FAQS on when to use Molecular Allergy Diagnosis (MD) in clinical practice

Short Title: Clinical uses of MD

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Abstract
In the last decades there has been a great progress in the field of molecular biology allowing the study of the sensitization to individual allergenic components of an allergenic source, a practice that has been termed Molecular Allergy Diagnosis (MD) or Component Resolved Diagnosis (CRD).

The purpose of the present review is to offer the clinician a practical approach to the use of MD by answering frequently asked questions among physicians on how MD can help us improve allergy diagnosis in our daily clinical practice.

The article is divided in three sections. First, a brief review on the importance for the clinician to know the main allergens of the different allergenic sources, their structure and their in vitro cross-reactivity before approaching MD (section A). Secondly the core of the review on the usefulness of MD in clinical practice (section B) answering FAQS on the subject, and finally a section (C) on the interpretation and integration of MD with the rest of available tools for allergy diagnosis.

Key words: Molecular Allergy Diagnosis (MD). Component Resolved Diagnosis (CRD). Allergen component. Genuine sensitization. Cross-reactivity.

Resumen
En las últimas décadas ha habido un gran avance en el campo de la biología molecular permitiendo el estudio de la sensibilización a componentes alergénicos individuales de una fuente alergénica. Dicha práctica se ha denominado Diagnóstico Molecular en Alergia (DM) o Diagnóstico por Resolución de Componentes (CRD, según las iniciales en inglés).

El propósito de la presente revisión es ofrecer al clínico un enfoque práctico para el uso del DM respondiendo preguntas frecuentes entre los médicos sobre cómo puede ayudarnos a mejorar el diagnóstico de alergia en nuestra práctica clínica diaria.

La revisión se divide en tres secciones. En primer lugar, se realiza una breve revisión sobre la importancia que tiene para el clínico conocer los principales alérgenos de las diferentes fuentes alergénicas, su estructura y su reactividad cruzada in vitro antes de abordar el DM (apartado A). En segundo lugar, está el núcleo de la revisión sobre la utilidad del DM en la práctica clínica (apartado B) respondiendo a las preguntas frecuentes sobre el tema, y finalmente se añade un apartado (C) sobre la interpretación e integración del DM con el resto de las herramientas disponibles para el diagnóstico de alergia.

A. BASIC KNOWLEDGE ON ALLERGENS, CROSS-REACTIVITY AND SPECIFIC IgE ASSAYS

It is beyond the scope of this review to analyze in detail every allergen, but before using MD physicians need to have a basic knowledge on the allergens that have been described so far, their main features and the commercially available assays for measuring specific IgE (sIgE).

An up-to-date list on allergens and protein families can be found at large allergen databases, including: http://www.allergen.org; http://www.allergome.org and http://www.meduniwien.ac.at/allergens/allfam/.

Table 1 summarizes the main allergens (grouped by protein families) and their common associated clinical features.

**Commercially available assays for quantitative measurement of specific IgE against individual components**

The main commercially available systems in Europe for individual allergen sIgE quantification include ImmunoCAP® (Thermofisher Scientific), Immulite® (Siemens) and Hytec®-288 (Hycor Biomedical).

The complete list of allergens (both native and recombinant) available for each assay can be found at their websites:

https://www.hycorbiomedical.com/noveosspecificigeallergens

There are several publications that have compared the different assays with relatively good correlation between them and with the clinical diagnosis, for the vast majority of allergens. In any case, it should be noted that although having high correlations the measurements obtained with the different assays are not interchangeable [1-6].

In addition to the determination of sIgE against components in singleplex, it is possible to determine sIgE to several allergenic components simultaneously: Commercially available multiplex microarrays in Europe include: ImmunoCAP ISAC®_112i (ThermoFisher Scientific Uppsala, Sweden), ALEX2® (MacroArray Diagnostics, Vienna, Austria) and EUROLINE ® (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany).

**ImmunoCAP ISAC_112i®** is an enzyme-linked immunoassay with a fluorochrome-labeled, solid phase secondary antibody of 112 allergenic components from 48 allergen sources. The results are presented in a semi-quantitative form (ISU-E) so, although they are related, they cannot be interchanged with those obtained with the platforms described below.


**ALEX2®** is a dot-blot (colorimetric) solid phase enzyme-linked immunoassay of 117 complete extracts and 178 allergenic components and includes an inhibitor of cross-reactive carbohydrate determinants (CCDs). The results are presented in a “quantitative” way by including an IgE curve (kU/L), although it is not...
interchangeable with those of other techniques. ([https://www.macroarraydx.com/products/alex](https://www.macroarraydx.com/products/alex))

**EUROLINE®** is a solid phase enzyme-linked immunoassay type “line-blot” (colorimetric) with various mixtures of complete extracts and allergenic components adapted to different geographical areas ([https://www.euroimmun.com/products/allergy-diagnostics/](https://www.euroimmun.com/products/allergy-diagnostics/)). The results are presented quantitatively in kU/L, and again, it is not interchangeable with those of other techniques.

