Precision medicine allergy immunoassay methods for assessing immunoglobulin E sensitization to aeroallergen molecules

Florin-Dan Popescu, Mariana Vieru

Florin-Dan Popescu, Mariana Vieru, Department of Allergology, “Carol Davila” University of Medicine and Pharmacy, Bucharest 022441, Romania

Florin-Dan Popescu, Mariana Vieru, Department of Allergology and Clinical Immunology, “Nicolae Malaxa” Clinical Hospital, Bucharest 022441, Romania

ORCID number: Florin-Dan Popescu (0000-0001-6316-3155); Mariana Vieru (0000-0001-7396-5688).

Author contributions: Popescu FD and Vieru M conceived the editorial and drafted the manuscript; both authors approved the final version of the article.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited Manuscript

Corresponding author to: Florin-Dan Popescu, MD, PhD, Associate Professor, Chief Doctor, Department of Allergology and Clinical Immunology, “Nicolae Malaxa” Clinical Hospital, Sos Vergului 12, Sector 2, Bucharest 022441, Romania. florindanpopescu@gmail.com
Telephone: +40-212-555405
Fax: +40-212-555275

Received: July 9, 2018
Peer-review started: July 9, 2018
First decision: August 2, 2018
Revised: August 9, 2018

Abstract
Molecular-based allergy diagnosis for the in vitro assessment of a patient immunoglobulin E (IgE) sensitization profile at the molecular level uses allergen molecules (also referred to as allergen components), which may be well-defined, highly purified, natural allergen components or recombinant allergens. Modern immunoassay methods used for the detection of specific IgE against aeroallergen components are either singleplex (such as the fluorescence enzyme immunoassay with encapsulated cellulose polymer solid-phase coupled allergens, the enzyme-enhanced chemiluminescence immunoassay and the reversed enzyme allergosorbent test, with liquid-phase allergens), multiparameter (such as the line blot immunoassay for defined partial allergen diagnostics with allergen components coating membrane strips) or multiplex (such as the microarray-based immunoassay on immuno-solid-phase allergen chip, and the two new multiplex nanotechnology-based immunoassays: the patient-friendly allergen nanobead array, and the macroarray nanotechnology-based immunoassay used as a molecular allergy explorer). The precision medicine diagnostic work-up may be organized as an integrated “U-shape” approach, with a “top-down” approach (from symptoms to molecules) and a “bottom-up” approach (from molecules to clinical implications), as needed in selected patients. The comprehensive and accurate IgE sensitization molecular profiling, with identification of the relevant allergens, is indicated within the framework of a detailed patient’s clinical history to distinguish genuine IgE sensitization from sensitization due to cross-reactivity (especially in polysensitized...
patients), to assess unclear symptoms and unsatisfactory response to treatment, to reveal unexpected sensitizations, and to improve assessment of severity and risk aspects in some patients. Practical approaches, such as anamnesis molecular thinking, laboratory molecular thinking and postmolecular anamnesis, are sometimes applied. The component-resolved diagnosis of the specific IgE repertoire has a key impact on optimal decisions making for prophylactic and specific immunotherapeutic strategies tailored for the individual patient.

Key words: Singleplex; Multiplex; Immunoglobulin E sensitization; Aeroallergens; Immunoassays

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Allergic respiratory diseases affect many people of all ages worldwide, showing increased prevalence, severity and complexity. New generation immunoassays using allergenic molecules represent a great precision medicine approach in research and clinical practice, allowing in vitro assessment of the immunoglobulin E (IgE) sensitization pattern at the molecular level, with favorable impact on allergy diagnosis and treatment, especially in selected patients with multiple aeroallergen sensitizations. The choice for a specific IgE immunoassay (singleplex, multiparameter or multiplex) for the allergenic extracts and molecular specificities, and the correct interpretation of the results, require optimal knowledge of the tests’ methodologies and characteristics, and good clinical judgments.

Popescu FD, Vieru M. Precision medicine allergy immunoassays methods for assessing immunoglobulin E sensitization to aeroallergen molecules. World J Methodol 2018; 8(3): 17-36 Available from: URL: http://www.wjgnet.com/2222-0682/full/ v8/i3/17.htm DOI: http://dx.doi.org/10.5662/wjm.v8.i3.17

INTRODUCTION

The precision medicine concept is both appealing and challenging\(^1\) for chronic allergic airway diseases, including allergic rhinitis and asthma, which are a major and growing global health problem. In this regard, it is worth mentioning that chronic respiratory and allergic diseases affect over one billion people of all ages worldwide, with increasing prevalence and severity. Precision medicine represents a novel, modern approach to the management of some of these patients, embracing as key features: Personalized care based on molecular, immunologic and functional endotyping, considering predictive and preventive aspects, with participation of the patient in the decision-making process. Implementation of precision medicine into clinical practice may help to combat allergies and chronic airways diseases. Significant healthcare system changes are required to achieve that\(^{6-5}\).

Assessing immunoglobulin E (IgE) sensitization to aeroallergens, in combination with detailed clinical history of the patients, represents the cornerstone for diagnosis of allergic airway diseases\(^5\). IgE sensitization and allergy, although very often correlated, are not always fully comparable. A positive IgE test result (IgE sensitization) is likely to correspond to a clinical reaction, but this cannot be considered universally valid because there are cases in which the clinical significance of some protein-IgE recognitions is not clear. Specific IgE, even in the absence of allergy, could be a risk factor for future clinical reactions or the memory of a previous allergic status\(^6,7\). International guidelines still indicate that clinical history and skin prick testing (SPT) are the first-level starting procedures of every allergy diagnosis (“top-down” approach). Specific IgE immunoassays with whole allergen extracts are considered a second-level diagnostic, and molecular allergy diagnosis a third-level one. Some authors suggest that a ”bottom-up” diagnostic approach with wide IgE profiling based on allergen micro- or macroarray-based immunodiagnostics may also have advantages\(^6,9\).

The methods usually applied in clinical practice to assess IgE-mediated sensitization to aeroallergens are skin prick tests and/or specific IgE immunoassays with allergen extracts. Skin tests represent the first diagnostic method in patients with a suggestive clinical history of allergic rhinitis/rhinoconjunctivitis and/or asthma\(^10\). SPT is a reliable method to diagnose IgE-mediated allergic disease in such patients\(^11\). There are European standards and North American practice parameters for a SPT panel and selection of key aeroallergens. Such extracts of plant, fungal and animal origin are used for the in vivo allergy assessment of patients with allergic rhinitis/rhinoconjunctivitis and/or asthma\(^11,12-13\). Intradermal skin tests are not useful for allergy diagnosis with aeroallergen extracts\(^10\). There are circumstances in which the in vivo and in vitro tests have their distinct advantages and limitations in the assessment process. In general, there is a good concordance between a positive skin test result and a positive blood test result for the most potent aeroallergens from house dust mites, cat and dog epithelia, and pollen of trees, grasses and weeds. Comparing evaluations of the two test methods mentioned, skin tests seem to be more sensitive (lower false-negative rate), while serum allergen-specific IgE immunoassays seem to be more specific (lower false-positive rate)\(^14,15\).

The skin prick tests and specific IgE immunoassays confirm sensitization to a specific aeroallergen; however, the clinical relevance must be interpreted based on medical history and clinical symptoms. Positive results to skin tests or specific IgE assays do not mean that an allergen is causing symptoms, and the relevance of allergen exposure and its relation with symptoms must be confirmed by the patient’s history\(^16\). Allergen provocation tests (such as local allergen challenge tests
or controlled exposure in allergen challenge chambers) can reproducibly confirm the clinical significance of a sensitized allergen, but may be difficult to perform and present limitations. Nasal and ocular challenges may be helpful as diagnostic tools for selected patients in clinical settings, and especially for research purposes[11,17,18]. Bronchial allergy challenge using an aerosol provocation system nebulizer or segmental allergen challenge using bronchoscopy are used only in research[19,20]. Few studies have assessed the basophil activation test (BAT) to determine the allergenicity of individual aeroallergens. Some researchers have concluded that BAT is not sensitive enough to be used for the routine diagnosis of individual pollen allergy, and they believe this may be due to a non-specific IgE cross-linking in the performance of BAT using CD63 expression[21]. In contrast, others have considered that BAT using CD203c expression is a reliable method in the diagnosis of pollen allergy[22]. It must be mentioned here that up-regulation of CD63 (lysosomal-associated membrane glycoprotein-3) is representative of anaphylactic degranulation, being expressed on the surface of degranulated basophils, while up-regulation of CD203c (glycosylated type II transmembrane molecule constitutively expressed in low levels on basophil surface) may be associated with piecemeal degranulation[23].

IgE is the least abundant human antibody, with approximately half being found as free IgE in the intravascular compartment and the other half being bound to IgE receptors of a various cells, especially mast cells and basophils, via the high-affinity IgE receptor (FcεRI). Although free serum IgE has a short half-life of approximately 2 d, FcεRI-bound IgE persists for about 2 mo. While serum IgE immunosay determination directly measures free IgE, SPT and BAT yield indirect information on mast cell- and basophil-bound IgE. Although all these methods offer qualitative diagnostic information, there are quantitative variations between the results, particularly due to different allergen sources and other methodological considerations[24]. The need for consistent quality is essential for immunology laboratories undertaking specific IgE antibody assays. External quality assessment is essential for approval by accreditation organizations[25].

Notwithstanding that the introduction of highly-purified natural and recombinant allergen molecular components represents an important improvement in the diagnosis of IgE sensitization to aeroallergens, the allergy skin testing cannot be completely replaced by molecular diagnosis in the near future. Besides costs and availability aspects, molecular allergy testing can be ordered by any physician; thus, patient selection and interpretation of results might not always be optimal. No allergy immunotherapy trial has yet shown efficacy in patients selected solely on the basis of molecular diagnosis. Moreover, molecular allergen treatment has still not been introduced in clinical practice[26]. Diagnostic molecular approaches are, however, currently revolutionizing the assessment of allergic patients[27]. Molecular allergen immunotherapy approaches have the potential to improve the treatment of allergic diseases and may be used as allergen-specific forms of secondary and eventually primary prevention for allergy[28].

