

Microarrayed Allergen Molecules for the Diagnosis of Allergic Diseases

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Abstract IgE-mediated allergic diseases are among the most prevalent diseases worldwide. The use of extracts in the skin test and the additional use of IgE testing still represent the current basis for the diagnostic work-up. During the past 30 years, knowledge of the molecular structure of allergens has increased dramatically, and the characterization and production of allergenic molecules, as natural purified compounds or recombinant products, is allowing us to approach the allergy diagnostic work-up differently. Much of this is based on the adoption of microtechnology since the first release of a biochip for IgE detection. Its use has prompted the development of new concepts linked to the diagnosis of allergic diseases. This review describes the background of allergy diagnosis and the tools currently used for specific IgE detection. It gives insight into the most recent advancement in the field of biotechnology leading to allergenic molecule availability, microtechnology leading to the routine use of protein biochips for IgE detection, and how they should be combined with information technology.

Keywords Allergy · Diagnosis · Molecules · Extracts · Biochip · IgE

Introduction

The overall allergic disease picture is not shared by any other disease in the general population. It affects all ages

and both genders to almost the same extent and is globally distributed, competing in prevalence with infective- and parasite-related diseases. Epidemiologic and diagnostic reports on allergy-related symptoms come from all countries, developed or developing. This holds true for asthma-related symptoms as well as for rhinoconjunctivitis or eczema. Pathogenic mechanisms, which can be immunologic or not, are the leading cause of clinical diseases such as asthma, urticaria, rhinitis, eczema, conjunctivitis, and anaphylaxis [1]. When an immunologic mechanism is suspected, IgE plays a fundamental role in the immunopathogenesis of the allergic disease [2]. In most cases, the IgE-mediated mechanism is the only mechanism involved. Thus, the first step in allergy diagnosis is to identify any allergen-specific IgE and to define whether it is related to the current disease or not.

This review describes established tools used for specific IgE detection and compares them with the most recent application of allergenic molecules and microtechnologies to IgE-mediated allergic diseases.

Current Tools: Skin Test and IgE Singleplex Detection Systems

Identification of the triggering allergenic sources is the basis for allergy diagnosis. The research on organisms and their tissues releasing allergenic compounds dates back more than 100 years and is still ongoing. Allergome, a Web-based platform (<http://www.allergome.org>) collecting information from international scientific literature, currently lists 1739 organisms and tissues from which allergenic molecules are released. Allergenic extracts, obtained from allergenic organisms or from one or more of their tissues, are applied in vivo to patients to stimulate an allergic

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reaction as a true provocation test (ie, food challenge or nasal/bronchial mucosa exposure) or as a skin test. Such tests can hardly be standardized in the way any laboratory test should be, largely due to issues with the main reagent material, the extract. Limitations in achieving such results have been reported and discussed in many reports even recently [3•].

Better results have been achieved in the quality of allergy testing with IgE laboratory-based detection systems, mainly after the release of second-generation in vitro detection methods [4••]. The time required for testing started to reach the range of hours, and automation reduced the need of human resources, thus reducing the costs and improving the overall quality of allergy testing. Studies run several times to comparatively evaluate skin test and in vitro specific IgE detection led to the conclusion that virtually the same results can be achieved by both methodologies, with advantages and disadvantages for each [5, 6]. Nevertheless, the skin test remains the preferred method for allergy diagnosis, as the cost incurred is much lower compared with the same number of tests applied to any in vitro system. Standard panels of food or inhalant allergenic sources required for a comprehensive screening of the allergic patient have limited the use of in vitro systems as the exception rather than the routine approach.

Allergens: Extracts or Molecules

Allergenic extracts are a heterogeneous and unpredictable mixture of allergenic and nonallergenic proteins. Papers reporting how the starting raw material or the final product can rarely be standardized are available in the scientific literature [7–9]. The need for improved allergenic extract standardization has led to the use of molecules as reference material [10, 11•]. Unless this approach produces a consistent step forward in improving extract quality, the extract per se cannot lead to the dissection of a patient's specific IgE allergenic molecule recognition patterns [12, 13].

During the 1980s, the availability of advanced biochemical and molecular biology techniques resulted in the characterization of the most important allergens and a dramatic increase in our knowledge about allergenic molecules. The increasing number of publications has led to a better understanding of the link between allergenic structures and allergic diseases [14]. Currently, 1783 allergens have been described, although different characterization levels have been achieved for each (<http://www.allergome.org/script/statistic.php>).

Many molecules have been used for skin testing, mainly to prove their biological activity in selected patients [15]. A few recent published reports have shown the feasibility of a molecule-based skin test for allergy diagnosis [16, 17•].