**When to use singleplex vs multiplex assays?**

The advantages and limitations of singleplex and multiplex assays have been extensively reviewed [7,8], a brief summary of the differences is presented in table 2. The decision of whether to use singleplex or multiplex testing should take into account various factors:

a) **Number of allergens to be tested**

If possibly a large number of allergens from one protein family are involved, multiplex testing might be preferred, especially for those allergen families with limited cross-reactivity, such as seed storage proteins like 2S-albumins, 7S-globulins and 11S-globulins.

b) **Purpose of the test. Preferred test sensitivity**

Singleplex testing offers an enhanced assay sensitivity and should be the preferred assay when the aim is to determine the extent of sensitization attributable to a given allergic component, ratio between the levels of sIgE to the component and the sIgE to the whole extract (we-sIgE/c-sIgE) [9], or when the purpose of the study to monitor a sensitization over time.

c) **Sample volume**

In situations in which the sample volume is limited, such as the management of some cases of pediatric patients, multiplex assays could be of choice of election.

d) **Availability. Costs**

Not all singleplex and multiplex allergen assays are available in every clinical setting nor in every country and thus it is sometimes not up to the clinician to decide which test to use. However, it is the clinician’s responsibility to know the advantages and disadvantages of each technique (table 2) and how to interpret them in each specific situation.

Regarding the costs of these assays, when the detection of more than 12 or 13 individual sIgE are needed, it has been suggested that the multiplex assay is more cost effective than the singleplex diagnostic approach and is therefore preferred [10].
B.-CLINICAL USES OF MOLECULAR ALLERGY DIAGNOSIS (MD)
B.-1- ALLERGEN IMMUNOTHERAPY (AIT)

Why should I perform MD before prescribing an AIT?
The general premise before considering AIT, is to prove that the patient is sensitized to major allergens of the allergenic source. This is one of the main benefits of Molecular Diagnosis (MD) in clinical practice, to discern between a genuine sensitization to an allergenic source from a cross-reactivity sensitization.

Recently, Barber D et al [11] have published an excellent and comprehensive review on the impact of molecular diagnosis in the prescription of AIT and have proposed diagnostic algorithms and decision trees driven by CRD for AIT with pollens, epithelia and some foods (non-specific Lipid transfer Protein; nsLTP; containing vegetables and peanuts).

Which allergens should be included in a pollen allergy study when considering AIT?
The panel of allergens should be chosen depending on the area and the availability of the components. The extended panel would include the marker of genuine sensitization to pollen (Phl p 1/5, Ole e 1, Par j 2, Cup a 1, Art v 1, Sal k 1, Pla a 1/2, Amb a 1, Pla l 1, Bet v 1), also, in areas of high olive pollen exposure Ole e 7 and Ole e 9 should be included. Sensitization to cross-reactive allergens: Bet v 2, Phl p12, Hev b 8, Mer a 1 (profilins) and Phl p 7 or Bet v 4 (polcalcins) should also be studied.

As a general concept, when the sensitization is clinically relevant and the patient is mainly sensitized to the major allergens of the pollen source, irrespective of the sensitization to cross-reactive allergens, the prescription of AIT is advisable. The general premise is that AIT product should be quantified and standardized for these major allergens.

If the patient is only or mainly sensitized to cross-reactive or minor allergens, AIT should not be prescribed since the content for minor allergens in AIT preparations is unknown and variable and there is no evidence of the efficacy of AIT products in patients only sensitized to minor allergens.

Which allergens should be included in a House Dust Mite (HDM) allergy study when considering AIT?
Component-resolved diagnosis using purified and/or recombinant allergens can improve the accuracy of specific IgE testing in HDM allergy but availability is limited worldwide. The WHO/IUIS allergen nomenclature sub-committee currently includes up to 31 D. pteronyssinus and D. farinae as well as 13 allergens from Blomia tropicalis and other allergens from storage mite species. The Dermatophagoides spp. group 1, group 2 and group 23 allergens, are the immunodominant allergens, the group 4, 5, 7 and 21 allergens exhibit medium or mid-tier allergenicity and the other groups minor or unknown allergenicity [12].
Similar to pollen AIT indication, when the HDM sensitization is clinically relevant in respiratory allergy and the patient is mainly sensitized to the major allergens; group 1: **Der p 1** or **Der f 1** and/or group 2: **Der p 2** or **Der f 2**, irrespective of the sensitization to other allergens, the prescription of AIT is advisable [13]. The general premise is that AIT commercial product should be quantified and standardized for these major allergens.

A particular case is when predominant sensitization or monosensitization to the major allergen **Der p 23** is detected. This allergen is present in the commercial extracts for AIT but until now only group 1 and 2 HDM allergens are quantified and standardized in these extracts, so we need more evidence to recommend HDM AIT in this particular case.

**Which allergens should be included in a epithelia pet allergy study when considering AIT?**

Regarding cat allergy, **Fel d 1** is a major allergen with sensitization rates up to 92% of cat-allergic patients as reported in a recent study on cat and dog AIT efficacy [14]. Thus, despite substantial differences in **Fel d 1** content among different immunotherapy extracts, it seems reasonable to confirm sIgE sensitization to **Fel d 1** before prescribing a cat AIT.

