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POSITION PAPER

Monitoring of occupational and environmental aeroallergens – EAACI Position Paper

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M. Raulf[1], J. Buters[2], M. Chapman[3], L. Cecchi[4], F. de Blay[5], G. Doekes[6], W. Eduard[7], D. Heederik[6], M. F. Jeebhay[8], S. Kespohl[1], E. Krop[6], G. Moscato[9], G. Pala[10], S. Quirce[11], I. Sander[1], V. Schlünssen[12], T. Sigsgaard[12], J. Walusiak-Skorupa[13], M. Wiszniewska[13], I. M. Wouters[6] & I. Annesi-Maesano[14,15]

[1]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Ruhr-Universität Bochum (IPA), Bochum; [2]ZAUM- Center of Allergy & Environment, Helmholtz Zentrum München/Technical Universität München, Christine Kühne Center for Allergy Research and Education (CK-CARE), Member of the German Center of Lung Research (DZL), Munich, Germany; [3]Indoor Biotechnologies Ltd, Cardiff, UK; [4]Interdepartmental Centre of Bioclimatology, University of Florence, Allergy and Clinical Immunology Section, Azienda Sanitaria di Prato, Prato, Italy; [5]Department of Chest Diseases, University Hospital Strasbourg, Strasbourg, France; [6]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, the Netherlands; [7]Department of Chemical and Biological Work Environment/National Institute of Occupational Health, Institute of Medical Biology, University in Tromsø, Tromsø, Norway; [8]Centre for Occupational and Environmental Health Research, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa; [9]Department of Public Health, Experimental and Forensic Medicine of the University of Pavia, Pavia; [10]Occupational Physician's Division, Local Health Authority of Sassari, Sassari, Italy; [11]Department of Allergy, Hospital La Paz Institute for Health Research (IdiPAZ) and CIBER of Respiratory Diseases CIBERES, Madrid, Spain; [12]Department of Public Health, Section for Environment, Occupation and Health, Aarhus University, Aarhus, Denmark; [13]Nofer Institute of Occupational Medicine, Lodz, Poland; [14]INSERM, Equipe Epidémiologie des allergies et des maladies respiratorires UMR-S 707, Paris; [15]EPAR, Faculté de Médecine Saint-Antoine, UPMC, Paris, France

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Correspondence
Monika Raulf, PhD, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Ruhr-Universität Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany.
Tel.: +49-(0)234-302-4582
Fax: +49-(0)234-302-4610
E-mail: raulf@ipa-dguv.de

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Abstract

Exposure to high molecular weight sensitizers of biological origin is an important risk factor for the development of asthma and rhinitis. Most of the causal allergens have been defined based on their reactivity with IgE antibodies, and in many cases, the molecular structure and function of the allergens have been established. Significant information on allergen levels that cause sensitization and allergic symptoms for several major environmental and occupational allergens has been reported. Monitoring of high molecular weight allergens and allergen carrier particles is an important part of the management of allergic respiratory diseases and requires standardized allergen assessment methods for occupational and environmental (indoor and outdoor) allergen exposure. The aim of this EAACI task force was to review the essential points for monitoring environmental and occupational allergen exposure including sampling strategies and methods, processing of dust samples, allergen analysis, and quantification. The paper includes a summary of different methods for sampling and allergen quantification, as well as their pros and cons for various exposure settings. Recommendations are being made for different exposure scenarios.

Key Message Box

- A key factor for the development of respiratory allergy is the contact between the respiratory organ and inhaled air containing the allergens; most airborne allergens are components of or carried by particles of 1–100 μm diameter.
- Aeroallergen monitoring is a stepwise process including selection of the suitable exposure assessment strategy, sampling methods, extraction procedure, allergen measurement, and data analysis.
- Choice of optimal procedures depends on the setting and objectives of allergen monitoring: in epidemiological (population) studies on exposure–response relation, in intervention studies, in diagnosis and follow-up of individual patients, in hazard identification for disease clusters, during identification of cases of 'new allergy' or as part of routine monitoring or health surveillance program.
- Ideally, allergen exposure assessment should be based on (active) measurement of airborne concentrations (outcome: allergen in ng (or pg) per m^3 or in particles per m^3). In occupational studies, low-flow personal airborne sampling in the breathing zone is the recommended standard procedure. For outdoor exposures, particularly for pollen and mold spores, stationary air sampling at high flow rates is commonly used. In the indoor (domestic) environment, the allergen content of settled or reservoir dust is typically used to provide estimates of exposure levels.
- Exposure assessment for individual patients or a specific location requires repeated measurements to account for temporal and spatial variations in airborne allergen levels. In population studies with larger numbers of subjects in exposure categories, repeated measurements are recommended in a limited subset to document the reproducibility of the procedure and the within-workers variation of exposure. In the diagnosis of individual patients, anamnesis must include attention to the exposure history, and if the combined evidence of anamnesis and diagnostic tests (e.g., skin prick test, specific IgE, provocation test) may be sufficient for a define diagnosis, management of the patient can be primarily focused on allergen avoidance without the need for allergen measurement.
- Allergen exposure assessment procedures should be adequately validated, and optimization of the elution and storage protocol is important. Immunoassays based on monoclonal or polyclonal antibodies are the method of choice for allergen quantification.
- Results from different immunoassays are not directly comparable and need to be standardized against known national or international standards.
- Effectiveness of interventions to reduce allergen exposure can only be evaluated if appropriate allergen measurements are included.
- More harmonized and validated exposure monitoring procedures to assess the relations between allergic outcome (sensitization, disease, efficacy of treatment, and preventive measurement) and allergen exposure are needed.

Exposure to airborne allergens of biological origin is an important risk factor for the development of allergic asthma and rhinitis (1). For this EAACI task force report, we defined 'allergens' as high molecular weight type I sensitizers; thus, macromolecules elicit specific immunoglobulin E (IgE) responses in humans and exclude microbial pro-inflammatory components such as endotoxin or β-glucans. Most of the thus-defined allergens are (glyco)proteins with molecular weights between 6 and 100 kD (refer to allergen databases in Table 1). Although low molecular weight sensitizers are of crucial importance for occupational airway disease, they are also excluded because nonimmunological methods are used for their air monitoring. In practice, however, the term *allergen* refers to either a single molecule, a mixture of molecules from the same source, or allergen-carrying particles such as grass pollen.

The most relevant route of exposure for developing respiratory allergy is the contact between the respiratory organ and inhaled allergens. Airborne allergens are not present as single molecules in the gas phase but components of identifiable particles such as pollen grains (10–60 μm) or mold spores (2–100 μm), or adsorbed to and carried on less well characterized and heterogeneous inorganic or organic dust particles as in house dust. As the site of deposition in the respiratory tract determines health outcomes (Fig. 1A,B), the aerodynamic size and shape distribution of the particles is of much relevance: inhalable, thoracic, and respirable fractions have been defined for occupational, indoor, and outdoor environments and should be measured depending on the outcome of interest.