Nevertheless, allergenic molecules have not yet entered into the routine diagnosis of allergy, mainly because very few commercial preparations are available for in vivo application. The cost is high because the molecule concentration used in the skin test ranges between 10 and 100 µg/mL and because of the huge number of preparations that need to be applied.

Furthermore, a relevant limitation to the in vivo use of allergenic molecules is the regulatory need to have all the preparations, natural or recombinant, approved as drugs used in humans. Nevertheless, the clinical relevance of an allergenic molecule-based diagnosis has become more and more evident by the use of small panels of allergenic molecules via established in vitro systems [18, 19]. An increasingly comprehensive panel of natural and recombinant allergens is currently available on singleplex IgE detection systems [4••]. Limitations to their use are chiefly a result of the proportional increase in the costs, as depicted in Fig. 1.

Specific IgE Detection Systems: Singleplex Versus Multiplex

Singleplex and multiplex testing refers to the selection and application of single reagents for detecting a specific analyte (ie, specific IgE) and the use of a predefined panel of reagents to be tested simultaneously in the same run, respectively. Thus, the difference between the two testing approaches is the number of results obtained by the application of a single specimen (ie, human serum, plasma, or any other body fluid) [4••]. The skin test could be considered a multiplex testing system, but the highly variable number of applied allergenic extracts makes the skin test method simply an addition of single diagnostic

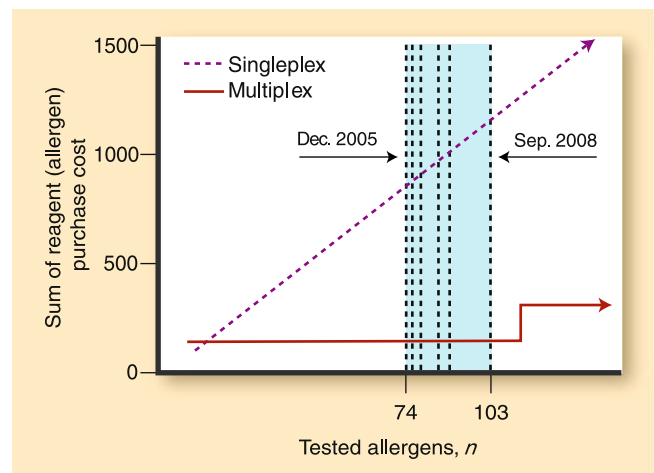


Fig. 1 Comparative schematic cost of singleplex and multiplex reagents to be used to yield the same number of diagnostic data

tests [20]. Multiplex testing systems are set to use exactly the same number of allergen preparations. There are in vitro systems in which a fixed panel of allergenic extracts are tested [21•]. To be fully comprehensive, they require multiple panels and a certain amount of specimen volume in the order of milliliters. They are considered multiplex systems because they produce multiple results following the application of a single specimen. The skin test and the in vitro test just mentioned do represent a “macro” version of allergy testing systems.

Fifteen years ago, genomics studies introduced the “micro” dimension into biomedicine, resulting in the genomics microarray [22]. Microarrays have been developed to detect the expression pattern of cellular transcripts in parallel. DNA microarrays have proven to be very useful tools for exploring differences in the expression of thousands of genes simultaneously [23]. Proteomics microarrays followed [24], and the very first proof of concept that they could be applied to allergy diagnosis came with the report by Hiller and coworkers [25] in 2002, followed by several papers using the same microarray [26–30], better known as the ISAC system (VBC-Genomics, Vienna, Austria). There are several other reports in the literature of micro in vitro devices for detecting IgE, most of which use allergenic extracts or other experimental compounds [31–38], while some others use allergenic molecules, such as the prototype mentioned previously that was created by Hiller and co-workers [39, 40–47, 48•, 49–51, 52•, 53•, 54]. These microarray prototypes were used experimentally, not routinely. Microarray format for IgE detection now fulfills the requirements for being classified as a multiplex in vitro diagnostic (IVD) [4•]. Combining allergenic molecules and the microarray format, now denoted as the *IgE biochip*, laid the basis for a dramatically new approach to the diagnosis of IgE-mediated allergic diseases.