Regarding dog allergy, the pattern of sensitization is more heterogeneous and a great variability in allergen content between different commercial extracts has been documented [15] which may explain poor and conflicting results of clinical efficacy of dog allergen immunotherapy in the medical literature [11, 16]. To date, dog allergens available for sIgE determination include **Can f 1**, **Can f 2**, **Can f 3**, **Can f 4**, **Can f 5** and **Can f 6**. Positive sIgE to **Can f 3** o **Can f 6** in the absence of sensitization to other dog allergens suggests cross-reactivity due to primary sensitization to other epithelia and AIT may not be advisable [11].

In cases of **Can f 5** monosensitization (reported in up to 37% of dog allergic patients [17] it has been recently described that, children show different reactions to male and female dog extract conjunctival provocation tests, suggesting tolerance to female dogs [18]. Therefore, in cases of **Can f 5** monosensitization it may be better to prescribe male dog avoidance rather than to prescribe an AIT with an unknown **Can f 5** content.

**Which allergens should be included in Alternaria allergy study when considering AIT?**

sIgE to **Alt a 1** should be assessed before considering the prescription of AIT in a patient with a clinically relevant sensitization to Alternaria. **Alt a 1** is a major allergen, recognized by more than 90% of Alternaria allergic patients, and a marker of primary sensitization to this fungus. A recent clinical trial has shown the efficacy and safety of allergen immunotherapy with a commercial extract of **Alt a 1** [19].
**What allergen profile should I request before prescribing hymenoptera venom immunotherapy (VIT)?**

Before prescribing VIT it is advisable to determine the levels of serum tryptase, total IgE (tIgE) and sIgE to the whole extract of all hymenoptera venoms relevant in your area (bee venom, common wasp venom or yellow jacket, paper wasp venom, mediterranean or european paper wasp, european hornet venom, asian wasp etc.) and sIgE to CCDs (MUXF3). The ratio between whole extract sIgE (we-sIgE) and tIgE (if detected with the same technique) inform us of the relevance of this allergenic source in the venom allergy of the patient and is especially important in cases of low levels of tIgE [9].

After having confirmed in vitro sensitization to the whole venom extract and ruled out a CCD sensitization, a study of sensitization to individual component should be carried out. In the case of bee venom allergy, sIgE to Api m 1, 2, 3, 5 and 10 (and Api m 4 if available) and in vespid venom allergy sIgE to Ves v 1, Ves v 5, Pol d 5 (and Pol d 1 if available) [20].

The ratio between the allergen component sIgE and whole extract sIgE (c-sIgE/we-sIgE) allows us to evaluate the extent of sensitization attributable to a given allergic component [9].

If genuine sensitization cannot be identified with these allergen profile, as described in the following question, the use of other techniques such as CAP inhibition may be a useful strategy [21].

**In cases of double or multiple positivity to hymenoptera venom can MD help me to determine which the genuine sensitizer is?**

MD can be a useful tool to discriminate a genuine sensitization from cross-reactivity only in some cases with double or multiple sensitizations to hymenoptera venoms.

The current panel of commercially available bee venom allergens considered as markers of genuine sensitization include Api m 1, Api m 3, and Api m 10. Api m 4 is not commercially available in Spain. These allergens can be also markers of bumble bee venom allergy [11, 20].

The hyaluronidase Api m 2 is a potential marker of bee venom allergy but shows limited cross reactivity with Ves v 2 and Pol d 2 in the absence of cross-reactive carbohydrate determinants or CCDs [11, 20].

Ves v 5 and Pol d 5 show a high in vitro cross-reactivity. Commercially available whole extracts from vespids venoms (common wasp venom or yellow jacket, paper wasp venom and mediterranean or european paper wasp are supplemented with antigen 5 and also present high in vitro cross-reactivity.

Api m 5, Ves v 3 and Pol d 3 belong to the family of Dipeptidyl peptidase-IV (DPPIV) and also have high in vitro cross-reactivity that prevents their use as markers of genuine sensitization. In fact, Api m 5 can be a marker of vespid venom allergy [11, 20].
The phospholipases A1 allergens Ves v 1 and Pol d 1 also present high in vitro cross-reactivity that prevents their use as markers of specific vespid venom allergy [11, 20].

When multiple in vitro sensitizations to whole hymenoptera venom extracts (especially to bee venom and common wasp venom extracts and less to paper wasp venom) a sensitization to CCD should be ruled out. The interference of CCD can be minimized by pre-incubation of the serum with a CCD inhibitor.

Are there sensitization profiles associated with a higher risk of adverse events during AIT?

There is evidence that some sensitization profiles are associated with risk of adverse events with grass and olive pollen subcutaneous immunotherapy (SCIT). Sastre J et al [22] have reported a significant association between the number of grass allergens (Phl p 1, Phlp 5 and Phl p12) that sensitized the patients and the total number of local and systemic adverse events with grass pollen SCIT.

In a trial on safety and efficacy of a grass sublingual immunotherapy (SLIT) tablet, the incidence of adverse events was correlated with the highest sIgE levels for Phl p 5 or Phl p 1 [23]. Sensitization to Ole e 7 (an olive pollen nsLTP) has been associated with severe clinical symptoms and systemic adverse reactions with AIT in regions with high levels of olive pollen exposure. In a recent algorithm to support the selection of olive pollen AIT, Barber D et al [24] recommend avoiding AIT prescription in those patients sensitized to Ole e 7, irrespective of Ole e 1 sensitization.