Although it has been shown that it would be possible to use molecular allergen components instead of allergen extracts for skin prick or intradermal testing and for topical mucosal provocations such as nasal challenge, these methods are not available for routine clinical applications. Their use is important, however, in the development of new hypoallergenic allergen immunotherapies[29,30]. The number of published studies using in vivo testing with recombinant allergens has declined substantially over the past years, due to implementation of regulations prohibiting the approval of clinical studies with non-Good Manufacturing Practice produced recombinant allergens[29]. Instead, over the recent years, significant technological developments allowed the use of such allergenic components in the in vitro measurement of allergen-specific IgE[30]. Thus, molecular technology has changed the way that clinical laboratories diagnose IgE sensitization to allergens in respiratory allergies.

Precision medicine is a structural model aimed at customizing healthcare, with medical products/decisions tailored to the individual patient at a highly detailed level. Precision medicine allergy immunoassays support the molecular-based allergy diagnosis. They also allow the accurate definition of the IgE sensitization profile of the patient (i.e., the patient’s IgE repertoire). Molecular-based allergy diagnosis, also known as “component resolved diagnostics”, is a patient IgE sensitization in vitro diagnostic approach at the molecular level using allergenic molecules, also referred to as “allergen components”. Two types of molecular allergen components are used in current immunoassays[31,32]: (1) Well-defined highly purified natural allergens (isolated and purified from natural allergen sources); and (2) Recombinant allergens (produced by recombinant DNA technology). The successful sequencing of the first allergen-encoding DNA kickstarted the era of molecular allergy diagnostics 30 years ago[31,36].

Allergen molecules or allergen components are highly defined, and purified proteins from a given allergen source. These molecular allergens in their native or recombinant forms are typically homogeneous, and have comprehensive quality control. By contrast, allergen extracts are crude, heterogeneous, unfractionated mixtures of many allergenic and nonallergenic proteins, polysaccharides and lipids obtained by extraction from an allergen source. Even if they are less expensive, due to easier preparation, such natural extracts are difficult to standardize by detailing their composition and allergenic potency. Moreover, the protein mixture complexity is a factor for low
specification, while endogenous degradation is a risk for low sensitivity\textsuperscript{[9]}. Regarding the preparation of allergen components, natural allergens are purified from different allergen sources (such as pollen grains or house dust mites) by chromatographic techniques. Recombinant allergens are mainly produced in prokaryotic expression systems (\textit{Escherichia coli}), with several exceptions produced in eukaryotic systems (the yeast \textit{Pichia pastoris}). Only few allergen molecules are produced under Good Manufacturing Practice conditions and are considered biologic reference preparations, due to their comprehensive characterization by physicochemical and immunological methods. Detailed physicochemical characterization includes protein identification and amino acid sequencing by mass spectrometry-based methods as well as quantification determined by amino acid analysis using reversed-phase high-performance liquid chromatography. Homogeneity is assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis silver-stained, isoelectric focusing Coomassie-stained and immunoblotting experiments, while folding and denaturation analysis by far-ultraviolet circular dichroism and Fourier-transform infrared spectroscopy. High-performance size-exclusion chromatography and dynamic light scattering are used for aggregation behavior and stability in solution. Biological activity is assessed \textit{in vitro} by BAT, with up-regulation of CD203c, and by enzyme-linked immunosorbent assay (ELISA) inhibition for batch-to-batch consistency regarding allergenic potency\textsuperscript{[37-39]}. 

Immunoadsays used for the detection of specific IgE to allergen components are either singleplex, multiparameter or multiplex assays (Table 1), according to the number of allergen extracts and molecular components used\textsuperscript{[9,40,41]}. 

### Table 1 Classification of immunoadsays used for the detection of specific immunoglobulin E against allergen components\textsuperscript{[9,40,41]}

| Type of immunoadsay | Description |
|---------------------|-------------|
| Singleplex          | Detect specific IgE against a single allergen component |
| Multiparameter      | Detect specific IgE against a few allergen components at once (usually - 10) |
| Multiplex           | Detect simultaneously specific IgE against many different allergen components (> 100) |

IgE: Immunoglobulin E.

**SINGLEPLEX IMMUNOADSAYS FOR SPECIFIC IgE TO ALLERGEN COMPONENTS**

Molecular-based singleplex specific IgE immunoassays refer to laboratory methods in which one analyte is measured per analysis. They are designed to detect and measure circulating IgE antibodies that can bind to one specific allergen or molecular component\textsuperscript{[9,42]}. The basic chemistry of such \textit{in vitro} IgE assays has remained essentially unchanged since their introduction more than 40 years ago. After serum specific IgE binding to solid- or liquid-phase allergen, bound IgE antibody is detected with a labeled anti-IgE reagent, with these reactions’ incubation periods being separated by buffer washes\textsuperscript{[43,44]}. Historically, the first generation solid-phase isotopic immunoadsay is the radioallergosorbent test (RAST), with allergens covalently coupled to a filter paper disc allergosorbent (solid-phase with low surface area), radioiodinated polyclonal anti-human IgE used as signal detection antibody, two overnight incubations needed, and bound radioactivity quantified in a gamma counter\textsuperscript{[24,44]}. This first \textit{in vitro} qualitative test for specific IgE-antibodies introduced in 1973 was used as a manual method for detection of serum specific IgE against various allergen extracts, but not against molecular components. It has been replaced by nonisotopic, more sensitive immunoadsays, which are in use currently; thus, the term RAST should be abandoned when referring to these \textit{in vitro} IgE testing methods\textsuperscript{[45,46]}. Automation of new generation, US Food and Drug Administration (FDA)-cleared, clinically used immunoadsays (such as ImmunoCAP® and Immulite® systems, which now hold the largest share of the global market) have optimized precision, reproducibility, and linearity to a performance standard of less than 15% coefficients of variation. These new generation immunoadsays must report comparable analytical sensitivity and calibration schemes traceable to the World Health Organization (WHO) IgE international standard. Interassay differences may exist, and have been attributed particularly to the differences in composition of the allergen extract-based reagents used and details of the calibration systems\textsuperscript{[44]}. 

Modern singleplex immunoadsays used to determine allergen-specific IgE antibodies comprise the following components\textsuperscript{[24]}: (1) Allergen-containing reagent: Solid-phase allergosorbent or liquid-phase labeled allergen; (2) Reaction compartment: Plastic capsule reserve with cellulose polymer; plastic reaction tube with dispersed assay-specific polystyrene bead, plastic microtiter plate with wells; (3) Human serum with specific IgE antibodies and negative serum controls; (4) Anti-IgE detection reagent: Monoclonal antibody specific to the constant Fc fragment of human IgE; and (5) Calibration and data processing systems. 

The total calibration curve used in most immunoadsay systems nowadays is linked to the WHO IgE standard and reported in arbitrary units, kU/L kilo mass units of allergen-specific antibody per unit volume of
sample, where the ‘x’ stands for allergen-specific. These are distinguished from internationally standardized units IU/mL or kU/L for total IgE measurement (1 kU/L = 0.994 kU/L), introduced to express the level of IgE in peripheral blood to alleviate the inconvenience in expressing the very low levels of serum IgE. The reference curve calibrated to the official WHO standard for total IgE is generated following each immunoassay run according to manufacturers’ specifications. The measurement signals obtained for allergen-specific IgE are converted into corresponding units (kU/L) with the help of total IgE reference curve as heterologous calibration[24].

Performance specifications for immunoassays are established for several characteristics, including the reportable range. Accuracy means trueness assessed by comparison-of-methods studies, while precision refers to the standard deviation or coefficient of variation (CV) estimated by replication studies. Analytical specificity is the ability to detect IgE, not antibodies of other classes, via interference studies, while analytical sensitivity, the limit-of-detection studies. Allergen molecules allow improved analytical specificity (selectivity) by binding a partial amount of the specific IgE repertoire. Analytical sensitivity is often improved (the lower Limit of Quantitation, commonly referred to as LoQ), when using allergen molecules, particularly if these are under-represented in the natural extracts or even entirely absent due to their instability. LoQ itself is the lowest concentration of specific IgE antibodies that can be reliably detected within a predefined precision. LoQ may be equivalent to or higher than the Limit of Detection (LoD), which refers to the weakest signal or lowest concentration of specific IgE antibodies reliably determined from the test, calculated using the Limit of Blank (the signal of a serum sample without allergen-specific IgE)[9,24,47,48].

Specific IgE immunoassays report results in a class system (classes 0-6) based on the amount of detected serum specific IgE. The higher the allergen-specific IgE level, the greater the likelihood of a patient to suffer from allergic symptoms caused by the exposure to the sensitizing allergen. These randomly assigned classes have evolved over time, to semiquantitatively and broadly categorize serum IgE concentrations. However, this class system has become obsolete with the quantitative reporting of specific IgE using kU/L[15,24]. The lower detection threshold for specific IgE determination was formerly 0.35 kU/L. Presence of specific IgE against a particular allergen above this level is deemed positive for that allergen, and a positive test (a level ≥ 0.35 kU/L) for aeroallergens generally correlates well with the clinical expression[42,50]. The sensitivity of new generation specific IgE immunoassays is now higher due to more sensitive calibration and improved resolution of low IgE values, being able to provide values below 0.35 kU/L, until down to 0.1 kU/L. This range is particularly informative and relevant when total IgE is extremely low (< 20 kU/L). Thus, the ratio of specific IgE to total IgE (referred to as antibody-specific activity) is particularly important in this case. The upper detection limit is 100 kU/L for most specific IgE immunoassays. Serum samples with higher specific IgE should be measured in diluted forms (1:10) in order to determine the actual value by multiplying with 10. Specific IgE/total IgE ratio is also important in samples with very high total IgE levels (> 1000 kU/L)[24,51]. Complete concordance between specific IgE immunoassays and SPT cannot be expected. An in vitro assay measures circulating allergen-specific IgE antibodies, whereas skin testing assesses cutaneous mast cell reactivity based on assumed cell-bound specific IgE[52].

The currently available singleplex immunoassays aim to determine serum specific IgE to molecular allergen components using either solid-phase coupled allergens (i.e., fluorescence enzyme immunoassay) or liquid-phase allergens (i.e., chemiluminescence immunoassay and reversed enzyme allergosorbent test). Advantages of singleplex assays for allergen-specific IgE testing with allergenic molecules/components, when compared with multiplex technology, include increased assay analytical sensitivity (lower LoQ) and greater sensitivity at low specific IgE levels. In addition, they have similar units for total IgE and allergen-specific IgE, due to heterologous calibration allowing calculation of allergen-specific IgE/total IgE-ratio, as well as more established quality control measures[9,33].