Table 1 Databases of type I allergens and other agents causing occupational respiratory diseases

Allergen databases
http://www.allergen.org; WHO International Union of Immunological Societies:
http://www.allergome.org; Allergome:
http://www.meduniwien.ac.at/allergens/allfam/; AllFam:
Causative Occupational Agents databases
http://eaaci.net/sections-a-igs/ig-on-occupational-allergy/allergen-list/235-occupational-allergens-list
http://www.asthme.csst.qc.ca/document/Info_Gen/AgenProf/Bernstein/BernsteinAng.htm
http://www.aoecdata.org/
http://hazmap.nlm.nih.gov/index.php

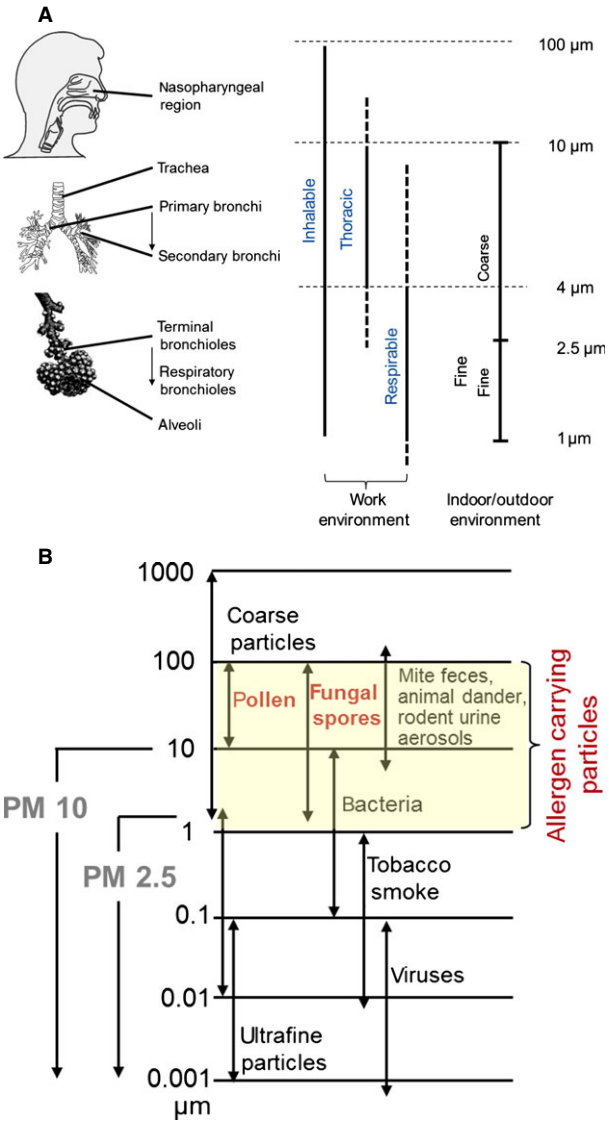

Figure 1 Definitions of dust fractions (A) Entry and interaction of particles with the respiratory tract. (B) particle size distribution of allergens.

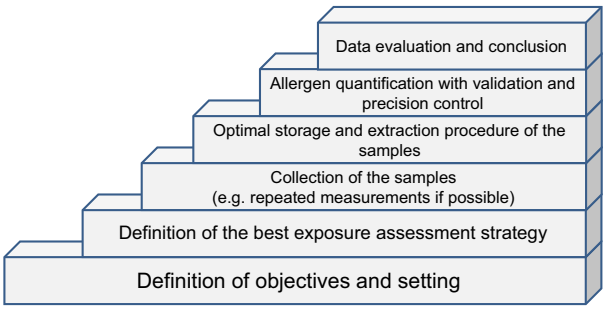

Figure 2 Stepwise selection process of methods and tools of allergen assessment. Depending on which exposure assessment strategy was chosen (e.g., measurement of inhalable particles), the sampling equipment (e.g., special filters mounted in a sampling head) as well as the following extraction procedures to recover the dust is needed to validate and optimize. It is not unlikely that optimal conditions for extraction may differ for each particular allergen and the type of samples from which it is to be recovered. Concerning allergen quantification, the specificity of antibodies just as the properties and purity of calibration standards are important. Results cannot be compared between studies when different standards are used. For more information on error rate see (9).

Knowledge of allergenic molecules has improved considerably in recent years. As a result, exposure–response relations with regard to allergic outcomes (sensitization, allergic rhinitis, allergic asthma, etc.) have been described for several allergens (2–6), but there is still a lack of information on exposure levels causing sensitization and allergic symptoms for many allergens. One reason is the complexity of airborne allergen monitoring, which is a multistep task requiring various tools and techniques and depending on the aim of the monitoring statistical methods (Fig. 2) (6, 7). Most of the available evidence comes from studies focusing on factors affecting the intensity of exposure to airborne allergens, as for house dust mite allergens in the home environment, pollen and mold spores in outdoor air, and a wide range of allergens from plants, animals, molds, or industrial products in the work environment; other studies have included allergen measurements when evaluating the effects of allergen avoidance measures.

The validity and accuracy of allergen exposure assessment in relation to the risk of allergies depends on well-validated methods to quantify airborne allergens. These procedures may consist of traditional microscopic methods to identify and count components or fragments containing allergens (e.g., mite, pollen or mold counting), or directly of immunological assays for which allergen molecules must be extracted from dust particles and/or filters (8). In some of the early measurement series, from epidemiological studies, exposure, for example, to wheat in bakeries has been assessed by traditional total dust measurements. This indirect approach is not meaningful to quantify potent allergens that sensitize workers at exposures in ng/m^3 range (9). Although in several epidemiological studies, for example, on seasonal allergy and asthma, monitoring of pollen count was used as proxy for environmental allergen exposure, the direct allergen measurements are needed to be validated against patients' symptoms. These examples demonstrate that for quantification of allergens, surrogates or proxies (such as gravimetric dust measurement, pollen counts, or enzymatic activity) are not recommended, because they only provide a crude approximation of actual exposure levels and they are often not sensitive or specific enough to determine allergen exposure responsible for sensitization or allergic symptoms.

The aim of this EAACI task force was to summarize information on allergen exposure assessment methods in the general indoor and outdoor and in the occupational environment and to appraise and assess their value for the establishment of exposure limits and implementation of allergen reduction strategies. The report includes exposure

assessment strategies, sampling methods, extraction procedures, allergen analyses, and epidemiological and clinical applications of allergen monitoring. It should be a reference document for researchers and occupational and environmental hygiene professionals (wish to use) using such methods and contribute to the quality of allergen exposure estimation and knowledge of allergen exposure–response relationships.

Methods

This consensus document was prepared by an EAACI task force panel consisting of experts in allergology and occupational hygiene and medicine, and researchers in method development for exposure assessment, as a concerted activity of members of the Interest Groups Occupational Allergy and Aerobiology & Air Pollution. The document was reviewed and accepted by the EAACI Executive Committee.

The literature was reviewed by the panel members for each section, and meetings were held to review findings and reach informal consensus. Further consensus was reached by an informal iterative process with input from all panel members into the draft document and summarized as the attached 'key messages' (see Key Message Box). The intention is not to provide a formal evidence-based guideline, but a document with practical information to support and assist allergologists, researchers, and professionals in the area of health and safety or occupational and environmental medicine, by summarizing the most important considerations that must be taken into account when planning and conducting allergen exposure assessment.

Exposure assessment strategies

Before starting allergen exposure assessment, the context in which the measurements are performed and the rationale must be clearly defined. Each setting has its own features, with consequences for selection of equipment and methods, for sampling and quantification of allergens, and for the design of the sampling strategy: that is, from which medium should be sampled, where and when, how often repeated, and with which time intervals? Answers may not only differ per allergen and type of environment, but also depend on the health effect for which allergen exposure would be a risk factor, and its presumed pathogenesis and kinetics.

It is important to recognize that the actual relevant allergen exposure can never be exactly determined. Exposure is defined as the intensity and frequency of physical contact between an agent and the relevant port of entry of the human body (10, 11) (Fig. 1). Thus, for induction of asthmatic reactions, the precise number of allergenic molecules deposited on the bronchial mucosae during a certain time interval preceding the symptoms should be determined, but this can only be estimated by a 'proxy' of exposure, such as allergen concentrations in the surrounding air – as in personal dust samples from the so-called breathing zone. For allergen exposure causing sensitization, one would like to know the average or cumulative levels of inhaled allergens deposited in the upper and lower airways during more prolonged periods. Airborne levels, however, show considerable temporal and spatial variation (12, 13). Results obtained on a single day or for one individual are usually not representative for exposure during more prolonged periods and cannot be generalized to a broader population with presumed similar exposure – such as all workers with the same job, inhabitants of the same home, or children of the same school class.

Outdoor allergen levels vary depending on climatic conditions, wind direction, and wind speed, and indoor exposures depend on home residents' activities, indoor sources, and on ventilation level and frequency. In work environments, exposure levels for the same environment or individual may vary across orders of magnitude within a few hours, as illustrated by job-task-related measurements (14–17). Such temporal and spatial variability is considerably larger than sampling (typically 10–50%) and analytical errors (<15–20%) for most allergen immunoassays.