The IgE Biochip: The Novelty in Allergy Diagnosis

The IgE biochip, as a new tool for IgE IVD, is showing promise in overcoming the reported limitations of “classical” allergy diagnostic tools. It has introduced new concepts to approaching patients with suspected IgE-mediated allergic diseases, as summarized in Table 1. Based on work done in our center [55•], routinely using the ISAC IgE biochip during the past 4 years, and on the basis of the research reported in the literature, novelties in the field of IgE IVD can be schematically described as follows:

The ISAC IgE biochip now bears 103 molecules from 43 different organisms, and 25 allergenic group markers. Food, inhalant, fungi, latex, and venom allergens are all present on ISAC. All allergens are spotted in triplicate on the biochip surface, thus offering an internal control in this IgE IVD. It

Table 1 IgE biochip features

Allergens can be obtained and spotted from all allergenic sources
Triplicate spotting increases the robustness of the biochip for in vitro diagnostics
No limit on the number of molecules that can be spotted on the biochip
Add-on strategy to have the most comprehensive allergen panel on the chip without clearing any of the previously spotted allergens
Natural and recombinant purified allergens can be used without limitation
Combined allergens from the same allergenic source can mimic that previously obtained by allergenic extract
Sensitivity and specificity of allergen-based biochip can be determined via comparison with molecule-based singleplex systems
No limit on the number of homologous molecules spotted on the biochip
Worldwide mapping and monitoring of allergen sensitization using the same standard tool
Patient's and allergist's independent screening of all potential sensitization
Clinically meaningful IgE testing using allergen markers
Low amount of required serum, 20 μ L, allows testing with the same allergen panel regardless of patient's age or other limiting conditions
Useful for experimental purposes (eg, SPHIAa)
Useful for detecting other specific immunoglobulins (eg, IgG, IgA, IgG ₄)

SPHIAa single-point highest inhibition achievable assay

becomes more robust compared with classical IVD, in which the test cannot be replicated without using a new reagent, which increases the costs.

An extract-based diagnosis can be obtained by using the most comprehensive allergenic molecule panel from a given allergenic source on the IgE biochip [26, 27], as previously reported by the use of singleplex methods [56]. Comparative evaluation of extracts versus allergens on the biochip belonging to the same allergenic source confirmed and unveiled unexpected problems related to the use of allergenic extracts from raw source material [57]. The allergenic extract is sometimes of very low quality because of missing allergenic molecules [58] and cannot be replaced by fresh sources because of their highly variable allergen content [59]. Such phenomena may lead to a false-negative result that puts the patient at risk of allergic reactions.

There are almost no physical or technical limitations in the number of allergenic molecules that can be spotted on the IgE biochip. From the very early experimental ISAC 29 (the number indicates allergenic molecules spotted on the biochip), there has been a great increase in this number, reaching what is now ISAC 103 (Fig. 1). Further increases are expected, depending on allergen availability.

Newly available allergenic molecules can be added to the current biochip without removing those previously spotted. All allergenic structures, regardless of their IgE reactivity prevalence, can be spotted on the biochip. During the past few years, we successfully applied this strategy by adding allergens to the ISAC IgE biochip as they were identified and became available both from our research laboratory and others (Fig. 1) [55•, 60–62].

The amount of preparation needed for spotting (0.1–1 ng/spot [63]) allows the use of allergenic molecules that have not yet been cloned. Thus, along with recombinants, highly purified natural allergens can be used even if they are present in the allergenic source material in very low amounts. Various companies (Bial Farmaceutica, Bilbao, Spain; Biomay AG, Vienna, Austria; Indoor Biotechnologies, Manchester, United Kingdom; Phadia AB, Uppsala, Sweden; Sigma-Aldrich, St. Louis, MO) or research laboratories can be sources of allergenic materials for spotting the biochip.

A thorough evaluation of the sensitivity and specificity of an allergenic molecule-based biochip for IgE detection has yet to be performed via molecule-to-molecule comparison [57]. If recombinant molecules are used, the isoform type should be known in order to correctly evaluate the counterpart. Panels of homologous molecules available on the biochip are useful to increase the sensitivity of the system and to explore a single patient's personal IgE profile.

There is no need to limit the number of homologous molecules belonging to the same group (eg, Bet v 1-like molecules, profilins, tropomyosins, parvalbumins, 2 S albumins, vicilins, 11 S albumins), as they cannot be so “highly” IgE co-recognized as can be predicted from their molecular structure [55•]. IgE co-recognition [14] of homologous molecules also can be affected by the nature of the primary sensitizer as determined by the patient's different environmental exposure. The use of homologous allergens would increase our knowledge regarding IgE epitope recognition, leading to a better understanding of allergen structures and, we hope, clinical phenotypes.

Epidemiology studies based on the IgE biochip were recently reported by Scala et al. [55•] in the first large-scale survey based on routine application of the ISAC biochip. This application could result in a common, shared, standard tool worldwide rather than being limited to geographical region-based allergenic extract panels [20, 64, 65]. Epidemiologic studies could increase our knowledge about allergy sensitization worldwide and provide us with data on allergenic molecule prevalence for future molecule-based immunotherapy [66]. Increasing the number of available makers of “genuine” exposure such as Phl p 1 or Par j 2 would also ease the collection of data informing us about the presence of the related allergenic sources in the environment [67, 68•]. The IgE immunologic

relationship of homologous molecules links allergenic species distributed in different geographical areas [55•]. This enables allergists to alert patients who are travelling to certain areas in which symptoms can be triggered by homologous allergens.

