m 1 (0.5 to 11.25 ng/mL), and Rat n 1 (1 to 90 ng/mL). Figure 1 displays a Levy-Jennings quality control plot for a representative Der f 1 IEMA in which the measured Der f 1 levels for the medium control are plotted for each of the assay dates.

Currently, no interlaboratory proficiency survey exists for indoor aeroallergen assays. Pate *et al.* [32••] investigated the feasibility of an interlaboratory proficiency survey for aeroallergens. Homogeneous batches of dust that were previously frozen following sieving were sent to eight laboratories for analysis by IEMA for Der p 1, Der f 1, Fel d 1, Can f 1, Bla g 1, and Mus m 1 IEMAs. Each laboratory used its standard procedures for sieving, extraction, and analysis of the allergen levels. Table 2 presents illustrative arithmetic means and intradust batch and interassay coefficients of variation for two quality control dust samples (*A* and *B*) prepared and analyzed in 2004. One hundred milligrams of each were weighed out three times (eg, *A1*, *A2*, *A3*), individually extracted, and analyzed by IEMA by one laboratory to investigate interdust batch variation and interassay variation. The two quality control dusts were matched such that Dust A had higher levels of Der p 1, Der f 1, Fel d 1, and Can f 1, whereas Dust B had higher levels of Bla g 1 and Mus m 1. The study concluded that the predicted confidence intervals for the allergen IEMAs were sufficiently precise to allow a laboratory to distinguish between residences with relatively low and high allergen levels. Importantly, identification of residences that were significantly above or below target risk

Table 2. Characterization of quality control dust aliquots for assessment of aeroallergen IEMA proficiency testing

		Dust mite allergens		Cat allergen	Dog allergen	Cockroach allergen	Mouse allergen
Code	N	Der f 1(μg/g)	Der p 1(μg/g)	Fel d 1(μg/g)	Can f 1(μg/g)	Bla g 1(U/g)	Mus m 1(μg/g)
A1	3	2.73 (19%)	56.9 (20%)	1500 (20%)	71.6 (18%)	0.4 (0%)	0.037 (35%)
A2	3	3.10 (11%)	41.5 (4%)	1460 (4%)	107 (4%)	<0.4	0.032 (99%)
A3	3	4.53 (9%)	41.5 (3%)	2130 (5%)	89.3 (6%)	<0.4	—
Pooled	9	3.46 (26%)	46.7 (21%)	1700 (21%)	89.3 (19%)	<0.4	0.034 (63%)
B1	3	0.333 (17%)	0.167 (35%)	35.5 (13%)	0.400 (87%)	13.3 (55%)	2.60 (68%)
B2	3	0.530 (25%)	0.184 (1%)	48.1 (2%)	0.378 (6%)	32.3 (58%)	1.84 (14%)
B3	3	0.520 (25%)	0.172 (10%)	54.0 (10%)	0.204 (13%)	NA	NA
Pooled	9	0.461 (30%)	0.174 (18%)	45.9 (19%)	0.327 (60%)	22.8 (73%)	2.22 (54%)

Aeroallergen levels are reported as arithmetic means and (percentage coefficient of variation) for each of two dust specimens (A and B) weighted and individually extracted three times each (A1, A2, A3 or B1, B2, B3). The pooled dust mite, cat, dog, cockroach, and mouse allergen data allow computation of the inter-batch coefficient of variation. Coefficients of variation less than 30% are considered acceptable, and those less than 15% are considered to be exceptional. Note the higher than desirable variation for the cockroach and mouse allergen IEMAs. IEMA—immunoenzymetric assay; NA—not applicable.
Adapted from Pate *et al.* [32••].

levels for sensitization and symptom induction (eg, 2 μg/g and 10 μg/g, respectively, for dust mite allergens) were distinguishable, with reasonable confidence. The study also confirmed the utility of frozen sieved house dust as a specimen for quality control and proficiency surveys. Interlaboratory variability was the dominant component of total variability in all IEMA analyses of the quality control dust extracts. Cited potential causes of this variability included technician error, limited shelf life of reagents, and differences in calibration of pipettes, extraction procedures, and operating conditions.

Conclusions

Accurate measurement of indoor aeroallergens that are known to drive asthma and allergy symptoms has become important, with an increased emphasis on avoidance of allergen exposure. Although the home environment has been the primary focus of most avoidance programs, work environments and, particularly, school environments are being increasingly examined for allergen exposures [36–38,39••]. The reservoir dust sample collected from all critical sites as a global specimen or from individual sites can be processed and extracted. Reproducible quantitative analytical measurements of dust mite, cat, dog, cockroach, mouse, and rat allergens allow accurate identification of environments that place sensitized individuals at risk for increased sensitization or allergic symptoms. Viable mold spore analyses can identify mold contamination within an indoor environment. With the knowledge of the allergen burden in a particular environment, remediation can be undertaken to reduce exposure and improve the quality of life for children and adults who suffer from asthma and allergies.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

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