In population studies, these problems can be resolved by grouping strategies and exposure modeling, in which individual exposure levels are not directly defined by measurements on the same subject but by the mean levels for the groups or categories to which he or she belongs (18). Such an approach is, however, not possible when dealing with individual patients or single locations. In these cases, a series of repeated measurements is required to reach sufficient accuracy.

Alternatively, a less variable but more remote 'proxy of exposure' can be used by measuring allergens in so-called dust reservoirs. Dust from floors, mattresses, or upholstered furniture mainly consists of particles with large aerodynamic diameter, too large to become and remain airborne for more than, for example, 15–20 min (5). Their allergen content may, however, be a reliable indicator of long-term airborne exposure levels. In fact, if reservoir dust allergen levels are relatively stable over time, the estimated – or only ranked and categorized – exposure may be more reliable than the more valid but imprecise airborne measurements at single moments (1).

In summary, the choice of methods, tools and the measurement strategy must be based on: a clear definition of the allergen exposure that is responsible for the induction of the health effect of interest and the known or presumed role of allergen exposure in its pathogenesis; validity; and precision of methods: Which provides the best proxy of exposure?

The sampling strategy must be such that replicate measurements at various moments and/or the same or different locations improve validity and precision: The best sampling and study design narrows the gap between the measured proxies and the actual exposure levels.

The main considerations of this paragraph are summarized in two decision trees (Figs 3 and 4) and will be discussed in the paragraph 6.

Sampling methods

Airborne allergen measurements make use of active volume-controlled sampling equipment, allowing results in allergen/m^3 level.

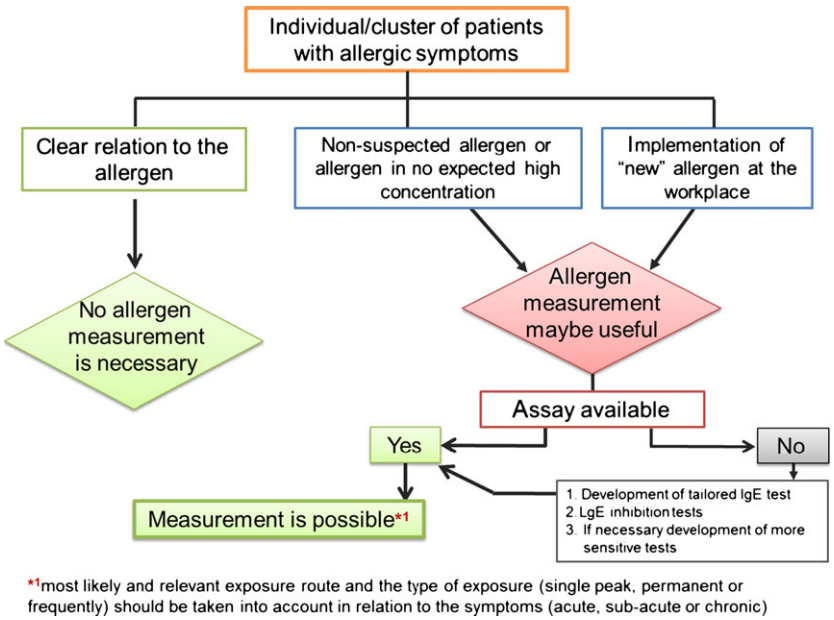

Figure 3 Decision tree of possible exposure assessment strategy for individuals or patients with allergic symptoms. If provocation tests, for example, with the suspected allergen source in addition to IgE measurement and skin prick testing, demonstrate sufficient evidence for a definite diagnosis, management of the patient can be primarily focused on allergen avoidance without the need for allergen measurements. For practical reason, allergen measurement in most idiopathic rhinitis patients with typical allergy symptoms seems to be not feasible, but should be an option if the symptoms persist. To avoid false-negatives and misclassification, elements of repetition should be implied, for example, by measuring on various days or taking samples for all or most of the patients.

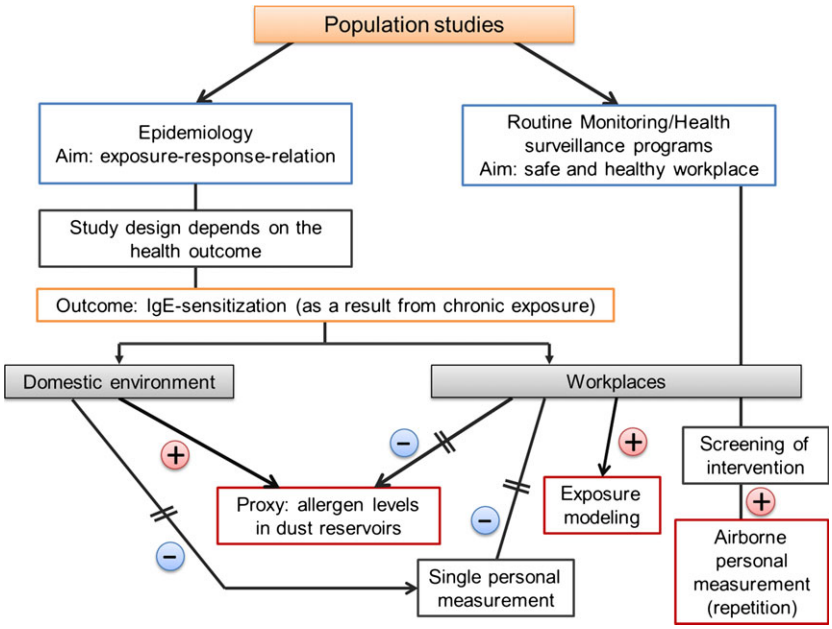

Figure 4 Decision tree of the exposure assessment strategy for population studies. Recommended or not recommended strategies are indicated by '+' or '−', respectively. Depending on the sampling place recommendation differs, for example, a regularly cleaned worksite should have no dust reservoirs with accumulated allergens.

Outdoor measurements are usually performed at a fixed location (stationary sampling), while in occupational environments, personal sampling is the first method of choice (Fig. 5) to assess what an individual is inhaling. In indoor environments with low air currents and turbulence, passive sampling of settled dust may be an alternative, resulting in

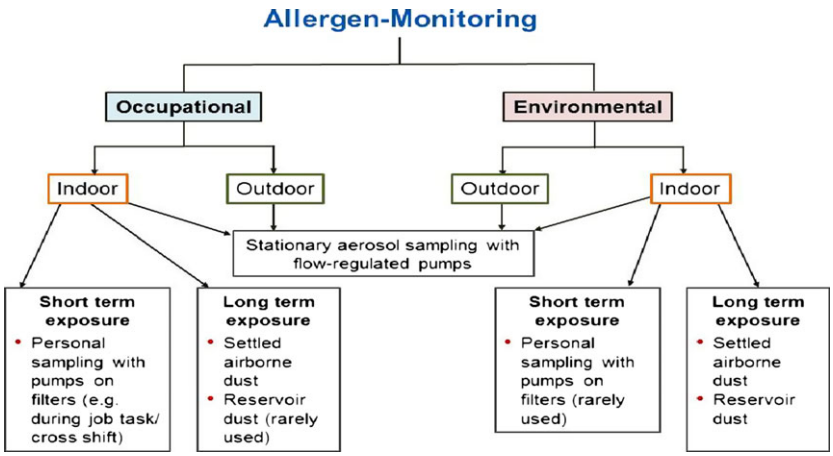

Figure 5 Options for allergen monitoring in occupational and environmental settings.

allergen levels in ng per m^2 and per day or week (19–22). Passively sampled airborne dust can be collected more easily over extended periods, thus reducing sampling errors due to temporal variability. The relation with airborne dust allergen levels is, however, complicated. Most of the settled particles must have been airborne, but smaller particles (<10 μm) settle with (much) lower efficiency, and the air volume from which settled particles are derived is unknown. Finally, during long sampling periods, allergens may either deteriorate or – if part of living (micro)organisms – be further produced (e.g., allergens derived from mites, cockroaches, etc. become airborne after fragmentation of mite bodies and fecal material).