A correct and comprehensive picture of the allergic patient can be obtained by applying all the available molecules, as IgE-negative results are as valuable as the positives for defining the patient's profile. There are many reports on the utility of allergenic molecules for describing the clinical phenotype and prognosis of the allergic patient [16]. Clinical pictures ranging from no clinical reactivity unless IgE are present [69] to severe generalized reactions [70–72] can be foreseen. The presence of allergenic molecules of any nature, regardless of the route and place of exposure, helps the patient and the allergist get a full and comprehensive clinical view of the patient's sensitization. It can be obtained even when the patient does not or cannot report his or her symptoms. It also applies to the allergist often lacking the time to collect a full medical history.

No differences exist among patients (eg, age or size) in exploring IgE sensitization, as the biochip-based IVD requires as little as 20 µl of serum [73]. An increase in serum should only occur when the allergenic molecules spotted on the biochip double, which doubles the needed slide surface. It is, of course, the opposite for singleplex allergy IVD, in which a direct proportional increase (1:1) of the serum volume is required when the number of allergens to be tested increases. The multiplex system allows the allergy diagnosis to be performed in exactly the same way in newborn babies (ie, limited body surface for skin testing and sample volume for blood testing) and aged patients [28], or when difficult or severely affected patients must be tested [74].

Although no studies have examined the cost of the IgE biochip approach to allergy diagnosis, no increases in the ISAC cost have been recorded during the 4 years of its routine use, except when 30% more molecules were spotted on the biochip (Fig. 1). As mentioned, the cost is determined by the very low amount of allergenic molecule preparations needed for spotting a single biochip. Furthermore, the cost of the most expensive allergens is generally well-balanced by the very low cost of others. It seems reasonable to assume that in moving from small-scale to large-scale production, the biochip cost would remain stable or even decrease. There is little chance that a singleplex system can compete with the IgE biochip IVD (Fig. 1). On the other hand, biochip-based allergy testing can compete strongly with skin testing, not only because of the different nature of the testing, but also because it reduces the number of steps needed before reaching the final diagnosis. Indirect costs of allergy diagnosis could be reduced significantly.

Several criticisms oppose the biochip-based allergy diagnosis (eg, the cost of the system, lack of automation, risk of detecting a huge number of sensitizations without actual symptoms, unwanted mixture of allergens coming from inhalants and foods, and the fact that not all the allergenic sources/molecules are represented in the current biochip). At the moment, the highest cost of the system is due to the laser scanner. Cheaper and affordable scanners are entering the market, thus reducing the overall cost of installation by a factor of two. Even with full automation, the system will be in the average/low cost range of any laboratory instrument. Applying a panel of allergenic extracts to the skin test, as is currently done by allergists, brings with it the recording of sensitizations rather than clinically meaningful tests [75]. The magnitude of such a phenomenon is strongly related to the number of reagents applied to the single patient, but whether it is a useless phenomenon will be a matter of clinical research, as not enough studies have been conducted on the topic. IgE production is the first step toward clinical allergy, and the biochip-based IVD could help enormously in the early detection of an ongoing process that leads to the diagnosis of allergy at the earliest stage of the disease. Furthermore, sensitization without symptoms sometimes can be present in some patients. It will be the role of molecular allergists to follow up with asymptomatic or mildly symptomatic patients and to suggest the appropriate therapeutic approach, if any is needed. The molecular evidence of a connection between food and inhalants has been reported clearly in the literature for many allergens. It is now difficult to discriminate a patient as belonging to one or the other category, because his or her allergic status can change over time. For many of the arguments reported above, such discrimination remains part of the “classical” diagnostic approach rather than being part of future technological

advances in allergy diagnostic tests. There is an ongoing process including identification, characterization, and production of compounds to be used in allergenic molecule-based IVD. While the number is still increasing, many fundamental allergenic compounds already have been discovered and fully described. The systematic search for allergens should be carried out within international projects by networks of basic and clinical research centers, as has been done during the EuroPrevall project [76]. Health authorities should allow biochip producers to have all the allergens available and facilitate access to these and other resources.

A comparative summary of all features of the three diagnostic approaches described above is presented in Table 2.

Conclusions

What dramatically changed the application of IVD methods was the allergenic molecule age. The microtechnology has evolved from basic research on allergens and translated them into meaningful markers for allergic diseases. These are the basis of unexpected new approaches to allergic diseases.