The fluorescence enzyme immunoassay (FEIA) with capssulated cellulose polymer solid-phase (ImmunoCAP™) coupled allergens is currently used to measure specific IgE antibodies to many allergen extracts (> 650) and 105 individual molecular allergens, in serum or plasma. Introduced as a second generation immunoassay in 1989, this ImmunoCAP technology has advantages regarding sensitivity and efficiency. Later generations of ImmunoCAP® specific IgE Phadia™ instruments (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden) with full automation, using the same test principle, brought further improvements in precision and reproducibility, more rapid procedure (100 min), higher capacity and continuous random access ability availability. Based on sandwich fluorescence immunoassay method, ImmunoCAP FEIA offers the opportunity to assess the patient’s allergic sensitization profile not only for natural extracts but also at molecular level. It has several steps[42,44,47,53–55], (1) Specific IgE binding to solid-phase step: Native purified or recombinant allergen component covalently coupled to a flexible solid-phase, with a large surface area, a highly branched, hydrophilic cellulose CNBr-activated polymer/sponge encased in a capsule or capsulated carrier polymer (ImmunoCAP with 1-2 µg allergen), reacts with the specific IgE from the patient plasma/serum sample; (2) Conjugate/labeled anti-
IgE detection antibody step: After washing away unbound antibodies, β-galactosidase-labeled anti-IgE mouse monoclonal antibody is added to form the so-called antigen-antibody immune complex; and (3) Fluorescent signal step: After the unbound enzyme-anti-IgE is washed away, 4-methylumbelliferyl-β-galactoside is used as a fluorogenic substrate, incubated with the bound complex to produce the fluorescent 4-methylumbelliferone. After stopping the reaction, the fluorescence measurement of the eluate is performed with a fluorocounter, and there is a correlation between fluorescence and the allergen-bound IgE established from a standard curve of concentration points.

The ImmunoCAP FEIA quantitative method delivers accurate results, as studies using mouse-human chimeric IgE antibodies to allergens have revealed. ImmunoCAP immunoassay needs only 40 µL serum or plasma per test, and the intra-assay precision is as good as standard clinical chemistry assays. This is important and should be emphasized considering the extremely low serum concentrations of IgE antibodies (µg/L). Moreover, each native allergen contains many protein components that may provoke an IgE antibody response, and there are possible interferences with immunoglobulins from other classes. Low CV (10%) translates into fewer replicates, avoiding unnecessary reruns and assay delays. LoQ for ImmunoCAP specific IgE is 0.1 kU/L. The detection limits are 0.10-100 kU/L. In the ImmunoCAP system, 1 kU/L specific IgE represents 0.994 kU/L total IgE, and is equal to 2.4 ng/mL specific IgE. Despite this good conversion from kU/L to ng/mL, it must be stressed that interlaboratory CVs were observed for both units of measurement. The conversion ratios have not been established with other immunoassay systems. Results from different specific IgE systems are not always comparable to each other even if they are provided in same units. Despite methodological differences, results obtained with ImmunoCAP solid-phase immunoassay and Immulite liquid-phase allergens immunoassay for specific IgE against molecular allergens are similar, but such results are not interchangeable by means of mathematical conversion.

The ImmunoCAP specific IgE classes are defined using six calibrators: 0, 0.35, 0.7, 3.5, 17.5 and 100 kU/L (Class 0: from 0 to < 0.35 kU/L; Class 1: from 0.35 to < 0.7 kU/L; Class 2: from 0.70 to < 3.5 kU/L; Class 3: from 3.50 to < 17.5 kU/L; Class 4: from 17.5 to < 50 kU/L; Class 5: from 50 to < 100 kU/L; and Class 6: from ≥ 100 kU/L).

The enzyme-activated chemiluminescence immunoassay with liquid-phase allergens is another advanced singleplex detection method that exploits liquid-phase kinetics in a bead format (3gAllergy™ Immulite® 2000 and Immulite® 2000 XPI immunoassay; Siemens Healthcare Diagnostics Inc., Erlangen, Germany). It is considered as a third generation assay to measure serum specific IgE antibodies against various allergen extracts and 21 individual molecular allergens. This new generation automated liquid-phase immunoassay was introduced in 2003. The use of fluid-phase allergens allows rapid binding kinetics between IgE and the allergic protein conformations, and a time-to-first-result of only 65 min, while enzyme-enhanced chemiluminescence is used for optimal accuracy. This automated quantitative chemiluminescent method can also be used in molecular allergy diagnostics. It has the following steps: (1) Specific IgE binding in liquid-phase step: Native purified allergen component covalently bound to soluble biotinylated polylysine polymer in a fluid phase binds to streptavidin-coated polystyrene bead (as solid-phase) in the reaction tube (through a streptavidin-biotin interaction) and reacts with specific IgE from the patient’s serum sample (during the incubation of streptavidin-coated bead, biotinylated liquid allergen, and patient sample); (2) Conjugate/labeled anti-IgE detection antibody step: After spin washing, alkaline phosphatase enzyme-labeled anti-human IgE monoclonal murine antibody is added to form the so-called antigen-antibody immune complex; (3) Chemiluminescent signal step: After the bead is washed again (efficient washing with spinning at high speed of the tube on its vertical axis), adamantyl 1,2-dioxetane aryl phosphate is added as chemiluminescent substrate. In the luminogenic reaction, the action of bound alkaline phosphatase on this stable substrate creates an unstable adamantly dioxetane anion, with its rapidly and spontaneously breakdown emitting a photon of light; and (4) Chemiluminescence measurement: Performed by a photon-counting photomultiplier tube/luminometer, and there is a correlation between the chemiluminescent signal and the allergen-bound IgE established from a standard curve of concentration points.

Defining features of such a third generation immunoassay include a true zero calibrator with a detection limit of 0.1 kU/L and functional sensitivity of 0.2 kU/L. The detection limits are 0.10-100 kU/L, and the sample volume is 50 µL. A high diagnostic accuracy of the specific IgE to allergen components measurement with this Immulite® 2000 system and a high agreement with ImmunoCAP® platforms were revealed. An important difference between these methods is the source and quality of the allergenic extracts used. Results of in vitro assessments for IgE sensitization using both new generation ImmunoCAP® and Immulite® systems show substantial correlation with respect to serum specific IgE detection for common aeroallergens. However, the results are not interchangeable. Although these two singleplex FDA-cleared assays have the same basic reaction sequence for IgE detection and report the results using the same units, methodological differences are important regarding allergen binding methods, signal detection methods (amplified chemiluminescence used in Immulite vs fluorescence in ImmunoCAP) and...
test running time (reduced from 100 min to 65 min).

The Immulite standard classification system uses eight calibrators (Class 0: from 0 to < 0.35 kU/L, but with the possibility to detect values from 0.1 to 0.35 kU/L; Class 1: from 0.35 to < 0.7 kU/L; Class 2: from 0.70 to < 3.5 kU/L; Class 3: from 3.50 to < 17.5 kU/L; Class 4: from 17.5 to < 52.5 kU/L; Class 5: from 52.5 to < 100 kU/L; and Class 6: from ≥ 100 kU/L).[55]

The reversed enzyme allergosorbent test (REAST) with liquid-phase allergens (Allerg-O-Liq™; Dr. Fooker-Achterrath Laboratorien GmbH, Neuss, Germany) is a reliable singleplex immunoassay using microwells, based on a sandwich ELISA, for the quantitative determination of specific IgE antibodies against about 500 allergen extracts and 50 individual highly-purified native and recombinant allergen components, in serum or plasma. Determination of specific and total IgE is possible in the same test run, with high sensitivity and specificity. Total of the incubation times are 3 h for the manual procedures. Fifty microliters of undiluted calibrators, controls and patient samples are needed, to be pipetted into wells. Fully automated microplate procedure for REAST is possible. It has low variations between different instruments and between manipulators. REAST Allerg-O-Liq has several steps[24,62-64]: (1) IgE binding to solid-phase step: All serum IgE antibodies are bound by immobilized anti-human IgE antibodies coating the microwells (microtiter plates); (2) Fluid-phase allergen binding step: After washing away unbound antibodies, biotinylated allergen is incubated in the microwells; (3) Detection conjugate step: After another washing procedure, the added horseradish peroxidase (commonly referred to as HRP)-conjugate forms a complex consisting of specific IgE/bound allergen/HRP-conjugate; (4) Chromogenic substrate step: After further washing, the substrate 3,3',5,5'-tetramethylbenzidine (commonly known as TMB) is added for colorimetric detection, resulting in the development of a blue color, and after stopping the enzymatic reaction with acid, the color changes to yellow; and (5) Optical density of the colored product is measured by spectrophotometry at 450 nm (reference wave length of 620 nm), with the specific IgE concentration of the patient sample being proportional to the optical density.

Calibrators with defined concentrations of IgE are assayed simultaneously with the patient samples to generate a calibration curve. IgE concentrations are calculated from this curve. This REAST immunoassay detects specific IgE concentrations between 0.35 IU/mL and 100 IU/mL. Specimens with higher specific IgE concentrations should be diluted and retested to determine the exact content. A level of specific IgE < 0.35 IU/mL is defined as positive. Although no single method has been officially designated as the gold standard for specific IgE detection, the worldwide spread ImmunoCAP® assay is commonly used for comparisons. The agreement between this FEIA and the Allerg-O-Liq method is good to excellent[62,64,65].

The chemiluminescence reverse sandwich immunoassay with liquid-phase allergens performed on the Advia Centaur® analyzer (Bayer Healthcare Diagnostics Division, Tarrytown, New York, United States) was also used as a fully automated, quantitative specific IgE in vitro test, using a calibration method based on a recombinant reference allergen. Monoclonal mouse anti-human IgE antibody covalently bound to paramagnetic particles in the solid-phase captures the sample specific IgE. Bound specific IgE reacts with liquid biotinylated allergen, which is detected as chemiluminescence using acridinium ester-labeled streptavidin. Specific IgE was considered positive at ≥ 0.35 kU/L. This immunoassay for determination of specific IgE to allergen components performed with good reproducibility. It also correlated well with the ImmunoCAP® system. A good agreement and correlation for some allergen components with the ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) microarray was revealed[66-68].