Regarding HDM markers of adverse reactions to immunotherapy, in a retrospective post-hoc analysis to evaluate if the sensitization profile to HDM was associated with the efficacy and safety of HDM SCIT, Gadermaier E et al [25] reported an association between sensitization to Lep d 2 and a higher rate of systemic reactions during the treatment. However, these results need to be validated in prospective studies.

No specific sensitization profile to individual components has been associated with the safety of SCIT with cat and dog extracts in two recent studies by Uriarte SA et al. using an ultrarush up-dosing phase protocol [26, 27].

Which hymenoptera allergens are associated with therapeutic failure or risk of adverse effects?

Api m 10 is an allergen with low abundance in bee venom (less than 1% of dry weight of venom) and apparently with unstable nature not only as native Api m 10 but also recombinant allergen.

There is evidence that suggests that patients with predominant Api m 10 sensitization may be at risk of therapeutic failure possibly due to its underrepresentation in some VIT preparations used for therapy because these commercially treatment extracts are lacking this allergen [28].
The risk of adverse events during bee venom immunotherapy in a Spanish population of bee venom allergic patients has been associated with presence of sIgE to \textit{Api m 4}, especially with levels of sIgE to \textit{Api m 4} higher than 0.98 kU/l [29, 30].

**A CRD-driven prescription of AIT will predict a better AIT efficacy?**

Different studies point towards a lower efficacy of AIT in those with a complex molecular spreading recognition. The use of allergenic molecules in various clinical studies [31-33] aimed at monitoring changes in the specific antibody repertoire of patients receiving AIT has shown good outcomes.

Specific studies designed to address the efficacy of molecular diagnosis driven AIT need to be performed since to date only post-hoc analysis have been performed with inconsistent results. While Chen KW et al [32] suggest that the use of molecular assays is a promising approach for predicting and monitoring HDM AIT efficacy, Arroabarren E et al [33] could not find a significant association between AIT efficacy and the HDM sensitization profile.

A recent study of Rodriguez-Dominguez A et al [13] on 24 HDM allergic patients who had received 1 year of HDM SCIT (Alutard® SQ) concluded that the stratification of patients with HDM allergy according to molecular sensitization profiles and molecular monitoring of AIT-induced IgG responses may enhance the success of AIT.

These recent studies emphasize that the use of molecular assays for the prediction and monitoring of AIT efficacy is a promising approach. However, prospective studies are needed to confirm that certain molecular IgE sensitization profiles are predictive biomarkers of AIT efficacy.

The clinician needs to be aware that other factors than the sensitization profile account for AIT efficacy, such as achieving the correct dose, the duration of the treatment and the compliance.

**Once knowing the patient’s sensitization profile can I choose an AIT extract accordingly?**

The heterogeneity of AIT preparation has conclusively been demonstrated for several allergen sources, including birch and grass pollen, house dust mite and insect venom preparations [34-37] so the recommendation is that you use commercial AIT extracts with quantified and standardized major allergens and evidence of efficacy.

**B.-2 POLISENSITIZATION / COMPLEX PATIENT**

**How can molecular diagnosis (MD) improve (add value) in the diagnosis of complex polysensitized patients?**

When a patient is sensitized to both food and respiratory allergens, there are two possible scenarios: either the patient has a genuine sensitization to both food and respiratory allergens, or the food allergy is caused by a phenomenon of cross-reactivity due to a primary sensitization to the inhalant allergen. The only way to
differentiate one situation from the other is to perform a MD, after an extensive clinical history and detection of sensitizations to whole allergen extracts.

In this scenario, the purpose of the MD study may be either to rule out a pollen-food syndrome, to help stratify the risk of severe reaction in case of food allergy or to guide food challenges or food avoidance advise.

**Which allergens should be included in the study of Pollen-food syndromes (PFS)?**

Pollen-food syndromes (PFS) refer to reactions with foods when the primary sensitization to the allergen has occurred through the respiratory route.

The main allergen families related to PFS are **Bet v 1 homologs (PR10)** and **profilins** [7], both protein families with extensive cross-reactivity in which the use of a single marker (**Bet v 1** for PR10 and **Bet v 2** or **Phl p 12** for profilins) may be sufficient to define the sensitization to the whole allergen family.

Further IgE-testing to food allergens belonging to the same family would potentially create many positive results with questionable clinical relevance.

Pollen food syndromes due to **nonspecific Lipid Transfer Proteins (nsLTPs)** cross-reactivity have also been described, classically between **Art v 3**, **Pla a 3** and **Pru p 3** [38,39], but recently [40] also between **Ole e 7** and **Pru p 3** explaining how **Ole e 7** could play a new role as primary sensitizer, leading to the peach nsLTP sensitization in regions with high olive pollen exposure. All these pollen LTPs are available for slgE determination both in singleplex and multiplex assays. In PFS due to cannabis LTP (**Can s 3**) cross-reactivity to multiple food-containing LTPs has been well stablised. However, its diagnosis relies in the determination of slgE to **Can s 3** which is not yet commercially available [41].