Moreover, advanced application of the biochip IVD can be foreseen by its use for IgE and IgG subclass detection in the same run [43]. The IgE biochip also could be used for experimental purposes, establishing a second level for allergy diagnosis using the panels of spotted molecules in inhibition/depletion assays with homologous compounds [77•, 78•, 79•].

The second edition of the *Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline* released by the Clinical and Laboratory Standards Institute

Table 2 Comparative summary of the main features of the three diagnostic approaches to IgE-mediated allergic disease diagnosis

	Skin test	IgE singleplex	IgE multiplex
Operator	Allergist	Lab personnel	Lab personnel
Allergenic extracts?	Yes	Yes	Technical limitation but possible
Allergenic molecules?	Yes, with technical and regulatory limitations	Yes, with cost limitations	Yes, with virtually no limitations
Time required for readout	20 min	3–6 h	3–6 h
Automation	Impossible	Achieved	Achievable
Quality control	Impossible	Currently run	To be established
Technique standardization	Hardly achievable or impossible	Achieved, better with allergenic molecules	Achievable
Cost of the full diagnostic process	Intermediate	High or very high	Intermediate or low
Clinical interpretation	Allergist	Allergist/molecular allergist	Molecular allergist

[4••] reports and “regulates” allergenic molecules and multiplex IVD for the first time.

In conclusion, scenarios of allergic disease diagnostic work-ups could change from current skin prick test supplemented by IgE singleplex testing to multiplex biochip testing supplemented by “classical” skin test and IgE singleplex testing to achieve full multiplex testing when all the possible allergens will be available. Not everything is fully established, and a great amount of work needs to be done by molecular allergists in the forthcoming years to provide a stronger clinical basis for this new allergic disease diagnostic age. One of the prominent challenges will be to develop bioinformatics tools to help molecular allergists in managing, visualizing, interpreting, and understanding this high-throughput IgE detection system. The Allergome platform and the recently released allergy electronic record “InterAll” (Allergy Data Laboratories, Latina, Italy) could be the basis for this research enterprise [80••].

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References

- Papers of particular interest, published recently, have been highlighted as:
- Of importance
 - Of major importance
- Johansson SG, Bieber T, Dahl R, et al.: Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004, 113:832–836.
 - Knudsen TB, Thomsen SF, Nolte H, Backer V: A population-based clinical study of allergic and non-allergic asthma. *J Asthma* 2009, 46:91–94.
 - Cox L, Williams B, Sicherer S, et al.: Pearls and pitfalls of allergy diagnostic testing: report from the American College of Allergy, Asthma and Immunology/American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force. *Ann Allergy Asthma Immunol* 2008, 101:580–592. *This is a revision of the currently used diagnostic tools and their indications and interpretations.*
 - Matsson PNJ, Hamilton RG, Esch RE, et al.: Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergen Specificities; Approved Guideline, edn 2. CLSI Document I/LA20-A2. Wayne, PA: CLSI; 2009. *This is the most comprehensive and up-to-date document on IVDs available for allergic diseases. This second edition of the CLSI guidelines includes information on allergenic molecules and microtechnologies for the first time.*
 - Nolte H, DuBusse LM: Performance characteristics of a new automated enzyme immunoassay for the measurement of allergen-specific IgE. Summary of the probability outcomes comparing results of allergen skin testing to results obtained with the HYTEC system and CAP system. *Ann Allergy Asthma Immunol* 1997, 79:27–34.
 - Kam KL, Hsieh KH: Comparison of three in vitro assays for serum IgE with skin testing in asthmatic children. *Ann Allergy* 1994, 73:329–336.
 - Ciardiello MA, Giangrieco I, Tuppo L, et al.: Influence of the natural ripening stage, cold storage, and ethylene treatment on the protein and IgE-binding profiles of green and gold kiwi fruit extracts. *J Agric Food Chem* 2009, 57:1565–1571.
 - Constantin C, Touraev A, Heberle-Bors E, et al.: Detection of antigens reactive to IgE and IgA during wheat seed maturation and in different wheat cultivars. *Int Arch Allergy Immunol* 2009, 149:181–187.
 - Hildebrandt S, Steinhart H, Paschke A: Comparison of different extraction solutions for the analysis of allergens in hen's egg. *Food Chem* 2008, 108:1088–1093.
 - Larenas-Linnemann D, Cox LS: European allergen extract units and potency: review of available information. *Ann Allergy Asthma Immunol* 2008, 100:137–145.
 - Lorenz AR, Luttkopf D, May S, et al.: The principle of homologous groups in regulatory affairs of allergen products—a proposal. *Int Arch Allergy Immunol* 2009, 148:1–17. *This article introduces the use of allergenic molecules for allergenic extract standardization.*
 - Mari A: Multiple pollen sensitization: a molecular approach to the diagnosis. *Int Arch Allergy Immunol* 2001, 125:57–65.
 - Tinghino R, Twardosz A, Barletta B, et al.: Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. *J Allergy Clin Immunol* 2002, 109:314–320.
 - Ferreira F, Hawranek T, Gruber P, et al.: Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004, 59:243–267.
 - Schmid-Grendelmeier P, Crameri R: Recombinant allergens for skin testing. *Int Arch Allergy Immunol* 2001, 125:96–111.
 - Asero R, Monsalve R, Barber D: Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen. *Clin Exp Allergy* 2008, 38:1033–1037.
 - Asero R, Jimeno L, Barber D: Component-resolved diagnosis of plant food allergy by SPT. *Eur Ann Allergy Clin Immunol* 2008, 40:115–121. *This article discusses approaching molecule-based diagnosis using the skin test.*
 - Mari A, Scala E, D'Ambrosio C, et al.: Latex allergy within a cohort of not-at-risk subjects with respiratory symptoms: prevalence of latex sensitization and assessment of diagnostic tools. *Int Arch Allergy Immunol* 2007, 143:135–143.
 - Lidholm A, Ballmer-Weber BK, Mari A, Vieths S: Component-resolved diagnostics in food allergy. *Curr Opin Allergy Clin Immunol* 2006, 6:234–240.
 - Heinzerling L, Frew AJ, Bindslev-Jensen C, et al.: Standard skin prick testing and sensitization to inhalant allergens across Europe—a survey from the GA2LEN network. *Allergy* 2005, 60:1287–1300.
 - Lee S, Lim HS, Park J, Kim HS: A new automated multiple allergen simultaneous test-chemiluminescent assay (MAST-CLA) using an AP720S analyzer. *Clin Chim Acta* 2009, 402:182–188. *This is an example of multiplex IgE testing using extracts.*
 - Schena M, Shalon D, Davis RW, Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995, 270:467–470.
 - Schulze A, Downward J: Analysis of gene expression by microarrays: cell biologist's gold mine or minefield? *J Cell Sci* 2000, 113:4151–4156.
 - MacBeath G, Schreiber SL: Printing proteins as microarrays for high-throughput function determination. *Science* 2000, 289:1760–1763.