MULTIPARAMETER IMMUNOASSAYS FOR SPECIFIC IGE TO ALLERGEN COMPONENTS

A multiallergen screen is not considered a true multiplex immunoassay despite the fact that it detects IgE antibodies to more individual allergens with a single serum addition[9]. Several immunoassays for the in vitro assessment of IgE sensitization to allergen molecules are considered multiparameter tests because they detect specific IgE against few allergen components at once, usually about 10 (2-11 recombinant or native molecules), along with specific IgE against several natural Aeroallergen extracts.

The multiparameter line blot immunoassay for defined partial allergen diagnostics with purified, biochemically-characterized allergen components coating membrane strips in thin parallel lines as line blots (Euroline™; EUROIMMUN AG, Lübeck, Germany) is used to measure simultaneously (on one test strip) specific IgE antibodies against few allergen extracts and several individual molecular allergens. This in vitro diagnosis with defined, in part recombinant partial allergens involves one panel with two pollen extracts plus eight pollen components, and another with one mold and five pollen extracts plus one mold and ten pollen components. Such a component-resolved multiparameter assay, based on immunoblot technology, uses defined proteins as single purified allergen components for IgE antibody detection along with whole
raw allergen extracts. It has several steps: (1) Specific IgE binding to solid-phase step: Recombinant or native purified allergen component coupled to blot strips as thin parallel lines at defined positions on the moistened solid-phase binds to specific IgE patient serum/plasma sample; (2) Conjugate/labeled anti-IgE detection antibody step: After washing away unbound antibodies, alkaline phosphatase enzyme-labeled mouse anti-human IgE monoclonal antibody is added to form the so-called antigen-antibody immune complex; and (3) Chromogenic substrate step: bound antibodies are stained with nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indoly phosphate (NBT/BCIP) for colorimetric detection; scanning is performed on the completely dried membrane strip line using a computer-linked flatbed scanner.

The Euroline immunoassay is a semiquantitative method. The incubation protocol includes an undiluted serum sample (0.4 mL) 60-min incubation, a 60-min incubation with conjugate and a 10-min incubation with substrate, all performed in a blot strip incubation channel on a rocking shaker and separated by washing procedures. The measurement range is given in the enzyme-allergo-sorbent test (EAST) system, in classes from 0-6, the detection limits being 0.35–100 kU/L. With respect to the concentration grades, the EAST is similar to the previously mentioned ImmunoCAP specific IgE classes: Class 1 (very low antibody titer, frequently no clinical symptoms where sensitization is present); Class 2 (low antibody titer, existing sensitization, frequently with clinical symptoms in the upper range of class); Class 3 (significant antibody titer, clinical symptoms usually present); Class 4 (high antibody titer, almost always with clinical symptoms); and Classes 5 and 6 (very high antibody titers). Introduced in clinical practice as a reliable and costly efficient IgE test in the molecular diagnosis of tree and grass pollen allergy, EAST classes of Euroline blot assay have acceptable correlation with ImmunoCAP®.

A similar manual or automated multiparameter technology-based enzyme immunoassay is performed with allergen extracts and components coated separately in lines on a carrier membrane fixed in the well of a cassette (Polycheck®; Biocheck GmbH, Münster, Germany). There are two panels containing recombinant allergens, one with 20 allergen extracts plus 2 recombinant pollen allergen components, and another with 2 Aeroallergen extracts with 4 allergen components. Only 200 µL of patient sera is needed. Anti-IgE antibody is the monoclonal murine antibody derived from over 51 allergen sources (2011 version), independently of the clinical history. The most important advantages of multiplex assays for allergen-specific IgE testing are the provision of an extended panel of specific and cross-reactive allergen molecules (widest allergen spectrum for in vitro allergen testing) for a broad IgE sensitization profiling, especially suited for patients with complex sensitization pattern or symptoms. Critical benefits include distinguishing genuine sensitization from sensitization due to cross-reactivity, assessing biomarkers for allergy phenotypes and allergen immunotherapy, and software interpretation of results. Other advantages over singleplex assays include conservation of sample volume, optimized costs due to fewer required reagents, and increased speed of analysis of the specific IgE repertoire.

Multiplex allergen microarray technology has been applied recently to the field of in vitro allergy diagnosis, being considered an extremely useful advanced diagnostic approach. The first report of an allergen chip-based microarray technology was published in 2002, and the chemistry of this immunoassay (called the ISAC™) was patterned. Ten years of rapid development, resulting in this first generation multiplex allergy test, increased the number of allergenic molecules from 74 to 112. Additional multiplex/oligoplex IgE immunoassays were used in the clinical setting or in research development strategies. Moreover, after the first generation of microarray-based tests, in 2016 and 2017 two new macroarray nanotechnology-based immunodiagnostics tools were launched. They captured the interest of allergists due to their direct impact on the management of patients with allergies in the context of a precision medicine approach.

Multiplex microarray-based immunoassays

A manual allergen microarray-based immunoassay on polymer-coated slide as solid-phase, the ImmunoCAP® ISAC™ (Thermo Fisher Scientific Inc., Phadia AB, Uppsala Sweden), is an European Conformity-marked (CE-marked), miniaturized multiplex in vitro molecular diagnostic test. It enables simultaneous measurement of specific IgE antibodies against a fixed selection of 112 recombinant or purified native allergen components, derived from over 51 allergen sources (2011 version), from a 30 µL of serum, plasma or capillary blood. Because the ISAC panel holds more than 100 allergen components and no allergen extract, it is a good mul-
mplex tool for a detailed exclusively molecular IgE profile assessment of patients \[^{36,77}\]. ISAC is a multiplex immunoassay with two reaction steps \[^{32,54,78,79}\]: (1) Specific IgE binding to solid-phase step: IgE antibodies from the patient sample bind to immobilized multiple allergen components spotted in triplicate onto a pre-activated amine reactive polymer-coated glass slide as solid-phase (100 pg allergen are immobilized on a single spot of the chip, spot size being 200 µm; each glass slide contains four microarrays giving results for four samples per slide); (2) Conjugate/labeled anti-IgE antibody binding step: After the nonspecific antibodies are washed away, fluorophore-labeled anti-human IgE monoclonal antibodies are added to promote the IgE-allergen complex formation; and (3) Fluorescence measuring and image processing: Performed after unbound labeled antibodies are removed by washing, using a confocal laser microarray scanner and microarray image analysis software.

ISAC test results are analyzed with microarray image analysis software and reported in arbitrary ISAC standardized units for IgE (ISU-E). The operating range is 0.3-100 ISU-E. This range is about the same as a concentration range of 0.3-100 kU/L (1 kU/L is equal to 2.4 ng/mL). The calibration curve is adjusted to approximately match the units in the ImmunoCAP singleplex method (kU/L). ISU-E measurements are assigned to one of four categories on a semiquantitative scale, ranging from undetectable or very low (< 0.3 ISU-E) to low (≥ 0.3 to < 1 ISU-E), moderate to high (≥ 1 to < 15 ISU-E) and very high (≥ 15 ISU-E). This miniaturized platform provides a relatively rapid and efficient means of assessing IgE sensitizations to a broad panel of allergen components. The immunoassay is performed in 4 h \[^{80,81}\]. In addition to the integrated Xplain software (Thermo Fisher Scientific Inc., Phadia AB), the AllerGenius expert system (ARMIA, Genova, Italy) was developed to support the interpretation of allergy tests obtained with the ISAC microarray technology \[^{82}\]. The average CV for all allergens tested in intra- and interassay comparisons is below 20%. The LoD of 0.3 ISU-E is assumed for all allergens tested. High concentrations of total IgE have no effect on test performance. The kinetics of ISAC 112 ensure that high-affinity specific IgE is preferentially bound, whereas the kinetics generated by the large excess of allergen in the ImmunoCAP singleplex assay allow binding of low-affinity specific IgE. The overall assay sensitivity (LoD and LoQ) of ISAC 112 is to be considered lower than that of the ImmunoCAP singleplex method. Although there are different epitope exposure on the solid-phase of the assay, due to immobilization on the polymer coating of the glass ISAC chips and covalent binding of allergens to the cellulose matrix in the ImmunoCAP system, a good to very good correlation of the ISU-E values with the ImmunoCAP-derived values (kU/L) is noted for the majority of Aeroallergens \[^{30}\]. Furthermore, the ISAC microarray platform has revealed comparable results to the traditional singleplex method ImmunoCAP and SPT \[^{83}\].

The Mechanisms for the Development of Allergies (MeDALL) allergen chip is a version of the ISAC platform developed for the European Union-funded project MeDALL. It is a research tool containing an expanded repertoire of 170 allergen molecules used for the sensitive detection of allergen-specific IgE against natural purified and recombinant allergen components. For each allergen protein, 50-200 fg, corresponding to 1-5 attomol, is spotted in triplicates onto the MeDALL chip, as compared to ImmunoCAP, in which one spot of the microarray contains 10000000 times less protein, influencing the microarray saturation levels \[^{32,84}\].

Additional multiplex/oligoplex IgE immunossays applied in the clinical setting or in development

An automated allergen microarray-based immunoassay on Microtest chip (Microtest Diagnostics, Worthing, United Kingdom) is a CE-marked, miniaturized in vitro diagnostic test to measure specific antibodies to allergen extracts and allergen components at the same time. The allergens used in the Microtest microarray can be customized. In contrast to the ISAC, it uses 100 µL of serum and may employ 19 allergen extracts and 16 allergenic molecules covering a total of 26 allergen specificities, according to published data, representing a simplified version of a proof-of-concept assay that used 95 allergen extracts and 8 recombinant proteins on aldehyde-activated glass microscope slides \[^{85}\]. Allergen molecules are immobilized covalently in triplicate or more onto a precoated slide as solid-phase in the form of a matrix with more than 150 spots, to which bind the specific IgE antibodies from serum or plasma. Each slide contains one matrix microarray. Up to five microarrays can be assayed at the same time on the platform. A HRP-conjugated antibody detects the IgE-allergen complex, and a detection solution is used to develop the fluorescent signal assessed by a fluorometer. The procedure is reported to take about 4 h. Results are reported in specific IgE classes (Class 0: < 0.35 kU/L; Class 1: 0.35-1 kU/L; Class 2: 1.01-15 kU/L; Class 3: > 15 kU/L). The Microtest allergen panel is much simpler than the ISAC panel but appears to give comparable results for specific IgE against some prevalent Aeroallergens \[^{81,83}\].