**Thaumatin-like proteins (TLPs)** are also responsible for PFS. Present in cypress, plane tree, artemisia pollens and in cannabis among other vegetables, they may cause cross-reactivity with fruits including rosacea, banana, kiwi, grape, melon and almond [42]. The limited number of TLP available for MD is still the main problem for studying this cause of PFS, since they are not available in singleplex assays and in multiplex assays only **Act d 2** (ImmunoCAP ISAC®) and **Mal d 2** (Alex2®) are available.

**Snakin/gibberellin-regulated proteins (GRPs)** have been recently recognized as responsible for PFS among cypress pollen and fruits (mainly peach, citrus fruits and pomegranate) [43]. Currently, only **Pru p 7** is available in singleplex of ImmunoCAP® assay but is very likely that **Cup a 7** will be soon commercially available.

Other allergen families responsible for less prevalent PFS include β-1,3 glucanases polygalacturonases and isoflavone reductases [7], and should be individually assessed according to the culprit allergenic sources.

Regarding, **latex-fruit syndrome** several allergen families have been involved, including class I chitinases (**Hev b 6**, **11** and **14**), beta 1,3 glucanases (**Hev b 2**), patatin like proteins (**Hev b 7**), nsLTP (**Hev b 12**) and acidic protein (**Hev b 5**). Singleplex slgE assays are available for some of these latex allergens [44].
Which allergens should be included in the study of respiratory and meat food cross-reactivity syndromes?

Serum albumins are also responsible for respiratory and meat cross-reactivity syndromes, including the pork-cat and the bird-egg allergy syndrome. Mammalian serum albumins are highly cross-reactive, thus determining sIgE to Can f 3, Fel d 2, Bos d 6 or Sus s 1 alone may be sufficient and should be guided by the suspected primary allergenic source according to the clinical history. When the clinical history suggests a bird-egg allergy syndrome, sIgE to Gal d 5 should be specifically determined, since the homology to mammalian albumins is very low [7].

How to interpret sensitization to Cross-reactive Carbohydrate Determinants (CCDs)?

The first description of the presence of IgE to Cross-reactive Carbohydrate Determinants (CCDs) was made in 1981 by Aalberse RC et al [45]. Now it is clear that anti-CCD IgE has little or no clinical relevance but it is a confusion factor for in vitro diagnosis [46].

sIgE to CCD bearing proteins mainly recognize a core of the amino sugar (α1,3 fucose) linked to N-acetylglucosamine (GlcNAc) named N-Glycans, the main structures related are MUXF and MMXF glycans.

IgE to CCD result in broad in vitro cross-reactivity especially among pollens, plant foods, latex and hymenoptera venoms. The overall prevalence of sIgE to CCDs is about 25% of patients arriving to 71% in patients with multiple pollen sensitizations. Given the low clinical relevance of CCDs sIgE antibodies there are two strategies to try to overcome their interference: one is to include a marker of CCD (MUXF3 into screening allergy panels) as an alert signal and the second one is to add a CCD-inhibitor to the detection method. A potential disadvantage of the latter approach is to decrease test sensitivity [47].

αGal (α1,3 galactose) also belong to N-glycans but in contrast to typical CCDs it has been reported to be associated with severe allergic reactions to meat [46].

B.-3.-RISK STRATIFICATION IN FOOD ALLERGY

CRD can improve diagnostic accuracy and help stratify clinical risk, but results must be interpreted always within the context of the patient’s clinical history.

The classic concept that remains valid is that sensitization to allergenic proteins with greater thermal stability and resistance to proteolysis and enzymatic digestion (storage proteins, nsLTPs, gliadins, thaumatin-like proteins, GRPs, tropomyosins, parvalbumins, caseins, and ovomucoid) is associated with a higher risk of systemic or severe reactions, while sensitization to gastro and heat-labile proteins (profilins or PR-10) is generally associated with mild symptoms or even lacking clinical relevance [48].
However, we would like to emphasize that there are exceptions to this rule, and severe reactions have been reported after ingestion of plant-derived foods in patients sensitized to PR10 proteins or profilins [49,50] in context of high doses of allergen exposure in the presence of augmentation factors (cofactors). Moreover, sensitization to stable allergens such as nsLTPs show a very heterogeneous clinical expression, being more frequently related to mild symptoms in the absence of cofactors [51].

**Can sIgE to “markers of severity” predict the clinical reactivity?**

Detection of specific IgE in a patient’s serum is strictly a marker of allergic sensitization and it alone cannot predict the probability of an allergic reaction. There are many variables that contribute to the clinical expression of a sensitization, some related to the allergen itself (such as the concentration of the protein in the edible food, the degree of homology and the stability to heat/digestion) but also others related to the immune response (IgE antibody concentration, specificity, affinity and effector cell reactivity) and host-dependent factors including augmentation factors (exercise, alcohol, intake of non-steroidal anti-inflammatory drugs (NSAIDs), illness).

Therefore, the clinical relevance of an allergic sensitization to an allergen molecule will always have to be interpreted in the context of the clinical history and a controlled food challenge (OFC) when needed.