25. Hiller R, Laffer S, Harwanegg C, et al.: Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002, 16:414–416.
26. Jahn-Schmid B, Harwanegg C, Hiller R, et al.: Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin Exp Allergy* 2003, 33:1443–1449.
27. Wohrl S, Vigl K, Zehetmayer S, et al.: The performance of a component-based allergen-microarray in clinical practice. *Allergy* 2006, 61:633–639.
28. Ott H, Schroder CM, Stanzel S, et al.: Microarray-based IgE detection in capillary blood samples of patients with atopy. *Allergy* 2006, 61:1146–1147.
29. Harwanegg C, Hutter S, Hiller R: Allergen microarrays for the diagnosis of specific IgE against components of cow's milk and hen's egg in a multiplex biochip-based immunoassay. *Methods Mol Biol* 2007, 385:145–157.
30. Untersmayr E, Lukschal A, Hemmer W, et al.: Exercise with latex sport bands represents a risk for latex allergic patients. *Immunol Lett* 2008, 115:98–104.
31. Musu T, Rabillon J, Pelletier C, et al.: Simultaneous quantitation of specific IgE against 20 purified allergens in allergic patients sera by checkerboard immunoblotting (CBIB). *J Clin Lab Anal* 1997, 11:357–362.
32. Fulton RJ, McDade RL, Smith PL, et al.: Advanced multiplexed analysis with the FlowMetrix system. *Clin Chem* 1997, 43:1749–1756.
33. Schweitzer B, Wiltshire S, Lambert J, et al.: Immunoassays with rolling circle DNA amplification: a versatile platform for ultrasensitive antigen detection. *Proc Natl Acad Sci U S A* 2000, 97:10113–10119.
34. Kim TE, Park SW, Cho NY, et al.: Quantitative measurement of serum allergen-specific IgE on protein chip. *Exp Mol Med* 2002, 34:152–158.
35. Bacarese-Hamilton T, Mezzasoma L, Ingham C, et al.: Detection of allergen-specific IgE on microarrays by use of signal amplification techniques. *Clin Chem* 2002, 48:1367–1370.
36. Matsubara Y, Murakami Y, Kobayashi M, et al.: Application of on-chip cell cultures for the detection of allergic response. *Biosens Bioelectron* 2004, 19:741–747.
37. Han MK, Seo MH, Lee D, et al.: Optimization of critical factors affecting the performance of an allergen chip for the analysis of an allergen-specific human IgE in serum. *Anal Sci* 2007, 23:545–549.
38. Hahn YK, Jin Z, Kang JH, et al.: Magnetophoretic immunoassay of allergen-specific IgE in an enhanced magnetic field gradient. *Anal Chem* 2007, 79:2214–2220.
39. Suck R, Nandy A, Weber B, et al.: Rapid method for arrayed investigation of IgE-reactivity profiles using natural and recombinant allergens. *Allergy* 2002, 57:821–824.
40. Fall BI, Eberlein-Konig B, Behrendt H, et al.: Microarrays for the screening of allergen-specific IgE in human serum. *Anal Chem* 2003, 75:556–562.
41. Alcocer MJ, Murtagh GJ, Wilson PB, et al.: The major human structural IgE epitope of the Brazil nut allergen Ber e 1: a chimaeric and protein microarray approach. *J Mol Biol* 2004, 343:759–769.
42. Shreffler WG, Lencer DA, Bardina L, Sampson HA: IgE and IgG (4) epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. *J Allergy Clin Immunol* 2005, 116:893–899.
43. Satinover SM, Reefer AJ, Pomes A, et al.: Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens. *J Allergy Clin Immunol* 2005, 115:803–809.
44. Ohyama K, Omura K, Ito Y: A photo-immobilized allergen microarray for screening of allergen-specific IgE. *Allergol Int* 2005, 54:627–631.
45. Lebrun SJ, Petchpud WN, Hui A, McLaughlin CS: Development of a sensitive, colorimetric microarray assay for allergen-responsive human IgE. *J Immunol Methods* 2005, 300:24–31.