A different fluorescence covalent microbead immunosorbent assay was assessed as a Lumexin bead-based suspension array technology test. It allowed the simultaneous quantitative detection of serum total and allergen-specific IgE against six natural purified and recombinant aeroallergen molecules. Multianalyte profiling fluorescent bead microspheres coupled with allergen molecules were used to bind antibodies from human serum and, once bound, the IgE antibody was subsequently detected with a biotinylated anti-human IgE and streptavidin-conjugated phycoerythrin,
fluorescence being measured by a multiplex microplate reader. The analytes were measured with very good interassay reproducibility, from a total serum volume of less than 20 µL and in 6 h. Total and allergen-specific IgE levels correlated with enzyme-linked and fluorescent enzyme immunoassay results[32,86].

A point-of-care fully automated fluorescence-based sandwich nanofluidic immunoassay is available in a disposable capsule containing nanofluidic biosensors with immobilized allergens for in vitro diagnostics (JVD Capsule Aeroallergens; Abionic SA, Biopôle, Switzerland). It is used for the quantitative determination of total IgE and specific IgE to five key aeroallergen components using the abioSCOPE® clinical analyzer (Abionic SA). A 50 µL capillary blood sample with specific IgE mixed with a solution composed of fluorescently labeled anti-IgE antibody to form a complex is drawn through a capsule by capillary action. Using diffusion phenomena, molecules interact together in the biosensors and form molecular complexes in case of specificity. IgE-anti-IgE antibody complex is bound by capsule allergens immobilized within the read-out area. The surface of each biosensor contains specific immobilized allergens (either from natural sources or purified allergens). The capsule with aeroallergens is placed into a disc mounting plate, which is then inserted into the abioSCOPE. These fluorescent immobilized complexes are optically measured by this reading unit, which contains a miniaturized fluorescent microscope. The measured fluorescence signal is reported in kU/L, according to the six classes: < 0.7 kU/L (absent, low or undetectable level of allergen-specific IgE); 0.7-3.4 kU/L (moderate level of allergen-specific IgE); 3.5-17.5 kU/L (high level of allergen-specific IgE); 17.6-50 kU/L (very high level of allergen-specific IgE); 51-100 kU/L (ultra-high level of allergen-specific IgE); > 100 kU/L (extremely high level of allergen-specific IgE). A good agreement was noted between the allergen-specific IgE values measured in ImmunoCAP® Phadia 250 and in the abioSCOPE. Nanofluidic-based biosensor containing a nanochannel that accelerates molecular interactions reduces the incubation duration and the immunoassay time to a matter of minutes[36,87,88]. Such a new disrupter nanotechnology-based diagnostic approach provides healthcare professionals with tools that help them to make a rapid point-of-care diagnosis.

New generation multiplex nanotechnology-based immunoassays

Protein arrays constitute a powerful tool for multiplexed protein analysis. Multiplex tests allow to detect specific IgE to many different preparations at once, assessing a patient’s IgE sensitization profile and allowing for tailoring of decisions for interventions[89]. The terms “microarray” and “macroarray” may be used to differentiate between spot size and the number of spots on the support. The ISAC microarray single spot size of the chip is 200 µm[78]. The term macroarray is usually used for the larger spot array. Multiplex macroarray- and nanotechnology-based immunoassays make available for the allergist an unprecedented quantity of data, which is very useful to explore polysensitized patients and to disclose unknown sensitizations. They offer, nowadays, the widest possible knowledge of the patient’s IgE sensitization profile[75,90,90].

The patient-friendly allergen nano-bead array (FABER®; MacroArray Diagnostics, Vienna, Austria, in collaboration with Centri Associati di Allergologia Molecolare-CAAM, Rome, Lazio, Italy, and its partners) is a new advanced multiplexed nanotechnology-based in vitro immunoassay for specific IgE measurement, having 122 molecular allergens and 122 allergenic extracts (FABER 244), coupled to chemically activated nanoparticles. The large majority of aeroallergens are represented. The inclusion of allergenic extracts is strategic to confirm or complement results obtained with the single allergenic molecules. Allergic preparations (either produced in-house or obtained from commercial providers) are individually coupled to nanobeads by means of optimized protocols in order to achieve maximum test performance and provide high diagnostic accuracy for each spotted allergenic item. Once coupled, they are arrayed to a solid-phase matrix to form a one-step comprehensive array-based testing approach using 100 µL of patient serum or plasma. FABER results expressed in arbitrary units (FIU) are considered negative (< 0.01 FIU/mL), doubtful (> 0.01 to < 0.30 FIU/mL) or positive (> 0.30 FIU/mL).

Test interpretation is supported by a center digital reporting system (an online dynamic visualization system). It provides real-time information and easier understanding of the test results, as a patient-friendly, multilanguage tool accessible from personal computers and mobile devices. The test is exclusively available from CAAM partner laboratory (Sermolab S.r.l., Italy), so the patients have to wait to get the results. FABER IgE measurements perform very well with most allergens, but improving the quality of some extracts will lead to better performances. FABER, ImmunoCAP® and Immulite® systems-having different reference standards-do not completely overlap each other[7,89,91,92].

The macroarray nanotechnology-based immunoassay used as a molecular allergy explorer (ALEX®; MacroArray Diagnostics) is the latest launched in vitro multiplex tool for precision medicine in allergy diagnosis. It is based on a state-of-the-art proprietary nano-bead technology. This new array contains 282 allergen reagents (157 allergenic extracts and 125 molecular components), with a large majority of aeroallergen families and cross-reactive food allergens being represented. This in vitro allergy explorer is the first in vitro multiplex allergy test allowing simultaneous measurement of total IgE and specific IgE against a plethora of allergen extracts and molecular allergens. The combination of second- and third-level assays in the same immunoassay allows to define the presence of IgE sensitization, whether it is...
genuine or cross-reactive, and saves time and costs, particularly in polysensitized patients and/or with pollen-food syndromes.

The ALEX® in vitro allergy test core technology is based on a two-phased manufacturing process and it represents a multiplex ELISA-based test with proven immunoassay chemistry and detection methods, as discussed below. Initially, allergens are coupled to activated nanoparticles, for coupling individual and combinatorial optimization. Each allergen is attached reflecting its biochemical properties and specific requirements for stability, thereby preserving the full epitope complexity. The nanoparticles multiply the surface of the solid-phase presenting the allergen during the immunoassay, enabling highly sensitive detection. In the next step, the allergen-bearing nanoparticles are deposited onto a solid-phase matrix, forming a macroscopic array of individual assay parameters. The different allergens and components, spotted onto a nitrocellulose membrane as immunosorbent in a cartridge chip, are incubated with 0.5 mL of a 1:5 dilution of serum under agitation, the serum diluent containing a Cross-reactive Carbohydrate Determinants (CCDs) inhibitor. After incubation for 2 h, the chips are extensively washed. A pretreated dilution of anti-human IgE labeled with alkaline phosphatase is added and incubated for 30 min. Following another washing cycle, the enzyme substrate is added, and after a few minutes, the reaction is complete. After the membranes are dried, the quantification of this colorimetric enzyme assay is performed with an easy-to-use and affordable image explorer. The image acquisition and analysis of a single test takes only a few seconds. The assay time is 3.5 h, and tests per run are up to 50 per operator, with manual processing.

The ALEX® immunoassay protocol integrates a powerful CCDs inhibitor during serum incubation, as previously mentioned. This reduces the interpretative burden for physicians of CCD-positive patients and increases the specificity of the test results. CCDs refer to a group of related glycans produced by invertebrates and plants but not by vertebrates. Induction of IgE antibodies against CCDs is thought to be driven in humans by pollen exposure and/or insect stings. Most natural allergen preparations originating from plants or insects contain CCDs, but CCDs do not behave as allergens in vivo and are clinically insignificant. IgE antibodies directed against CCDs cross-react with all proteins containing these CCDs epitopes. Therefore, they are an important cause of cross-reactivity for in vitro specific IgE assays regarding CCD-containing allergens from pollen, plant foods and insect venoms if a CCDs inhibitor is not used. A notable 22% of serum samples from patients with suspected sensitizations to pollen, foods or insect venoms were detected to have anti-CCD IgE antibodies, the incidence reaching 35% in the teenage group. A similar overall prevalence of 23% of positive IgE to CCD was recorded in a previous study in subjects with suspected allergic respiratory disease. The prevalence varied when subsets of non-allergic (5%), non-pollen allergic (10%), and pollen allergic (31%) subjects were considered, and further increased in subsets with multiple pollen sensitization (71%) because many patients have anti-CCD IgE antibodies, there are a significant number of positive specific IgE results without the use of a CCDs inhibitor. The presence of such anti-CCD IgE could be misleading for the in vitro reactivity in the case of extract-based testing or when using many CCD-containing natural purified glycoproteins from pollen grains. Recombinant proteins produced in Escherichia coli bacteria are not affected by CCD recognition, because of the lack of the posttranslational glycosylation of proteins. nAna c 2 (MUXF3) is a purified N-glycan from Ananas comosus bromelain (nAna c 2) able to detect IgE against N-glycans in most pollen sources, anti-CCD IgE being a biomarker of reactivity to carbohydrate moieties of glycoproteins.

The ALEX measuring range for specific IgE is 0.3-50 kU/L (quantitative) and for total IgE is 1-2500 kU/L (semiquantitative). The sample requirement is 100 μL serum or plasma. The results are expressed as Class 0 (< 0.3 kU/L), Class 1 (0.3-1 kU/L), Class 2 (1-5 kU/L), Class 3 (5-15 kU/L), and Class 4 (> 15 kU/L). ALEX is commercially available, having attained CE certification, which assures that the quality of the assay, regarding LoD, precision and repeatability as well as specificity and linearity, is in line with in vitro diagnostic features. There is no significant interference from high total IgE, hemoglobin, bilirubin or triglycerides. A flexible Raptor analysis software (specifically designed for ALEX®) allows to analyze tailored allergen panels, as considered fit for clinical needs (multiplex on-demand).