Reservoir dust from mattresses, pillows, or carpets, as used in house dust allergen studies, is usually collected with a conventional vacuum cleaner using special sampling devices (see Settled and reservoir dust sampling), resulting in allergen levels in ng per μg dust (or μg/g dust).

A specific type of outdoor allergen measurement is pollen counting. The interest in pollen as allergen carriers has led to the development of monitoring networks in USA (23), Europe (www.ean.polleninfo.eu), and other parts of the world. Minimum requirements and guidelines for pollen counting are available from the European Aerobiology Society (24).

Air sampling

Particle size

Allergens from pollen, molds, plants, or animals can be found in all inhalable size fractions (diameter up to 100 μm), and inhalable dust is therefore commonly collected in workplace monitoring. The thoracic and respirable fractions consist of particles penetrating the airways and alveoli, respectively. Particles larger than 10-μm deposit in the nose and may induce hay fever, particles of 4- to 10-μm deposit in the lower airways and may cause asthma, and <4-μm particles can reach the alveoli and may cause allergic alveolitis. The size limits are, however, not absolute but defined as 50% deposition efficiency cutoffs (25). In the public health domain, similar particle size definitions (PM10 and PM2.5) are used to distinguish fractions depositing mainly in the airways and in the alveoli (26) (Fig. 1).

Pollen grains are typically >10 μm, and >90% of their allergens are detected in this >10-μm fraction (27), but under special weather conditions, for example, thunderstorms, pollen allergens can also be detected in the PM10 fraction (28, 29). Fungal spores are typically <10 μm, but often occur as larger aggregates, and therefore may cause both nasal symptoms and effects in the lower airways (30). Therefore, for allergen exposure, the larger particulate fractions are the most relevant ones, thus inhalable dust in the work environment and PM10 or larger for outdoor air. Most pollen grains and fungal spores are isometric particles (all three dimensions are of similar size), and their deposition in the airways is adequately described by their aerodynamic diameter, which includes impaction and sedimentation mechanisms. However, spore aggregates and fungal mycelial fragments may be more elongated, and skin scales have a flake-like structure, which increased their deposition due to interception. Such nonisometric particles have not only aberrant deposition, they have also aberrant dispersal due to floating behavior. These particles deposit to a greater extent than indicated by their aerodynamic size and are more likely to expose the upper airways.

Samplers

The methods and their application field are depicted in Fig. 5 and some technical details of equipment in Table 2. Equipment used in work environments differs because exposure levels are usually higher than in the general indoor and outdoor environment. Personal sampling is performed in the breathing zone of the worker with low-flow portable sampling pumps, while outdoor sampling is performed with larger stationary pumps at high flow rates (up to 60 m^3/h, see Table 2).

Aerosol samplers range from impaction devices (impactors and impingers with and without size separation) to cyclones, filter cassettes, or sedimentation devices. Inhalable, thoracic, and respirable dust sampling may be performed in parallel to assess health-relevant particle size fractions and provide a

Table 2 Some common samplers used for sampling of allergens

	Sampler name	Sampling principle	Sampled particles	Flow rate	Setting/usage	Analysis
Cyclone	Burkard (79)	Cyclone	>5 μm	16.6 l/min		Microscopy, counting, culturing, dust weighing, extraction for allergen quantification
Impactors	Harvard impactor (80)	Pump, cyclone preseparator, impaction on filter	PM10 or PM2.5 sampling	10–16 l/min	Outdoor and indoor	Microscopy, counting, culturing, dust weighing, extraction for allergen quantification
	Hirst automatic volumentric spore trap (81)	Impaction		1–10 m^3/h	Outdoor, 1–7 days, pollen and fungi	Microscopy, counting,
	Rotorod (82)	Impaction	>10 μm		Outdoor, pollen and fungi	Microscopy, counting
	Andersen, Chemivol (31)	Cascade impaction	Size separation <0.4 μm-≥10 μm-	0–60 m^3/h	Outdoor and indoor	Microscopy, counting, culturing, dust weighing, extraction for allergen quantification
	Respicon (83)	Virtual impactor, additional scattered-light measurement	Size separation, <4 μm, <10 μm, >10 μm	3.1 l/min	Outdoor, indoor, work environment, personal-related sampling	Photometric measurement of dust fractions, dust weighing, extraction for allergen quantification
	Gravikon PM 4 (84), Gravikon VC 25 (85)	Filtration in combination with impaction	Inhalable dust, respirable dust	4 m^3/h 22.5 m^3/h	Indoor, work environment	Dust weighing, extraction for allergen quantification
Filter sampling	IOM (86), PAS6, GSP (87), Millipore		Inhalable dust	2–10 l/min	Indoor, work environment, often personal sampling	Dust weighing, extraction for allergen quantification
Settled dust	Petri dishes, pizza box, EDCs (88–90)	Sedimentation of dust		–	Domestic or occupational indoor	Dust weighing, extraction for allergen quantification
	Durham (91)	Passive, gravity	>10 μm		Outdoor, pollen and fungi	Microscopy, counting, culturing
Reservoir dust	ALK device, Duststream Collector (Indoor) sock samples, wipes	Vacuum cleaning of a surface		–	Domestic indoor	dust weighing, extraction for allergen quantification
Nasal sampler	Inhalix (7, 92)	Impaction by person's own respiration		–	Outdoor and indoor	Extraction for allergen quantification

simple characterization of the allergen distribution over various particle sizes. More elaborate measurements make use of impactors for size-dependent fractionation, but few impactors are suitable for personal sampling.

A very specific type of sampler is the nasal sampler that collects particulates directly from the air inhaled by the test person (7). Theoretically, this should be a highly valid and relevant method, but it has some major drawbacks: Collection efficiency of particles is poor for particles <5 μm, sampling time is limited, the necessity of nasal breathing complicates application during heavy work, and particle collection on sticky substrates may complicate further analysis.

Sampling materials

Most airborne sampling is performed with capillary pore or fiber filters from which allergens can be eluted. The use of capillary pore filters has the advantage of easy access, better recovery, and direct (electron) microscopic study of particles. Alternatively, dust can be impacted in fluids (in impingers) or in dry tubes when using cyclones. Impactors have the advantage that the filters do not clog, as no air passes through the 'filters'. However, particle bounce can be a problem that is reduced using sticky substrates or porous 'filter' material.

Settled and reservoir dust sampling

Passive sampling of settled dust makes use of specific materials such as aluminum foil-covered pizza boxes (21), three-dimensional polyurethane foam (31), or electrostatic dust clots and wipes (20, 32).

Surface reservoir dust is collected on cellulose or glass fiber filters or in nylon bags ('socks'), from which the dust can either be easily removed or be extracted together with the filter or nylon sock (33). The major 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 (34). Most large-scale studies use the ALK device (ALK Allergologisk Laboratorium A/S, Horsholm, Denmark), MITEST (Indoor Biotechnologies, Charlottesville, VA, USA), or Dustream® collectors (Indoor Biotechnologies) for vacuum sampling of reservoir dust. They can be used in domestic and occupational settings.