46. King EM, Vailes LD, Tsay A, et al.: Simultaneous detection of total and allergen-specific IgE by using purified allergens in a fluorescent multiplex array. *J Allergy Clin Immunol* 2007, 120:1126–1131.
47. Gaudin JC, Rabesona H, Choiset Y, et al.: Assessment of the immunoglobulin E-mediated immune response to milk-specific proteins in allergic patients using microarrays. *Clin Exp Allergy* 2008, 38:686–693.
48. •• Cerecedo I, Zamora J, Shreffler WG, et al.: Mapping of the IgE and IgG4 sequential epitopes of milk allergens with a peptide microarray-based immunoassay. *J Allergy Clin Immunol* 2008, 122:589–594. *This study shows advanced microtechnology applied to measurement of different immunoglobulin isotypes toward peptides to describe milk allergen epitope recognition.*
49. Heyries KA, Loughran MG, Hoffmann D, et al.: Microfluidic biochip for chemiluminescent detection of allergen-specific antibodies. *Biosens Bioelectron* 2008, 23:1812–1818.
50. Gadermaier G, Wopfner N, Wallner M, et al.: Array-based profiling of ragweed and mugwort pollen allergens. *Allergy* 2008, 63:1543–1549.
51. Ott H, Baron JM, Heise R, et al.: Clinical usefulness of microarray-based IgE detection in children with suspected food allergy. *Allergy* 2008, 63:1521–1528.
52. • Matsumoto N, Okochi M, Matsushima M, et al.: Development of peptide arrays for detection of IgE-binding epitopes in cow's milk allergens. *J Biosci Bioeng* 2009, 107:324–330. *Microtechnology is used to measure IgE response to peptides identifying epitopes.*
53. • Cretich M, Di Carlo G, Giudici C, et al.: Detection of allergen specific immunoglobulins by microarrays coupled to microfluidics. *Proteomics* 2009, 9:2098–2107. *This is an example of a further microtechnology, microfluidics, applied to in vitro detection of IgE.*
54. Constantin C, Quirce S, Poorafshar M, et al.: Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis. *Allergy* 2009, 64:1030–1037.
55. •• Scala E, Alessandri C, Bernardi ML, et al.: Cross-sectional survey on immunoglobulin E reactivity in 23 077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* 2010, 40:911–921. *This is the first high-throughput study ever published in the allergy/immunology field based on electronically collected routine diagnostic data that were analyzed as an epidemiologic survey. Prevalence of IgE recognition, demographic data distribution, along with new clustering analysis representation were reported.*
56. Mari A: Skin test with a Timothy grass (*Phleum pratense*) pollen extract vs IgE to a Timothy extract vs IgE to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12: epidemiological and diagnostic data. *Clin Exp Allergy* 2003, 33:43–51.
57. Zennaro D, Palazzo P, Pomponi D, et al.: Retrospective comparative analysis of skin test and IgE reactivity to extracts, and singleplexed or multiplexed allergenic molecules. *Allergy* 2007, 62:S153.
58. Akkerdaas JH, Wensing M, Knulst AC, et al.: How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? *Int Arch Allergy Immunol* 2003, 132:132–140.
59. Zuidmeer L, van Leeuwen WA, Kleine Budde I, et al.: Allergenicity assessment of apple cultivars: hurdles in quantifying labile fruit allergens. *Int Arch Allergy Immunol* 2006, 141:230–240.
60. Tuppo L, Giangrieco I, Palazzo P, et al.: Kiwellin, a modular protein from green and gold kiwi fruits: evidence of in vivo and in