**Which allergens present potential clinical cross-reactivity?**

Molecule-based sensitization tests may be helpful to explore the degree and potential clinical relevance of further cross-reactivities to related molecules of a protein family.

In case of **protein families with highly cross-reactive allergens** (*Bet v 1*-homologs, profilins, nsLTPs, polcalcins (calcium-binding proteins), serum albumins, grass pollen major group 1 and 5 allergens, parvalbumins, tropomysosins...) it is sufficient to test only one member of the family and then conduct a thorough clinical work-up to identify relevant clinical cross-reactions.

In case of **allergens of limited cross reactivity** (seed storage proteins like 2S-albumins, 7S-globulins (vicilins), 11S-globulins (legumins); lipocalin subfamilies...) an appropriate panel of related allergens (from the same protein family) could be used to demonstrate or exclude subsequent (serological) cross-reactivities. Usually, the allergen with the highest sIgE antibody level will represent the primary sensitizer. In the case of nut allergies in patients sensitized to seed storage proteins, the highest clinical reactivity has been found related to botanical family relationship, with strong correlations between cashew-pistachio, walnut-pecan, and walnut-pecan-hazelnut-macadamia [52,53].

However, it is again important to underscore that a serological cross-reactivity is not equivalent to clinical cross-reactivity as it has been proven that rates of sensitization significantly outnumber clinical allergy. Although molecular diagnosis may be helpful in assessing the possibility of clinical cross-reactivities, the correlation with the clinical history and OFC remains the gold standard.
Could we use sensitization to some allergens for guiding challenge tests?

Using the sensitization profile can help characterize the patient’s risk of a severe reaction and who should undergo an OFC, but to date there are no well-defined cut-offs for most allergens.

The CRD diagnostic value for predicting positive OFC has been established mainly for peanut and tree nuts.

Ara h 2 is the most important predictor of symptomatic peanut allergy and different recommended sIgE Ara h 2 cut-offs have been proposed, with important variations between different populations [54-56].

Cor a 9 and Cor a 14 have also been associated with systemic reactions upon hazelnut ingestion. Various diagnostic cutoffs have been proposed for both allergens [52,57].

Levels of sIgE to Jug r 1 or 4 greater than or equal to 0.35 kUA/L have been reported to provide the best diagnostic method for identifying walnut-allergic patients [58].

Cut-off points have also been defined for predicting positive OFC for cashew Ana o 3, soy Gly m 8 and wheat Tri a 19 (reviewed by Foong RX et al) [59].

Regarding milk and egg allergy, component testing has not consistently been shown to predict baked milk or baked egg tolerance.

Could we use sensitization to some allergens for guiding avoidance measures?

When the MD study yields a positive result to cross-reactive food allergens, the advisable approach is not to indiscriminately remove tolerated foods from the diet just because they are related to an allergen that caused a reaction.

Some important aspects of clinical cross-reactivity that may assist in deciding on undertaking the OFC or pursuing diet expansion have been recently addressed by Cox AL et al [60].

B.4 FAQS in component-resolved diagnosis (CRD) in idiopathic anaphylaxis (IA)

Is MD useful in the study of an idiopathic anaphylaxis (IA)?

Idiopathic anaphylaxis (IA) is an exclusion diagnosis when all specific possible trigger of recurrent anaphylaxis has been ruled out by a step up standard allergic diagnosis.

MD has been useful to identify causes of anaphylaxis previously labelled as idiopathic. In the case of singleplex assays, the clinician may choose the single components to be tested as for example is the case of delayed meat anaphylaxis, which can be diagnosed by detecting sIgE to α-gal (galactose-α-1,3-galactose)
or sIgE to ω-5-gliadin (Tri a 19) in cases of suspected wheat dependent exercise-induced anaphylaxis or other known allergens [61].

Another approach is the use of multiplexed assays, in which a high number of individual allergen molecules are tested simultaneously. Molecular diagnosis arrays allow their use as a form of ‘screening’ tool in anaphylaxis to assess sensitization of the patient identifying the potential triggers [61].

It is important to highlight that in the diagnosis of IA it is necessary to carefully assess possible concurrent conditions such as cofactors (NSAIDs, exercise, alcohol etc.) and potential mast cell disorders.

Which allergens are not well represented in commercial whole extracts for prick test or sIgE and therefore should be assessed in any anaphylaxis study?

Not all allergens are well represented in commercial assays; the allergist has to know which allergens are underrepresented in clinical practice. For example, years ago, the latex allergen Hev b 5, vespid antigen 5 were poorly represented in commercially whole extracts. The manufacturers have resolved this question supplementing whole extracts with some relevant allergens as whole extract of latex with Hev b 5 or whole vespid extracts with Ves v 5 or Pol d 5. Similar situation has been currently detected for or ω-5-gliadin (Tri a 19) in whole wheat extracts but is not yet resolved.

Other case is allergen extracts used for in vivo as well as in vitro diagnostic tests do not contain lipophilic allergens as the oleosins because these proteins are lipophilic and nearly insoluble in aqueous solutions. Oleosins of sesame, peanut, and hazelnut have been registered as allergens responsible of anaphylaxis so negativity of sIgE whole extract does not discard implication of these specific foods.