**PRECISION MEDICINE IMMUNOASAYS FOR ASSESSING IgE SENSITIZATION TO AEROALLERGENS**

Molecular allergy diagnosis work-up may be organized as an integrated “U-shape” approach, with a classical “top-down” approach (from symptoms to molecules, using extract-based skin prick tests and/or IgE singleplex assays) combined with a “bottom-up” approach (from molecules to clinical implications, using multiplex assays), as needed in selected patients. This is done in order to explain various allergic phenotypes or diseases, by exploring in detail the individual serum IgE profile or repertoire.

The selection of IgE immunoassays, allergenic extract and molecular specificities, and the interpretation of the results assessing in vitro IgE sensitization to aeroallergens require knowledge of test principle, methods and characteristics, and clinical judgments based on carefully collected history and physical exa-
Table 2 Allergen molecules of house dust and storage mites origin\(^1\) used in singleplex and multiplex immunoassays\(^{[9,74,102,103]}\)

| Allergen molecule | Biological function, comments, CR |
|-------------------|----------------------------------|
| nDer p 1          | Cysteine-protease, cleavage of regulatory IgE synthesis CD23, CD25 |
| rDer f 1          | Midgut (colon, intercolon, postcolon) and mite fecal pellets |
| rDer p 2          | Functional homologue of adaptor MD-2, TLR4 coreceptor |
| nDer p 2          | Midgut (ventriculus, paired ceca), male reproductive tract, fecal pellets |
| rEur m 2          | Group 2 major mite allergen, NPC2 family, CR Der p 2, Der f 2, homologue Lep d 2 |
| rDer p 4          | Group 4 major mite allergen |
| rDer p 5          | Group 5 major mite allergen, homologue fllo t 5 |
| rDer p 7          | LPS-binding protein, stimulation TLR2, group 7 mite allergen |
| rDer p 9          | Serine protease, group 9 mite allergen |
| rDer p 10         | Invertebrate panallergen tropomyosin muscular protein from mite locomotory muscles |
| nDer p 11         | Group 10 CR mite allergen, invertebrate panallergen |
| rDer p 14         | Apolipophorin-like allergens, lipid transport particles |
| rDer p 15         | Chitinase-like allergens |
| rDer p 18         | Chitinase-like allergens |
| rDer p 20         | Arginine kinase, CR shrimp Penaeus monodon Pen m 2 |
| rDer p 21         | Group 21 mite allergen, gut and fecal particles |
| rDer p 23         | Peritrophin-like protein (8 kDa), group 23 mite major allergen |
| Storage mites (Glycyphagus domesticus, etc.) | Peritrophic lining of mite gut, fecal pellets |
| rGly d 2          | Group 2 mite allergens, NPC2 protein |
|                   | CR Lep d 2, homologue Der p 2 (limited CR) |

\(^1\) n: Native purified; r: Recombinant; Per a: Cockroach periplaneta americana allergen molecule; Mite Dermatophagoides pteronyssinus (Der p), Dermatophagoides farinae (Der f), Glycyphagus domesticus (Gly d), Lepidoglyphus destructor (Lep d), Blomia tropicalis (Blo t) allergen molecules; Nematode Anisakis simplex (Ani s) allergen molecule; Shrimp Penaeus aztecus (Pen a), Penaeus monodon (Pen m), Litopenaeus vannamei (Lit 1), clam Venus gallina (Ven g), squid Ureothrixius duvauceli (Uro du) and snail Helix aspersa (Hel as) allergen molecules; CD: Cluster of Differentiation; CR: Cross-reactivity; LPS: Lipopolysaccharide; NPC2: Niemann-Pick type C2 protein; TLR: Toll-like receptor.

\(^{[9,74,102,103]}\) Although some multiplex assays, such as ISAC 112, may be correlated to singleplex assays, such as ImmunoCAP, from the point of view of the results for important corresponding molecular allergens, the tests’ results are not interchangeable because of the different applied technologies. Due to the underlying different methodological backgrounds, it is not surprising that differences appear between different immunoassays. They may be caused by differences in method sensitivity, the use of native or recombinant allergens and the representation of the sensitizing molecule in the testing procedure.\(^{[9,74,102,103]}\)

Specific and cross-reactive aeroallergen components of animal (e.g., house dust mites, cockroaches, mammalian pets), fungal (molds) and plant (pollen) origin used in allergy immunoassays are either well-defined highly purified natural or recombinant molecules. They are utilized in singleplex and multiplex immunoassays for the detection of IgE sensitization (Tables 2-7). Such precision medicine immunoassays used for in vitro assessment of the IgE sensitization to aeroallergen components\(^{[9,33,109-111]}\) are: (1) Indicated within the framework of a patient’s detailed clinical history, because IgE sensitization to a given allergen does not necessarily imply clinical significance and comprehensive case history alone may overlook relevant aeroallergens, especially in multisensitized patients, but also in the case of unclear symptoms and/or sensitization patterns or for assessing unsatisfactory response to treatment. Some history-related information may indicate certain underlying allergenic molecules in the process of anamnesis molecular thinking (e.g., apple oral allergy syndrome with symptoms of rhinoconjunctivitis during birch pollen season suggests Bet v 1 involvement); (2) Essential for comprehensive and accurate IgE sensitization profiling with identification of the clinically relevant allergens, especially when using the multiplex approach, distinguishing genuine IgE sensitization from sensitization due to cross-reactivity in polysensitized patients, and revealing unexpected sensitizations or helping rule out potential triggers by delivering IgE results for a broad spectrum of aeroallergens. Some data correlations may be referred as laboratory molecular thinking (e.g., high levels of specific IgE to all pollen species without plant food allergic reactions may be related to sensitization to polcalcins) or postmolecular anamnesis (e.g., mite-shrimp syndrome related to the presence of a sensitization to Der p 10 tropomyosin); (3) Particularly useful for IgE sensitization profiling in several groups/situations, including small children with limited skin area, elderly with less reliable skin tests, all settings of
inflamed or atopic skin or when medications interfering with SPT cannot be discontinued; (4) Equipped with the ability to improve assessment of severity of reactions associated with allergens. Multisensitization to several different allergen components from a single allergen source may increase symptom severity. Combination of several sensitizing aeroallergen exposures at a given time—the allergen load—is related to disease severity, pushing the patient over the symptom-onset threshold, particularly during viral infections. Moreover, molecular allergy diagnosis may reveal potential risk for food-related reactions; and (5) Posing a key impact on the optimal decision making for prophylactic measures and specific immunotherapy. The detailed assessment of the molecular pattern of IgE sensitization to aeroallergens may improve interventions to reduce allergen exposure or for allergen avoidance, may decrease the need for provocation testing and be useful to guide prescription of allergen immunotherapy, with a better selection of patients and immunotherapy products, potential prediction the efficacy and, in some cases, prediction of the adverse reactions risk.

**CONCLUSION**

Precision medicine is increasingly recognized as the way forward for optimizing patient care. Introduction of the new generation immunodiagnostics, with well-defined purified natural and recombinant allergens,
Table 5  Allergen molecules of fungal origin\(^1\) used in singleplex and multiplex immunoassays\(^{[9,74,106-108]}\)

| Allergen molecule | Biological function, comments, CR |
|-------------------|----------------------------------|
| Ascomycetes fungi (Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus) | Fungal beta-barrel protein, detected in spores before germination. Involvement in Alternaria-spinach syndrome. |
| rAlt a 1 | Fungal beta-barrel protein, detected in spores before germination. Involvement in Alternaria-spinach syndrome. |
| rAlt a 6 | Fungal enolase, panallergen CR Cla h 6, Asp f 22, Pen c 22. |
| rCla h 8 | Fungal mannitol dehydrogenase, major allergen, CR Alt a 8. |
| rAsp f 1 | Fungal ribotoxin, specific major allergen, member of the mitogillin family. Not present in spores, but produced after germination and growth. Genuine exposure and IgE sensitization to A. fumigatus germinated in the respiratory tract. |
| rAsp f 2 | Species-specific allergen component, with high frequency of sensitization among patients affected by ABPA. |
| rAsp f 3 | Fungal peroxisomal protein, CR Pen c 3. |
| rAsp f 4 | Fungal protein, highly specific allergen. With high frequency of sensitization among ABPA patients. |
| rAsp f 6 | Fungal Mn-SOD, CR Alt a 14, Mal d 11. |
| n: Native purified; r: Recombinant; Fungi Alternaria alternata (Alt a), Cladosporium herbarum (Cla h), Aspergillus fumigatus (Asp f), Penicillium citrinum (Pen c), Malassezia sympodialis (Mal a) allergen molecules; ABPA: Allergic bronchopulmonary aspergillosis; CR: Cross-reactivity; Mn-SOD: Mn superoxide dismutase. |