Sample processing: storage, transport, and extraction

Before further analysis in, for example, immunoassays, sampled reservoir or airborne dust has to be converted into a particle-free supernatant, by elution from a filter or other collecting device during incubation and agitation in a well-defined medium, after which extracted aliquots can be stored frozen until analysis (Table 3). In the case of reservoir dust specimens that commonly contain a myriad of particulates other than allergenic proteins that need to be removed prior to extraction, for example, by sieving (34). The large majority of known IgE-binding allergens can be easily extracted in conventional buffers at physiologic ionic strength and pH, but may also be partially lost by adsorption to the filter, pipette tips, or the wall of the extraction tube. The addition of a mild detergent such as Tween-20 to the medium is therefore recommended, although its positive effects on allergen yield have been demonstrated systematically in only a few studies, mainly on occupational allergens (Table 4). Experiments to compare extraction procedures of airborne dust need at least two parallel filters with same amounts of dust and allergens. Recovery of allergens has also been studied with filters onto which allergen solutions had been spotted (35), but such experiments do not account for interference by other dust particle components during and after elution of the allergen. The use of side-by-side parallel airborne dust samples from a representative sample location much better reflects the actual monitoring situation (36). As dust load of parallel samples may still show substantial variation, special units for parallel sampling have been developed to obtain series of filters with nearly identical inhalable dust and allergen load (37, 38). With the use of such equipment in previous EU projects focusing on occupational allergens, only limited effects were found for most varying parameters in extraction procedures, such as use of different extraction tubes, incubation time, agitation method – whereas for both bakery and laboratory animal allergens, recovery was consistently better in media with a low concentration of Tween-20 (0.05%) (36, 39, 40). However, for other agents and assay procedures, the optimal detergent concentration may differ and other parameters can be relevant as well and should thus in principle be tested for each new type of allergen.

Allergen analysis

Standard methods for allergen quantification are immunoassays (7, 41, 42). A range of assays to detect environmental and occupational allergens have been described and applied, but only few of them are commercially available (Table 5).

The reagents may be antibodies from serum of specifically sensitized patients or from immunized animals. Animal antibodies can be polyclonal, recognizing a range of epitopes on various allergen molecules from the same source, or selected monoclonal antibodies against single epitopes on a well-defined allergen molecule. Polyclonal antibodies may thus be used to measure a mixture of allergenic molecules that nominally all belong to the same 'allergen', such as proteins from wheat flour, natural rubber latex, or molds, of which many contribute to the total allergenicity. Some assays, especially in a sandwich design, are sensitive enough to quantify airborne allergens, at least in occupational environments (43). Monoclonal antibody (mAbs)-based assays have a higher specificity, by measuring one specific allergen molecule, usually a so-called major allergen, and have been widely used as a proxy for exposure to a complete allergen source (e.g., assays for Der p 1 or Der f 1 to assess indoor environmental exposure to house dust mite allergens; for complete lists see Table 5). Both for the application polyspecific antisera or for the monoclonal antibodies, potential pitfalls should be taken into consideration. Polyspecific antisera obtained by immunization with crude antigenic mixtures will often react to more than a single antigen (e.g., an antiserum to mouse urine reacting not only with Mus m 1, but also with the mouse serum albumin). If the dust extract contains both antigens,

Table 3 Commonly used extraction protocols consist of five steps

1. Transfer to extraction tube Careful transfer of the dust-loaded filter, tissue, or other sampling equipment to an extraction tube of suitable size.
2. Addition of extraction medium Addition of an appropriate volume (from 5 to 20 or, in case of >1 g dust, up to 50 ml) of an extraction medium with physiologic salt concentration (0.15 M) and pH (7,8), usually with added detergent (0.05–0.5% Tween-20), such that the filter, cloth, etc., and the dust are completely immersed
3. Elution of allergens by agitation Incubation on a shaking platform, or in an end-over-end roller, during 30–120 min at ambient (room) temperature; extra modifications may be intermittent vigorous vortexing, sonification, or homogenization in case of fungal spores, to release dust particles from the filters and to disrupt spores or larger particle aggregates
4. Centrifugation Separate the solution of extracted components from the nondissolved dust, centrifugation at ~1000–1500 × g for 10–15 min is usually sufficient to obtain a dust particle-free supernatant
5. Harvesting and storage Harvesting the supernatant and storage in aliquots, at −20°C/−80°C, until later analyses. In some cases, addition of 'nonspecific' proteins, such as bovine serum albumin or casein, increases stability of aliquots

and the reference material contains only Mus m 1, or contains both antigens in a ratio different from the dust sample, a potential quantitation problem has to be considered. In addition, polyspecific antisera may contain highly cross-reactive antibodies, which require a careful control of the specificity of the test. MAbs directed to allergens are highly specific tools in allergen standardization and quantification. Their mono-specificity allows sensitive detection of individual allergens. It is the same quality that asks for caution (44): mAbs can be too specific and therefore isoforms of allergens can be missed. On the other hand, mono-specific antibodies can also be cross-reactive, possibly resulting in overestimation of allergen content. More details have been reviewed by international workshops on indoor allergens (1).

Immunoassays may be competitive or noncompetitive, with the essential antigen–antibody binding reaction occurring in the liquid or solid phase, respectively. Measuring airborne allergens, with levels in the pg to ng/m^3 range, requires high sensitivity, and therefore usually a two-sited sandwich enzyme immunoassay (EIA) with capture antibodies and labeled detection antibodies are used. Such assays can be 10- to 1000-fold more sensitive than an inhibition assay using the same antibodies (41, 42). Assay sensitivity can be further enhanced by signal amplification kits that utilize enzyme cascade or cycle systems, or conjugates with multiple enzymes (43, 45).

Recently, monoclonal antibodies have been used in fluorescent multiplex arrays that allow detection of multiple allergens in a single test. This principle has been successfully applied in a test system for up to eight allergens, which can be measured simultaneously under the same assay conditions (Multiplex Array for Indoor Allergens; MARIA™ system, Indoor Biotechnologies Inc, Charlottesville, VA, USA), thus improving standardization and reproducibility, and increasing the efficiency of measurements (46–48).

Other exposure assessment methods determine and count microscopically visible material from which allergens are released such as pollen, mold spores, or mite bodies. It is also possible to count allergen-bearing particles with the so-called halogen immunoassay (49, 50). Lateral flow immunoassays (LFIA) allow direct semiquantitative identification of allergens in a test solution or dust extract, as shown for alpha-amylase (51), laboratory animal allergens (52), and house dust mite (53); LFIA are particularly useful to quickly show the presence of allergens in a specific situation.

Unknown optical density results of the EIA are interpolated from the calibration curve in mass units per ml or units per ml, but the potency of the reference materials in mass units is often uncertain, for example, even for Der p 1. The factor used for the conversion from potency relative to the reference material into mass units has a substantial (<2-fold) uncertainty in the current literature. It is very important to recognize that measurement data produced by different immunoassays can only be compared if assay calibration standards are validated and tested against known national or international standards, where these are available (CREATE; 47, 54). The aims of the CREATE project were to identify candidate reference materials and ELISA reagents that would subsequently be prepared by regulatory authorities. This has indeed proved to be the case. The European Directorate for Quality of Medicines (EDQM) carried out a follow-up study (BSP090) to produce recombinant allergen reference preparations for Bet v 1 and for Phl p 5 (55). These references have been adopted by the European Pharmacopoeia Commission as Recombinant Major Allergen rBet v 1 CRS (Chemical Reference Substance) and Recombinant Major Allergen rPhl p 5a CRS, respectively. They are intended for use as reference preparations for determination of the Bet v 1 and Phl p 5a content, respectively, in allergen extracts and recombinant Bet v 1 and Phl p 5a preparations by ELISA and are publicly available (http://www.edqm.eu/site/News-EDQM-standards-1572.html). The EDQM is engaged in a program to develop other allergen standards, including Der p 1, Der p 2, and other major allergens.