B.-5 What does molecular diagnosis provide in occupational allergy?

sIgE reactivity to occupational allergen components has been poorly investigated, with the notable exception of latex allergy and cereal flour responsible for baker’s asthma. For other occupational allergens, it remains necessary to evaluate the relevance of single allergen molecules for the sensitization induced by occupational exposure [62].

Twenty-seven wheat allergens are listed in the WHO/IUIS allergen nomenclature database, but only a few of them are commercially available for testing individually. Tri a 19 (ω-5-gliadin) is not relevant for the diagnosis of baker’s asthma but is involved in wheat-dependent, exercise-induced anaphylaxis (WDEIA) and also for the early childhood type-I wheat allergy. In Spanish bakers, wheat lipid transfer protein (nsLTP) Tri a 14 was described as a major allergen in baker’s asthma [62].
In the 1980s, latex allergy emerged as an epidemic in places where powdered natural rubber latex gloves were used, exposed health care workers and some patients (e.g., patients with spina bifida) were affected. There are two main problems in latex allergy: systemic type I reactions (anaphylactic shock in anesthetized patients were the most frequent manifestation) during medical or surgical procedures due to mucosal or parenteral release of allergens and cross reactivity with vegetables: latex fruit syndrome answered with pollen food syndrome.

The systemic type I reaction can occur after sensitization to the any latex allergen except isolated Hev b 8 (profilin) There are at least 15 latex allergens identified: Hev b 1 to Hev b 15 belong to different allergen families [44] but only Hev b 1, Hev b 3, Hev b 5 and Hev b 6, Hev b 8 and Hev b 11 are commercially available.

For patients who have latex allergy and may need surgery or other specialized procedures in operating/procedure rooms the recommendation are that subjects be cared for as the first case of the day and in latex free surgical room. Patients who presented isolated sensitization to Hev b 8 (profilin) [63] and/or CCDs with positive sIgE to latex whole extract without other latex allergens negative do not need to avoid latex in surgical procedures [64].

C.-INTERPRETATION OF MD

Is the generation of an extensive IgE sensitization profile a disadvantage of multiplex assays?

One of the main criticisms that have been made to the MD by multiplex assays is the detection of unexpected sensitizations that may confuse the clinician when interpreting the results.

However, this is not an uncommon situation in routine clinical practice in which, on many occasions, skin tests are performed with extensive panels of whole allergenic extracts, sometimes yielding unexpected results. Therefore, it seems reasonable to interpret these results in the same way as with other clinically irrelevant sensitizations to food or respiratory allergens: perform a meticulous medical history to assess the clinical relevance of the sensitization and controlled challenges when needed.

The detection of silent sensitivities may give the clinician the chance to investigate other hypersensitivities and to alert the patient of possible risks, but sensitization itself (without a concordant clinical history or positive challenge tests) should not drive avoidance measures.

Considering all the benefits of MD in the clinical practice, can I skip “conventional” allergy diagnosis tests?

It may be tempting to only test the levels of sIgE to the major allergens from the suspected allergenic source when we know the most common sensitization profile in our area. However, this practice could lead to misinterpretation of the results.
Recently, Pascal et al have proposed the use of ratios of the sIgE of a given specific allergen component (c-slgE) to the levels of sIgE to its whole extract (we) (c-slgE/ we-sIgE) to evaluate the extent of sensitization attributable to this specific allergic component [9].

Besides, the number of allergen components available for diagnostics is still limited and detecting sensitization to certain allergenic sources is only possible using whole allergen extracts.

Probably there is nothing more “traditional” than the medical history and this should never be replaced by any diagnostic tests that, let's not forget, will only detect sensitization. The allergy workup should always be directed by a meticulous clinical history aimed at identifying the culprit allergen and the clinical relevance of the sensitizations detected.

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Funding
None
Bibliography


Figure 1. Summarized the main clinical uses of molecular allergy diagnosis (MD).

IA: Idiopathic Anaphylaxis; AIT: Allergen Immunotherapy; sIgE: specific IgE, whole extract; SPT: Skin Prick Test; PFS: Pollen Food Syndrome.
Table 1. Main allergens families and associated clinical features