Table 6  Main species-specific allergen molecules of pollen origin\(^1\) used in singleplex and multiplex immunoassays\(^{[9,74,93]}\)

| Plant source | Allergenic molecule | Biological function, comments |
|--------------|---------------------|------------------------------|
| Specific allergen components of tree pollen origin | rBet v 1 | PR-10 protein, heat sensitive. CR with Bet v 1-like proteins Cor a 1, Mal d 1. Involved in birch-hazelnut-Rosaceae fruit syndrome. |
| Birch Betula verrucosa (Bet v) | rBet v 1 | PR-10 protein, heat sensitive. CR with Bet v 1-like proteins Cor a 1, Mal d 1. Involved in birch-hazelnut-Rosaceae fruit syndrome. |
| Oleaceae family | rOle e 1 | Trypsin inhibitor |
| Platanaceae family | rPla a 1 | Invertase inhibitor |
| Cupressaceae family | nCup a 1 | Pectate lyase |
| Specific allergen components of grass pollen origin | nPhl p 1 | Beta-expansin |
| Poaceae family | nPhl p 5 | Ribonuclease; other allergen molecules: rPhl p 2, rPhl p 6. |
| Specific allergen components of weed pollen origin | nCup a 1 | Pectate lyase |
| Chenopodiaceae family | rChe a 1 | Ole e 1-like protein |
| Beta-expansin | rArt a 1 | Defensin-like protein. |
| Asteaceae family | nArt a 1 | Defensin-like protein. |
| nAmb a 1 | Pectin lyase, CR Art a 1. |
| Urticaceae family | rPar j 2 | Lipid transfer protein |
| Plantaginaceae family | rPla l 1 | Trypsin inhibitor |
| Amaranthaceae/Chenopodiaceae family | rSal k 1 | Pectin methylesterase |
| nSal k 1 | Pectin methylesterase |
| rChe a 1 | Ole e 1-like protein |
| n: Native purified; r: Recombinant; Fruit hazelnut Corylus avellana (Cor a) and apple Malus domestica (Mal d) allergen molecules; Pollen Betula verrucosa (Bet v), Olea europaea (Ole e), Platanus acerifolia (Pla a), Cupressus arizonica (Cup a), Phleum pratense (Phl p), Cynodon dactylon (Cyn d), Artemisia vulgaris (Art v), Ambrosia artemisiifolia (Amb a), Parietaria judaica (Par j), Plantago lanceolata (Pla l), Salsola kali (Sal k), Chenopodium album (Che a) allergen molecules; CR: Cross-reactivity. |

in the field of allergy research and clinical practice represents a great precision medicine approach. Modern IgE immunoassays allow for accurate and affordable patient detailed IgE sensitization profiling and identification of the clinically relevant allergens, with potential favorable impact on the allergy diagnosis and treatment, especially in selected patients, including refined useful information regarding clinically relevant allergens, allergenic risks, early interventions, effective and optimized management. Collectively, this will lead to improvements in patient health, quality of life and overall costs.
Table 7  Cross-reactive allergen components of pollen origin\(^1\) used in singleplex and multiplex immunoassays\(^{9,74}\)

| Allergen molecule | Biological function, comments, CR |
|-------------------|----------------------------------|
| Polcalcin         | Calcium-binding protein, polcalcin biomarker |
| rPhl p 7          | Minor allergen CR with other polcalcin contained in pollen grains: grasses: *Poaceae* and non-*Poaceae*, e.g., Bermuda grass (Cyn d 7) trees: e.g., birch (Bet v 3), alder (Aln g 4), olive (Ole e 3), juniper (Jun o 4) weeds: e.g., mugwort (Art v 5), short ragweed (Amb a 10) Polcalcin, which contains only two calcium-binding domains (rBet v 3, which contains three calcium-binding domains) Polcalcin biomarker |
| rBet v 4          | Involved in mugwort-plant association, *Asteraceae*-hazelnut association |
| nArt v 3          | PR-14 protein, CR with peach Pru p 3 from, hazelnut Cor a 8 Involved in mugwort-plant association, *Asteraceae*-hazelnut association |
| nOle e 7          | PR-14 protein, CR with peach Pru p 3, pear Pyr c 3, melon Cuc m LTP, kiwifruit Act d 10, Involved in olive pollens-fruit syndrome Involved in Platanaus pollens-fruit/vegetables association |
| rPla a 3          | Involved in *Platanus* pollens-fruit/vegetables association |
| Profilin rPhl p 12| Plant parallergen actin-binding protein (cytoskeleton dynamics) Profilin biomarker with great sequence identity with other profilins CR with profilins from: grass pollen: *Poaceae* and non- *Poaceae*, e.g., Bermuda grass (Cyn d 12) tree pollen: i.e., birch (Bet v 2), olive (Ole e 2), date palm (Pho d 2) weed pollen: i.e., ragweed (Amb a 8) and mugwort (Art v 4); sunflower (Hel a 2) CR with profilin in latex (Hev b 8) and exotic fruits, involved in pollen-latex-fruit syndrome: profilins from ananas (Ana c 1), banana (Mus xp 1), kiwi (Act d 9) and olive pollens (Ole e 2) CR with profilins from various plant foods: CR protein v 4 CR with Dau c 4, Api g 4, involvement in celery-mugwort-spike syndrome profilin Amb a 8 CR with Cuc m 2, Mus xp 1, involved in ragweed-melon-banana association Cross-reactive biomarker, important for the profilin group CR profilins in Apiaceae (Dau c 4, Api g 4) Involvement in birch-Apiceae vegetables association |
| rBet v 2          | CR profilin in pollen grains; CR profilins from ananas (Ana c 1), banana (Mus xp 1), kiwi (Act d 9) and olive pollens (Ole e 2) CR with profilins from various plant foods: CR protein v 4 CR with Dau c 4, Api g 4, involvement in celery-mugwort-spike syndrome profilin Amb a 8 CR with Cuc m 2, Mus xp 1, involved in ragweed-melon-banana association Cross-reactive biomarker, important for the profilin group CR profilins in Apiaceae (Dau c 4, Api g 4) Involvement in birch-Apiceae vegetables association |

\(^{1}\) Native purified; r: Recombinant; Fruit hazelnut Corylus avellana (Cor a), Prunus persica (Pru p), Pyrus communis (Pyr c), Cucumis melo (Cuc m), Actinidia deliciosa (Act d), Musa acuminata (Mus a), Ananas comosus (Ana c) allergen molecules; Latex *Hevea brasiliensis* (Hev b) allergen molecule; Pollen *Bétula verrucosa* (Bet v), Alnus glutinosa (Aln g), Olea europaea (Ole e), Platanus acerifolia (Pla a), Juniperus oxycedrus (Jun o), Phoenix dactylifera (Pho d), Phileum pratense (Phl p), Cynodon dactylon (Cyn d), Artemisia vulgaris (Art v), Ambrosia artemisiifolia (Amb a), Helianthus annuus (Hel a) allergen molecules; Vegetables *Apium graveolens* (Api g), Daucus carota (Dau c) allergen molecules; CR: Cross-reactivity; PRA: Pathogenesis-related plant proteins.

REFERENCES

1. Agache I, Rogozea L. Endotypes in allergic diseases. *Curr Opin Allergy Clin Immunol* 2018; 18: 177-183 [PMID: 29561354 DOI: 10.1097/ACI.0000000000000434]
2. Agache I, Akdis CA. Endotypes of allergic diseases and asthma: An important step in building blocks for the future of precision medicine. *Allergy* 2016; 65: 243-252 [PMID: 27282212 DOI: 10.1016/j.aller.2016.04.011]
3. Hellings PW, Borrelli D, Pietikainen S, Agache I, Akdis C, Bachert C, Bewick M, Botjes E, Constantinidis J, Fokkens W, Haahr H, Hopkins C, Illario M, Joos G, Lund V, Muraro A, Pugin B, Seys S, Somekh D, Stårman P, Valvuiste A, Vorös F, Bouquet J. European Summit on the Prevention and Self-Management of Chronic Respiratory Diseases: report of the European Union Parliament Summit (29 March 2017). *Clin Transl Allergy* 2017; 7: 49 [PMID: 29229920 DOI: 10.1186/s13601-017-0186-3]
4. Muraro A, Fokkens WJ, Pietikainen S, Borrelli D, Agache I, Boissier J, Costigliola V, Joos G, Lund VJ, Poulsen HK, Price D, Rolland C, Zuberbier T, Hellings PW. European Symposium on Precision Medicine in Allergy and Airways Diseases: Report of the European Union Parliament Symposium (October 14, 2015). *Allergy* 2016; 71: 583-587 [PMID: 26660269 DOI: 10.1111/ all.12819]
5. Muraro A, Steelant B, Pietikainen S, Borrelli D, Childers N, Callebaut I, Kortekaas Krohn I, Martens K, Pugin B, Popescu FD, Vieru M, Jutel M, Agache I, Hellings PW. European symposium on the awareness of allergy: report of the promotional campaign in the European Parliament. *Asthma Allergy* 2017; 72: 173-176 [PMID: 27696452 DOI: 10.1111/all.13058]
6. Hamilton RG, Kleine-Tebbe J. Molecular Allergy Diagnostics: Analytical Features That Support Clinical Decisions. *Curr Allergy Asthma Rep* 2015; 15: 57 [PMID: 26233428 DOI: 10.1007/s11882-015-0565-7]
7. Alessandri C, Ferrara R, Bernardi ML, Zennaro D, Toppo L, Giangreco I, Tamburrini M, Mari A, Ciardillo MA. Diagnosing allergic sensitizations in the third millennium: why clinicians should know allergen molecule structures. *Clin Transl Allergy* 2017; 7: 21 [PMID: 28725346 DOI: 10.1186/s13601-017-0158-7]
8. Kowalski ML, Ansetegui I, Abner W, Ahmadi M, Akdis M, Ballmer-Weber BK, Beyer K, Blanca M, Brown S, Bunnag C, Hulet AE, Castells M, Cing HH, De Blay F, Ehsasowa F, Fineman M, Golden DB, Haahr H, Kallner M, Kadertis C, Lee BW, Makowska J, Muller U, Mullol J, Oppermann J, Park HS, Parsonkar J, Passalacqua G, Pawanaka R, Renz H, Ruff K, Sanchez-Borges M, Sastre J, Scadding G, Scharer S, Tantilipikorn P, Tracy J, van Kempen V, Bohle B, Canonica GW, Caraballo M, Ballmer-Weber BK, Beyer K, Blanca M, Brown S, Bunnag C, Hulet AE, Castells M, Cing HH, De Blay F, Ehsasowa F, Fineman M, Golden DB, Haahr H, Kallner M, Kadertis C, Lee BW, Makowska J, Muller U, Mullol J, Oppermann J, Park HS, Parsonkar J, Passalacqua G, Pawanaka R, Renz H, Ruff K, Sanchez-Borges M, Sastre J, Scadding G, Scharer S, Tantilipikorn P, Tracy J, van Kempen V, Bohle B, Canonica GW, Caraballo M, Gomez M, Ito K, Jensen-Jarolim E, Larche M, Mellion G, Matricardi PM, et al. Allergy Organization Statement. *Asthma Allergy* 2017; 7: 21 [PMID: 28725346 DOI: 10.1186/s13601-017-0158-7]
9. Mattiacci P, Kleine-Tebbe J, Hoffmann JH, Valenta R, Hilger C, Hofmaier S, Aalberse RC, Agache I, Asero R, Ballmer-Weber
Disease. Microarrays (Basel) 2017; 6: 3 [PMID: 28134842 DOI: 10.3390/microarrays6010003]