Some further unresolved issues have to be kept in mind for quantification of the allergens: With regard to the validation of the lower limit of the detection of an allergen in a sample, not only the dose–response curve of a purified allergen is

Table 4 Published results on elution methods to obtain maximal yields of allergen

Antigen/Allergen	Assay types	Sample type	Varied parameters	Relevant parameters	Optimal condition	References
Phleum pratense pollen	Inhibition, human IgG_4	Spotted allergen on glass fiber filters	Detergent, agitation	Detergent, agitation	0.5% Tween-20 filter homogenization	(35)
House dust mite Der p 1	Sandwich, mAbs	Vacuumed dust	Buffer, extraction time, temperature	Buffer, temperature	Borate-buffered saline pH 7.4, 24°C	(93)
Rat urinary protein	Inhibition, human IgE	Airborne dust	Filter type, detergent agitation	Detergent	0.5% Tween-20	(94)
Rat urinary protein	Sandwich, pAbs	Airborne dust	Tube, detergent, agitation, centrifugation	Detergent	0.05% Tween-20	MOCALEX
Rat n 1	Sandwich, mAbs					
Mouse urinary protein	Sandwich, pAbs Inhibition, pAbs				0.05% Tween-20	
Mus m 1	Sandwich, pAbs					
Soy flour antigen	Inhibition, human IgG_4 Sandwich, pAbs				0.05% Tween-20	
Complete soy hull proteins	Inhibition, pAbs				0.05%/0.5% Tween-20	
Low molecular weight soy hull proteins	Inhibition, pAbs Sandwich, pAbs				0.05% Tween-20	
Wheat flour antigen	Inhibition, pAbs Inhibition, human IgG_4 Sandwich, pAbs	Airborne dust	Tube, detergent, agitation, centrifugation, storage	Detergent	0.05% Tween-20	(95)
α-Amylase Asp o 21	Sandwich, pAbs Sandwich, mAbs			Detergent, storage	0.05% Tween-20, addition of 0.1% casein	(40)
Potato antigen	Inhibition, human IgG_4	Airborne dust	Filter type, buffer, detergent, agitation	Detergent, agitation method	0.5% Tween-20, sonication plus vortexing	((38)
Aspergillus fumigatus antigens	Sandwich, pAbs	Airborne dust	Homogenization	Homogenization	Homogenization with glass/ceramic beads	(96)
Penicillium chrysogenum antigens	Sandwich, pAbs					
Cladosporium herbarum antigens	Sandwich, pAbs					

MOCALEX, Measurement of Occupational Allergen Exposure; http://ec.europa.eu/research/environment/pdf/env_health_projects/air_pollution/ap-mocalex.pdf.

Table 5 List of available allergen tests

Detected allergens	Standard test antigen	Type of assay	Antibodies	Detection limit	Company/Institute	References
Plants						
Wheat flour	Wheat flour protein mix	Inhibition ELISA	'human IgG4 or rabbit IgG'	20 ng/ml	IRAS	(51)
Wheat flour	Wheat flour protein mix	Sandwich ELISA	pAb	0.2 ng/ml	IRAS	(51)
Wheat alpha-amylase inhibitor Tri a 15	Wheat alpha-amylase inhibitor	Sandwich ELISA	mAb	0.1 ng/ml	Health and Safety Laboratory, UK	(97)
Rye flour	Rye flour protein mix	Sandwich ELISA	pAb	0.2 ng/ml	IPA	(66, 98)
Soy hull	Soy hull protein mix	Sandwich ELISA	pAb	0.04 ng/ml	Laboratorios Clinicos-Barcelona (IRAS)	(45)
Pollen – *Paritaria judaica*	Par j 1 and Par j 2	Sandwich ELISA	mAb	2 ng/ml	Bial-Aristegui	
Pollen – *Paritaria judaica*	Par j 1 (Par o 1/Par m 1)	Sandwich ELISA	mAb	0.2 ng/ml	Abello	
Pollen – *Plantanus acerifolia* Pla a 1	Pla a 1	Sandwich ELISA	mAb	3 ng/ml	Bial-Aristegui	
Pollen – *Plantago lanceolata* Pla l 1	Pla l 1	Sandwich ELISA	mAb	0.4 ng/ml	ALK-Abello	
Pollen – *Olea europea* Ole e 1	Ole e 1	Sandwich ELISA	mAb	0.1 ng/ml	Bial-Aristegui	(99)
Pollen – *Olea europea* Ole e 9	Ole e 9	Sandwich ELISA	mAb	20 ng/ml	ALK-Abello	
Pollen – Betula verrucosa Bet v 1	Bet v 1 (purified)	Sandwich ELISA	mAb	0.4 ng/ml	ALK-Abello	
Pollen – *Betula verrucosa* Bet v 1	rBet v 1	Sandwich ELISA	mAb	2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Pollen – *Cupressus arizonica* Cup a 1	Cup a 1 (purified)	Sandwich ELISA	mAb	62.5 ng/ml	Bial-Aristegui	(100)
Pollen – *Cupressus sempervirens*	Cup s 1 (purified)	Sandwich ELISA	mAb	31.25 ng/ml	Bial-Aristegui	
Pollen – *Phleum pratense* (group 1 grass allergens)	Phl p 1 (purified)	Sandwich ELISA	mAb	30 ng/ml	ALK-Abello	
Pollen – *Phleum pratense* (group 4 grass allergens)	Phl p 4 (purified)	Sandwich ELISA	mAb	150 ng/ml	Allergopharma	

Table 5 (Continued)

Detected allergens	Standard test antigen	Type of assay	Antibodies	Detection limit	Company/Institute	References
Pollen – *Phleum pratense* Phl p 5	rPhl p 5	Sandwich ELISA	mAb	4 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Pollen – *Cynodon dactylon* Cyn d 1	Cyn d 1 (purified)	Sandwich ELISA	mAb/pAb	1.1 ng/ml	ALK-Abello	
Pollen – *Lolium perenne* Lol p 1	Lol p 1 (purified)	Sandwich ELISA	mAb	125 ng/ml	Bial-Aristegui	
Pollen – *Artemisea vulgaris* Art v 1	Art v 1 (purified)	Sandwich ELISA	mAb	0.2 ng/ml	ALK-Abello	
Pollen – *Ambrosia artemisiifolia* Amb a 1	Amb a 1 (short ragweed extract)	Sandwich ELISA	pAb	0.002 U/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Latex *Hevea brasiliensis*	Latex protein mix	ImmunoCAP Inhibition	human IgE	200 ng/ml	IPA	(101)
Latex Hev b 1	Hev b 1 (purified)	Sandwich ELISA	mAb	2 ng/ml	IPA	(102)
Latex Hev b 1	Hev b 1 (purified)	Sandwich ELISA	mAb	1.2 ng/ml	Indoor/Quattromed	
Latex Hev b 3	Hev b 3 (purified)	Sandwich ELISA	mAb	2.3 ng/ml	Indoor/Quattromed	
Latex Hev b 5	Hev b 5 (purified)	Sandwich ELISA	mAb	0.5 ng/ml	Indoor/Quattromed	
Latex Hev b 6	Hev b 6.02 (purified)	Sandwich ELISA	mAb	0.1 ng/ml	Indoor/Quattromed	
Wood – obeche	Obeche wood protein mix	Inhibition ELISA	pAb	30 ng/ml	IPA	(103)
Molds						
Aspergillus fumigatus	*Aspergillus fumigatus* protein mix	Sandwich ELISA	pAb	0.15 ng/ml	IPA	(96)
Aspergillus fumigatus Asp f 1	Asp f 1 (purified)	Sandwich ELISA	mAb/pAb	0.32 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Aspergillus versicolor	*Aspergillus versicolor* protein mix	Sandwich ELISA	mAb	16 U/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Aspergillus versicolor Asp v 13	Asp v 13 (purified) or *Aspergillus versicolor* spores extract	Sandwich ELISA	pAb	0.0024 ng/ml	Ottawa-Carleton Institute of chemistry	(104)
Cladosporium herbarum	*Cladosporium herbarum* protein mix	Sandwich ELISA	pAb	0.05 ng/ml	IPA	(96)
Penicillium chrysogenum	*Penicillium chrysogenum* protein mix	Sandwich ELISA	pAb	0.15 ng/ml	IPA	(96)
Stacchybotrys chartarum	Protein mix SchX	Sandwich ELISA	mAb	16 U/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Stacchybotrys chartarum	Protein mix SchY	Sandwich ELISA	mAb	31 U/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Alternaria alternata Alt a 1	rAlt a 1	Sandwich ELISA	mAb	0.4 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits

Table 5 (Continued)