- vitro processing and IgE binding. *J Agric Food Chem* 2008, 56:3812–3817.
61. Ciardiello MA, D'Avino R, Amoresano A, et al.: The peculiar structural features of kiwi fruit pectin methylesterase: amino acid sequence, oligosaccharides structure, and modeling of the interaction with its natural proteinaceous inhibitor. *Proteins* 2008, 71:195–206.
 62. Bernardi ML, Palazzo P, Tuppo L, et al.: Pectin methylesterase (Act d 7) and pectin methylesterase inhibitor (Act d 6): two new kiwi fruit allergens. *Allergy* 2008, 63:S585.
 63. Deinhofer K, Sevcik H, Balic N, et al.: Microarrayed allergens for IgE profiling. *Methods* 2004, 32:249–254.
 64. Rona RJ, Keil T, Summers C, et al.: The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007, 120:638–646.
 65. Zuidmeer L, Goldhahn K, Rona RJ, et al.: The prevalence of plant food allergies: a systematic review. *J Allergy Clin Immunol* 2008, 121:1210–1218.
 66. Valenta R, Niederberger V: Recombinant allergens for immunotherapy. *J Allergy Clin Immunol* 2007, 119:826–830.
 67. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, et al.: Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006, 118:481–488.
 68. • Barber D, De la Torre F, Feo F, et al.: Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008, 63:1550–1558. *This was a comprehensive survey in a geographical region using singleplex allergens on a standard IVD system.*
 69. Mari A, Ooievaar-de Heer P, Scala E, et al.: Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. *Allergy* 2008, 63:891–896.
 70. Kleine-Tebbe J, Vogel L, Crowell DN, et al.: Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in soybean, SAM22. *J Allergy Clin Immunol* 2002, 110:797–804.
 71. Ballmer-Weber BK, Holzhauser T, Scibilia J, et al.: Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol* 2007, 119:1489–1496.
 72. Zuidmeer L, van Ree R: Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. *Curr Opin Allergy Clin Immunol* 2007, 7:269–273.
 73. Harwanegg C, Hiller R: Protein microarrays for the diagnosis of allergic diseases: state-of-the-art and future development. *Clin Chem Lab Med* 2005, 43:1321–1326.
 74. Zennaro D, Scala E, Pomponi D, et al.: Defining IgE specificities in reported cases of IPEX syndrome by means of ISAC proteomic microarray system. *Allergy* 2008, 63:S41.
 75. Bousquet J, Anto JM, Bachert C, et al.: Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. *Allergy* 2006, 61:671–680.
 76. Vieths S, Hoffmann-Sommergruber K: EuroPrevall food allergen library. *Mol Nutr Food Res* 2008, 52(Suppl 2):S157–S158.
 77. • Ciardiello MA, Palazzo P, Bernardi ML, et al.: Biochemical, immunological and clinical characterization of a cross-reactive nonspecific lipid transfer protein 1 from mulberry. *Allergy* 2010, 65:597–605. *This was a combined application of microarrayed IgE-tested allergens for patient selection plus population survey plus inhibition experiments.*
 78. • Gadermaier G, Harrer A, Girbl T, et al.: Isoform identification and characterization of Art v 3, the lipid-transfer protein of mugwort pollen. *Mol Immunol* 2009, 46:1919–1924. *Microarrayed allergens were used for single-point highest inhibition achievable assay to define features of isoforms produced as recombinant allergen.*
 79. • Krause S, Reese G, Randow S, et al.: Lipid transfer protein (Ara h 9) as a new peanut allergen relevant for a Mediterranean allergic population. *J Allergy Clin Immunol* 2009, 124:771e5–778.e5. *A single-point highest inhibition achievable assay was used to define features of newly characterized allergen and IgE response to peanut allergen survey of a population for defining clinical relevance of allergens in causing the clinical disease.*
 80. • Mari A, Rasi C, Palazzo P, Scala E: Allergen databases: current status and perspectives. *Curr Allergy Asthma Rep* 2009, 9:376–383. *This review examined current information technology-based tools to be used in combination with microarray-based IVDs for allergic diseases.*