33 Canonica GW, Anzotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, Melioli G, Nunes C, Passalacqua G, Rosenowasser L, Sampson H, Sastre J, Bousquet J, Zuberbier T, WAO-ARIA-GA2LEN Task Force: Katrina Allen, Riccardo Asbera, Barbara Bohle, Linda Cox, Frederic de Blay, Motohiro Ebisawa, Rene Maximiliano-Gomez, Sandra Gonzalez-Diaz, Tari Haahstela, Stephen Holgate, Thilo Jakob, Mark Larche, Paolo Maria Matricardi, John Oppenheimer, Lars K Poulsen, Harald E Renz, Nelson Rosario, Marc Rothenhe, Mario Sanchez-Borges, Enrico Scala, Rudolf Valenta. A WAO - ARIA- GA2 LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J 2013; 6: 17 [PMID: 24093938 DOI: 10.1186/1169-3455-41-6.17]

34 Riccio AM, De Ferrari L, Chiappori A, Ledda S, Passalacqua G, Melioli G, Canonica GW. Molecular diagnosis and precision medicine in allergy management. Clin Chem Lab Med 2016; 54: 1705-1714 [PMID: 26985687 DOI: 10.1518/cmcl.2016-0007]

35 Breiteneder H, Hassfeld W, Pettenburger K, Jarolim E, Hassfeld W, Pettenburger K, Jarolim E, Butzke J, Sander C, Kremer M, Schuhmacher BM, Stoecker W, Weimann A, Koch M, Ahrens E, Breitenbach M, Rumpold H, Kraft D, Scheiner O. Isolation and characterization of messenger RNA from male inflorescences and pollen of the white birch (Betula verrucosa). Allergy 2014; 69: 480-488 [PMID: 24002513 DOI: 10.1111/all.12324]

36 Chapman MD, Ferreira F, Villalba M, Cromwell O, Bryan D, Becker WM, Fernandez-Rivas M, Durham S, Viets S, van Ree R; CREATE consortium. The European Union CREATE project: Part 1: the development of a fully automated multianalyte allergen component-specific IgE antibody serology: a primer for the practicing North American allergist/immunologist. J Allergy Clin Immunol 2010; 126: 53-63 [PMID: 20451984 DOI: 10.1016/j.jaci.2010.03.014]

37 Johannsson SG, Bennich H, Foucard T. Quantitation of IgE antibodies and allergens by the radioallergosorbent test, RAST. Int Arch Allergy Appl Immunol 1973; 45: 55-56 [PMID: 4580379 DOI: 10.1159/0002231001]

38 NIAID-Sponsored Expert Panel, Boyce JA, Assa’ad A, Burks AW, Jones SM, Sampson HA, Wood RA, Plaut M, Cooper SF, Fenton MJ, Arshad SH, Banaa SL, Beck LA, Byrd-Bredbenner C, Camargo CA Jr, Eichenhen L, Furuta GT, Hanifin JM, Jones C, Kraft M, Levy BD, Lieberman P, Luccioni S, McCall KM, Schneider LC, Simon RA, Simons FE, Teach SJ, Yawn BP, Schwaninger JM. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. J Allergy Clin Immunol 2010; 126: 51-58 [PMID: 21134576 DOI: 10.1016/j.jaci.2010.10.007]

39 Armbuster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 2008; 29 Suppl 1: S49-S52 [PMID: 18852857]

40 Bud EM. Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev 2010; 23: 550-576 [DOI: 10.1128/CMR.00074-09]

41 Amarasekera M. Immunoglobulin E in health and disease. Asia Pac Allergy 2011; 1: 12-15 [PMID: 22053291 DOI: 10.5415/apallergy.2011.1.12]

42 Johannsson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, Motala C, Ortega Martella JA, Platts-Mills TA, Ring J, Thien F, Van Cauwenberge P, Williams HC. 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 [PMID: 15131563 DOI: 10.1016/j.jaci.2003.12.591]

43 Kleine-Tebbe J, Matricardi PM, Hamilton RG. Allergy Work-Up Including Component-Resolved Diagnosis: How to Make Allergen-Specific Immunotherapy More Specific. Immunol Allergy Clin North Am 2016; 36: 191-203 [PMID: 26617235 DOI: 10.1016/j.iac.2015.08.012]

44 Ollert M, Weissenbacher S, Rakoski J, Ring J. Allergen-specific IgE measured by a continuous random-access immunonalyzer: interassay comparison and agreement with skin testing. Clin Chem 2005; 51: 1241-1249 [PMID: 15903313 DOI: 10.1373/ clinchem.2004.046565]

45 Goikoetxea MJ, Sanz ML, Garcia BE, Mayorga C, Longo N, Gamboa PM; Immunology Committee of SEAIc, Barber D, Caballero Molina T, de la Calle Toral A, Escribano Mora L, Garcia Martinez JM, Labrador M, Lopez Hoyos M, Martinez Quesada J, Montesinre Mateo J. Recommendations for the use of in vitro methods to detect specific immunoglobulin E: are they comparable? J Investig Allergol Clin Immunol 2013; 23: 448-454; quiz 2 p preceding 455 [PMID: 2465308]

46 Millesi G, Bonifazi F, Bonini S, Maggi E, Mussap M, Passalacqua G, Rossi ER, Vaeca A, Canonica GW; Italian Board for ISAc (IBI). The ImmunoCAP ISAc molecular allergology approach in adult multi-sensitized Italian patients with respiratory symptoms. Clin Biochem 2011; 44: 1005-1011 [PMID: 21627961 DOI: 10.1016/j.clinbiochem.2011.05.007]

47 Park KH, Lee J, Sim DW, Lee SC. Comparison of Singleplex Specific IgE Detection Immunoassays: ImmunoCAP Phadia 250 and Immulite 2000 3gAllergy. Ann Lab Med 2018; 38: 23-31 [PMID: 29071815 DOI: 10.3343/alm.2018.38.1.23]

48 Wood RA, Segall N, Ashlett S, Williams PB. Accuracy of IgE antibody laboratory results. Ann Allergy Asthma Immunol 2007; 99: 34-41 [PMID: 17650827 DOI: 10.1016/S1081-1206(06)00816-7]

49 Szecsi PB, Studier S. Comparison of immunoglobulin E measurements on IMMULITE and ImmunoCAP in samples consisting of allergen-specific mouse-human chimeric monoclonal antibodies towards allergen extracts and four recombinant allergens. Int Arch Allergy Immunol 2013; 162: 131-134 [PMID: 23921375 DOI: 10.1159/000353276]

50 Vignati G, Pastori E, Portalupi S, Temporiti R. In vitro allergy diagnosis: comparison of a new method of fully automated
determination of specific IgE, using Immulite 2000 compared with UniCAP 100. Eur Ann Allergy Clin Immunol 2003; 35: 285-294 [PMID: 14633047]

59 López-Herreño M, Lázaro MT, Rodríguez JJ, Sanz ML, Labrador-Herrero M, Ramos R, Martín-Estebaranz M, Pastor R, Hernández MD, Casas ML, Peláez A, García BE. Quantitative measurement of allergen-specific immunoglobulin E levels in mass units (ng/mL): an interlaboratory comparison. J Investig Allergol Clin Immunol 2012; 22: 387-389 [PMID: 23101323]

60 Schulte J, Leberkuhne L, Salzmann-Manrique E, Schubert R, Zielen S, Rosewich M. Comparison of two different assays and the predictive value of allergen components in house dust mite allergy. Immunotherapy 2017; 9: 1253-1262 [PMID: 29130795 DOI: 10.2217/intm-2017-0084]

61 Villalta D, Da Re M, Conte M, Martelli P, Uasuf CG, Barralle M, La Chiara SM, Brusca I. Allergen component specific IgE measurement with the Immulite™ 2000 system: diagnostic accuracy and intermethod comparison. J Clin Lab Anal 2015; 29: 135-141 [PMID: 24797249 DOI: 10.1002/jcl.21741]

62 Fokee M. Novel approaches for the in-vitro diagnosis of type I allergies. In: Gao ZS, Zheng ML, Gilissen L, Shen HH, Frewer LJ. Multidisciplinary approaches to allergies. Zhejiang University Press, Hangzhou and Springer-Verlag Berlin Heidelberg 2012; 193-201 [DOI: 10.1007/978-3-642-31609-8]

63 Worm M, Jappe U, Kleine-Tebbe J, Schäfer C, Reese I, Saloga J, Treudler R, Zuberbier T, Walthmann A, Fuchs T, Dölle S, Ratthel M, Ballmer-Weber B, Niggemann B, Werfel T. Food allergies resulting from immunological cross-reactivity with inhalant allergens: Guidelines from the German Society for Allergology and Clinical Immunology (DGAKI), the German Dermatology Society (DDG), the Association of German Allergologists (AeDA) and the Society for Pediatric Allergology and Environmental Medicine (GPA). Allergy J Int 2014; 23: 1-16 [PMID: 26120513 DOI: 10.1007/s40629-014-0004-6]
Potential reactivity.

...distribution and appraisal of the in vivo and in vitro determinants enhances diagnostic selectivity.

...structures present in common allergen sources on specific IgE (A1045) [DOI: 10.1111/all.13539]

...based on new state-of-the-art multiplex nano-bead technology in different clinical contexts.

...quantification of allergen-specific IgE. Clin Transl Allergy 2016; 6 Suppl 2: 6 [DOI: 10.1186/s13061-016-0123-x]

...test for allergy diagnosis: food molecule- and extract-based allergenic preparations in the newest and broadest nanotechnology IgE test. Clin Transl Allergy 2017; 7 Suppl 1: 11 [DOI: 10.1186/s13061-017-0142-2]

...challenges. Allergol Int 2016; 109: 871-876; quiz 877-878 [PMID: 26563614 DOI: 10.1002/acr.22073]
Helliens PW, Fokkens WJ, Bachert C, Akdis CA, Bieber T, Agache I, Bernal-Sprekelsen M, Canonica GW, Gevaert P, Joos G, Lund V, Muraro A, Onerci M, Zuberbier T, Pugin B, Seys SF, Bousquet J; ARIA and EPOS working groups. Positioning the principles of precision medicine in care pathways for allergic rhinitis and chronic rhinosinusitis - A euforea-aria-epos-airways ICP statement. Allergy 2017; 72: 1297-1305 [PMID: 28306159 DOI: 10.1111/all.13162]