Detected allergens	Standard test antigen	Type of assay	Antibodies	Detection limit	Company/Institute	References
Aspergillus oryzae alpha-amylase Asp o 21	Asp o 21 (Fungamyl 1600S)	LFIA†	pAb/mab	1 ng/ml	IRAS/IPA	(51)
Aspergillus oryzae alpha-amylase Asp o 21	Asp o 21 Alpha-amylase (Megazym)	Sandwich ELISA	mAb	0.2 ng/ml	Health and Safety Laboratory, UK	(105)
Aspergillus oryzae alpha-amylase Asp o 21	Asp o 21 (Fungamyl 1600S)	Sandwich ELISA	pAb	0.02 ng/ml	IRAS	(40)
Aspergillus oryzae alpha-amylase Asp o 21	Asp o 21 (Fungamyl 800L)	Sandwich ELISA	mAb	0.08 ng/ml	IPA/Karolinska Institute	
Animals						
Cow hair	Cow hair protein mix	Sandwich ELISA	pAb	0.1 ng/ml	IPA; Germany	(19)
Cow dander Bos d 2	20 kDa allergen from bovine dander Bos d 2	Sandwich ELISA	mAb	1 ng/ml	University of Kuopio, Finland	(106)
Cow dander Bos d 2	rBos d 2	Sandwich ELISA	mAb	0.4 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Horse Equ c 4	Equ c 4 in extract	Sandwich ELISA	mAb	0.16 Units/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Dog Can f 1	Can f 1*	Sandwich ELISA	mAb/pAb	2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Cat Fel d 1	Fel d 1*	Sandwich ELISA	mAb	0.8 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Mouse urinary protein	Mouse urinary protein mix	Sandwich ELISA	pAb	0.075 ng/ml	IRAS; NL	(107)
Mouse urinary protein	Mus m 1*	Sandwich ELISA	pAb	0.2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Mouse urinary protein	Mus m 1 (purified from Indoor)	Sandwich ELISA	pAb	0.012 ng/m^3	University of Kuopio; Finland	(108)
Mouse urinary protein	Mus m 1 (purified)	LFIA†	pAb	semi-quantitative	Karolinska Institute, Sweden	(52)
Rat urinary protein	Rat n 1 (purified from Indoor)	Sandwich ELISA	mAb	0.098 ng/m^3	University of Kuopio, Finland	(108)
Rat urinary protein Rat n 1	Rat n 1*	Sandwich ELISA	mAb	0.8 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Rat urinary protein	Rat urinary protein mix	Sandwich ELISA	pAb	0.075 ng/ml	IRAS	(107)
Rat urinary protein Rat n 1	Rat n 1 (purified)	LFIA	mAb	semi-quantitative	Karolinska Institute, Sweden	(52)

Table 5 (Continued)

Detected allergens	Standard test antigen	Type of assay	Antibodies	Detection limit	Company/Institute	References
Fish (Pilchard and anchovy)	Fish protein mix	Inhibition ELISA	Human IgE pool	500 ng/ml	NHLS (IIDMM)	(109, 110)
Fish (Pilchard and anchovy)	Fish protein mix	Inhibition ELISA	pAb	200 ng/ml		(67, 111)
Fish (whiff and hake)	Fish protein mix	Inhibition ELISA	Human IgE pool	200 μg/ml		(112)
Crab	Crab protein mix	Inhibition IgE test	Human IgE pool	100 ng/filter 5–50 ng/m³		(113, 114)
Cockroach – *Blattella germanica* Bla g 1	rBla g 1 (purified)	Sandwich ELISA	mAb/pAb	0.08 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Cockroach – *Blattella germanica* Bla g 2	Bla g 2*	Sandwich ELISA	mAb/pAb	2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Domestic mites	*D. farinae* protein mix	Sandwich ELISA	pAb	0.05 ng/ml	IPA, Germany	(43)
Mite – *D. pteronyssinus*	*D. pteronyssinus* protein mix	Sandwich ELISA	pAb	0.6 ng/ml	IPA, Germany	(32)
Mite – *Acarus siro*	*Acarus siro* protein mix	Sandwich ELISA	pAb	0.15 ng/ml	IPA, Germany	
Mite – *Tyrophagus putrescentiae*	*Tyrophagus putrescentiae* protein mix	Sandwich ELISA	pAb	0.3 ng/ml	IPA, Germany	
Mite – *Lepidoglyphus destructor*	*Lepidoglyphus destructor* protein mix	Sandwich ELISA	pAb	0.8 ng/ml	IPA, Germany	
Mite – *Lepidoglyphus destructor Lep d 2*	rLep d 2	Sandwich ELISA	mAb	3.7 ng/ml	Karolinska Institute, Sweden	(115)
Mite – *D. pteronyssinus Der p 1*	Der p 1*	Sandwich ELISA	mAb	2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Mite – *D. farinae Der f 1*	Der f 1*	Sandwich ELISA	mAb	2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Mite – Mite group 2	Der p 2* Der f 2	Sandwich ELISA	mAb	0.8 ng/ml/2 ng/ml dependent of the standard	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Mite – *Blomia tropicalis Blo t 5*	rBlo t 5	Sandwich ELISA	mAb	2 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/ELISA-Kits
Allergen mixture mites, cat, cockroach, dog	Der p 1, Der f 1, Bla g 2, Fel d 1, Can f 1 *	MARIA™‡; 5-plex (AS-MRA-P5)	mAb	0.02 ng/ml	Indoor Biotechnologies	(47)
Allergen mixture mites, cat, dog, cockroach, rat, mouse	Der p 1, Der f 1, Mite group 2, Fel d 1, Can f 1, Bla g 2, Rat n 1, Mus m 1*	MARIA™‡, 8-plex (AS-MRA-P8)	mAb	0.01 ng/ml	Indoor Biotechnologies	

Table 5 (Continued)

Detected allergens	Standard test antigen	Type of assay	Antibodies	Detection limit	Company/Institute	References
Allergen mixture 1–11	Der p 1, Der f 1, Mite group 2, Fel d 1, Can f 1, Bla g 2, Rat n 1, Mus m 1, Bet v 1, Phl p 5, Alt a 1§	MARIA™‡, *PickYourPlex* (AS-MRA-C1-11)	mAb	0.01–0.98 ng/ml	Indoor Biotechnologies	http://inbio.com/EU/Products/MARIA-Kits

IRAS, Institute for Risk Assessment Sciences, University Utrecht, the Netherlands; IPA, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Ruhr-University Bochum, Germany.
*Universal Allergen Standard (UAS) containing: Der p 1, Der f 1, Der p 2, Fel d 1, Can f 1, Rat n 1, Mus m 1, and Bla g 2.
†LFIA, lateral flow immunoassay.
‡MARIA™, Multiplex Array for Indoor Allergens.
§Universal Allergen Standard containing: Der p 1, Der f 1, Mite group 2, Fel d 1, Can f 1, Rat n 1, Mus m 1, Bla g 2 plus individual allergen standards of Bet v 1, Phl p 5, Alt a 1.

important but also spike recovery in actual dust samples is an essential part of the protocol – also the parallelism of dose–response relation between sample and standard is essential – EIA based on monoclonal as well as on polyclonal antibodies has its specific features and therefore its specific limitations (e.g., with highly specific mAbs, underestimation of allergen variants with structural changes is possible and polyclonal polyspecific antibodies are more difficult to reproduce and define). Whether the application of other techniques such as PCR assays or mass spectrometric methods is suitable for allergen detection and quantification in environmental or occupational samples should be validated in the future.

Epidemiological and clinical aspects of allergen monitoring

Allergen measurements are applied in a range of settings, including the following:

- Diagnosis and management of individual patients.
- Population studies on exposure–response relationships.
- Intervention studies.
- Routine surveillance, mainly in the work environment.

This section discusses strategies and selection of tools and procedures in these settings, as summarized in the two 'decision trees' (Figs 3 and 4). In addition, allergen measurements are an essential component in allergen avoidance intervention studies and recommended in clinical trials.

Diagnosis of individual patients

Anamnesis of the allergic patient must include attention to the exposure history to identify allergenic exposures and trigger factors, their suspected source, intensity, peak concentrations, and frequency. Therefore, anamnesis items, for example, asking about the definable event after the allergic symptoms began such as moving into a new home or school environment or workplace or the implementation of 'new substances' at work, are helpful. In addition, asking whether changing their living or working space by going on an extended vacation diminished their allergic symptoms is also informative. A walk-through visit to the home or worksite can be useful, and industrial hygienists will also look at product inventory lists and material safety data sheets (56) and compare with compendia of environmental and workplace allergens and asthma-causing agents (see Table 1). When skin prick or IgE test results are positive, further conclusions will depend on the sensitizing potency of the allergen, duration and intensity of exposure, and existing control measures (57). Provocation tests with the suspected allergen source have to be included in the diagnosis procedure. If the combined evidence may be sufficient for a definite diagnosis, management of the patient can be primarily focused on allergen avoidance without the need for allergen measurements (Fig. 3, left column). But in symptomatic and sensitized patients without clear exposure, hidden allergen exposure can be detected by allergen measurement. (e.g., cat allergen can accumulate in homes that do not contain cats and can cause symptoms in allergic individuals.)

If exposure measurements are considered necessary for further confirmation, and assays to quantify the suspected allergen are available, an industrial or environmental hygienist may conduct area or personal sampling in the home or in high-risk departments at work. If suitable at workplace, 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. In some occupational settings, however, the allergen and its concentration may not be known when a new work process or product is introduced into the environment. This will require demonstration of allergens in the source material prior to airborne allergen measurements (58). If the suspected offending agent is a 'new' type of allergen, it may be necessary to first develop a specific assay, for example, using the patient's allergen-specific IgE (Fig. 3, right column).

Patients with a suspected indoor (e.g., house dust mite, pets, cockroach) allergy may also benefit from evaluation of aeroallergen exposure (34). If the question is the presence of dust mite contamination, carpeted and upholstered surfaces in bedrooms, living rooms, and recreation rooms are key areas for sample collection. Nevertheless locations for ideal sample collection vary with the specific circumstances associated with the habits of the allergic individual. Demonstrating the presence of the allergen in their home environment and identifying sources of high-risk exposure with the assistance of medical indoor environment counselor will convince the patient to pursue allergen reduction methods (59, 60).

A specific application in individual diagnosis is airborne allergen measurement during specific bronchial challenge in the diagnosis of occupational asthma (56). Allergen challenge chambers are also used for evaluating pollen-allergic rhinitis patients. Apart from careful compliance with standardized protocols, it is important to control and document the airborne and inhaled allergen concentrations during the challenge (61, 62).

Exposure–response relationships in population studies

Home environment

Many studies have demonstrated quantitative relationship between the risk of mite, cockroach, and/or pet allergy and the levels of these allergens in reservoir dust samples from floors or mattresses (2, 63). The actual shape of the dose–response curves – monotonously positive or bell-shaped optimum curves – and the (non-)existence of 'safe' threshold levels have, however, been a matter of longstanding and as yet unresolved debate (64). It further must be emphasized that only few studies have managed to relate allergen levels in reservoir dust to the actually inhaled airborne concentrations (2).

Occupational environment

Allergen exposure assessment in the workplace is increasingly used in epidemiological studies (65), especially in environments with dominant exposures to particular allergens such as bakeries, laboratory animal facilities, detergent enzyme manufacturing, seafood-processing environments, wood handling, and healthcare settings. In a dusty workplace such as bakeries, the flour allergen levels show a high correlation with dust concentrations (66), but for most other allergens and worksites, the allergen content of the airborne dust is highly variable, and dust is a very poor proxy of allergen exposure (67). Detailed exposure characterization studies are helpful in identifying high-risk groups of workers, work processes, or tasks, and the use of such information in exposure modeling has allowed the establishment of dose–response relationships for various occupational allergens (4, 68–71).

There do not seem to be threshold exposure levels below which risks are negligible, and the slope of the curves often suggests that to achieve a substantial – for example, 30–50% – risk reduction, the exposure should be lowered by at least one or even two orders of magnitude (68, 72).

Surveillance and routine allergen exposure monitoring

Workplace exposure surveillance can reveal when exposure controls are not functioning properly and quantify allergen levels associated with new onset or recurrence of allergic symptoms in exposed individuals (58). Similarly, domestic exposure monitoring can identify inadequate control or new allergen sources in symptomatic individuals on treatment (34, 63). Although results of routine monitoring cannot be compared with absolute standards or legally imposed threshold values, well-performed series of repeated measurements can be very useful to monitor longitudinal changes in exposure levels, especially when new equipment and procedures are introduced at the workplace, allergen avoidance measurements are introduced, or other major changes may affect exposure levels.

Intervention studies

Allergen avoidance

Aeroallergen avoidance in the home environment has been studied widely, especially for house dust mite and pet allergens. Prospective longitudinal studies of cohorts of newborns, descriptive or interventional, show the complexity of primary prevention of allergy development. Several studies have reported that primary prevention of allergic disease is also possible by strict allergen avoidance from birth, but in other cohort studies, no preventive effects of active interventions could be demonstrated. The role of allergen exposure as a primary cause of asthma or allergy incidence is still a matter of controversy and rethink of strategies for mite allergen avoidance including determination of when and how personal airborne exposure to allergen occurs is necessary (64, 73).

Cessation or reduction of exposure is also a key to the prevention and management of occupational asthma and allergy (56, 74, 75). The most convincing example of its effectiveness has been the substitution of powdered latex gloves with low-protein nonpowdered gloves (76).

Obviously, the eventual outcome of interest regarding avoidance studies is the clinical improvement of the patient (s) or a significantly reduced incidence of new allergies. However, interpretation of positive or negative results is only possible when the studies are complemented with exposure measurements before and after the introduction of the avoidance measures.

Clinical trials

Clinical trials to assess the efficacy of anti-allergic drugs and specific immunotherapy must take into account as potential confounders any changes in exposure levels during the trial. Outdoor ambient air pollen counts are considered the most reliable proxy for airborne pollen allergen exposure, and daily symptom scores for allergic rhinitis depend on airborne allergen exposure. Outdoor pollen counts should thus be used in clinical trials and documented, according to guidelines for Europe (EMEA, 2005), the USA (FDA, 2000), and the WAO (77). New methods accounting for the allergen content of pollen (27) may provide even better exposure estimates, but studies are still lacking in this regard. Regarding drug and immunotherapy trials in house dust mite allergy, EMEA has underlined the importance of validated methods to measure the exposure to these allergens serially (EMEA, 2005), even while levels of mite allergens often decrease during the trial (78).

Conclusion

Airborne exposure to high molecular weight sensitizers of biologic origin is a clear determinant of risk for the development of rhinitis and asthma. Therefore, the knowledge of exposure levels is helpful to estimate the risk of sensitization or induction of symptoms in occupational or environmental settings. Allergen monitoring is an important tool for exposure assessment and especially for intervention control. The stepwise process of aeroallergen monitoring, which is summarized in the paper includes the selection of the suitable exposure assessment strategy, sampling methods, extraction procedure, allergen measurement, and data analysis. Nevertheless, it is clear that measuring allergen concentration is only a proof of exposure and not a definite proof of, for example, occupational airway disease. Although more allergen exposure data are becoming available, more methods of allergen monitoring have been described and methods are becoming more accurate, there is a clear need for standardization of sampling and analytical procedures. Using different protocols for the sampling, elution and different immunoassays complicate the comparison of results of different studies. The development of consensus protocols based on validation studies and using them in studies with exposure assessment can be helpful in the future for a better comparison of data from different studies estimating the exposure dose–response interaction between sensitization and allergic symptoms.

Author contributions

This position paper is the result of the collaboration of a panel of experts who contributed to the document according to their different experiences and competences, coordinated by Monika Raulf, PhD.

Conflicts of interest

M. Chapman has a financial interest and ownership position in Indoor Biotechnologies. All other authors have no conflict of interest to declare.

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