<table>
<thead>
<tr>
<th>Allergen Families</th>
<th>Examples</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-10 or Bet v 1-homologous</td>
<td>Bet v 1.-Birch pollen, Pru p 1.-Peach, Cor a 1.-Peach, Mal d 1.-Apple, Ara h 8.-Peanut, Gly m 4.-Soy, Act d 8.-Kiwi, Api g 1.-Celery, Dau c 1.-Carrot</td>
<td>High number of sensitizations in the northern area of Spain and central and northern Europe. Present in pollens and plant foods. Sensitization to birch pollen and other pollens from <em>Fagales</em> trees leads in some cases to plant food allergy due to extensive cross-reactivity (pollen-food syndrome). Heat and digestion labile allergens and usually associated with mild reactions such as oral allergy syndrome upon ingestion of fresh plant derived foods, although anaphylactic reactions have been described in certain circumstances (eg cofactor related). Extensive <em>in vitro</em> cross-reactivity.</td>
</tr>
<tr>
<td>Profilins</td>
<td>Bet v 2.-Birch pollen, Ole e 2.-Olive Pollen, Phl p 12.-<em>Phleum pratense</em> pollen (grass), Mer a 1.-<em>Mercurialis annua</em> pollen, Hev b 8.-Latex, Pru p 4.-Peach, Bad d 4.-Apple, Cuc m 2.-Melon</td>
<td>Panallergens, present in pollens, latex and plant foods. The route of sensitization is (in Spain) usually through sensitization to grass or olive pollen leading in some cases to mild symptoms upon ingestion of raw vegetables due to cross-reactivity. Heat and digestion labile allergens and in case of food allergy they are usually associated with mild reactions such as oral allergy syndrome, if any, although anaphylactic reactions have been described in certain circumstances (extreme pollen counts). It usually causes reactions with raw foods. Extensive <em>in vitro</em> cross-reactivity.</td>
</tr>
<tr>
<td>nsLTP specific lipid</td>
<td>Pru p 3.-Peach</td>
<td>High number of sensitizations in Spain and the Mediterranean area. They are</td>
</tr>
</tbody>
</table>
transfer proteins or PR-14) | Mal d 3.-Apple  
| Jug r 3.-Walnut  
| Ara h 9.-Peanut  
| Cor a 8.-Hazelnut  
| Lac s 1.-Lettuce  
| Len c 3.-Lentil  
| Tri a 14.-Wheat  
| Pla a 3.-Plane tree pollen  
| Art v 3.-Artemisia pollen  
| Ole e 7.-Olive pollen  

| TLP (Thaumatin like proteins or PR-5) | Act d 2.-Kiwi  
| Mus a 4.-Banana  
| Pru p 2.-Peach  
| Bad d 2.-Apple  
| Cor a TLP.-Hazelnut  
| Lac s TLP.-Lettuce  
| Tri to TLP.-Wheat  
| Pla a TLP.-Plane tree pollen  
| Cup a 3.-Cupressus arizonica pollen  

| Chitinases and other latex-related | Hev b 5.-Latex  

Present in pollens and plant foods. The most frequent onset is sensitization through the digestive tract, although pollen sensitization has also been described. Allergens resistant to heat and digestion and in case of food allergy a wide spectrum of symptoms has been described, from asymptomatic and mild reactions to anaphylactic reactions (especially in the presence of cofactors). May cause reactions with both raw and cooked foods. High cross-reactivity both in vitro and in vivo.

High number of sensitizations in Spain, although there are few commercial methods of measure. Present in pollens and plant foods. Both pollen and digestive sensitization have been described. Resistant to heat and digestion and in case of food allergy a wide spectrum of symptoms has been described, from asymptomatic and mild reactions to anaphylactic reactions (especially in the presence of cofactors). May cause reactions with both raw and cooked foods. Intermediate cross reactivity. Limited in vivo cross-reactivity studies.

High number of sensitizations in Spain and worldwide, although they have been
<table>
<thead>
<tr>
<th>Proteins (PR-3, PR-4 and PR-11)</th>
<th>Hev b 6.-Latex</th>
<th>Hev b 7.-Latex</th>
<th>Hev b 11.-Latex</th>
<th>Mus a 2.-Banana</th>
<th>Cas s 5.-Chestnut</th>
<th>Pers to 1.-Avocado</th>
<th>Act d chitinase.-Kiwi</th>
<th>Sola l 1.-Tomato</th>
<th>Sola t 1.-Potato</th>
<th>Bra r 2.-Mustard</th>
<th>Man e 5.-Yucca, Cassava</th>
<th>Proteins decreasing in recent years. Present in latex and plant foods. Both respiratory and digestive sensitization has been described. Resistant to heat and digestion and in case of food allergy, anaphylactic reactions have been described. May cause reactions with both raw and cooked foods. They are associated with the so-called latex fruit syndrome. High cross-reactivity both in vitro and in vivo within each protein family.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snakin/gibberellin-regulated proteins (GRPs)</td>
<td>Cit s 7.-Lemond</td>
<td>Pru p 7.-Peach</td>
<td>Pru av 7.-Cherry</td>
<td>Pru m 7.-Japanese apricot</td>
<td>Pun g 7.-Pomegranate</td>
<td>Cup s 7.-Cupressus sempervirens pollen</td>
<td>Cry j 7.-Cryptomera japónica pollen</td>
<td>Recently described in Japan and Mediterranean area (France). Present in pollens (cupressaceae) and plant foods. Both pollen and digestive sensitization have been described. Resistant to heat and digestion and in case of food allergy anaphylactic reactions have been described, although the spectrum is very broad. May cause reactions with both raw and cooked foods. Limited in vivo cross-reactivity studies.</td>
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<tr>
<td>Storage proteins of legumes, nuts, seeds and cereals</td>
<td>Ara h 1, 2, 3, 6.-Peanut Jug r 1, 2, 4, 6.-Walnut Cor a 9, 14.-Hazelnut Ana or 2, 3.-Cashew Gly m 5, 6.-Soy Ses i 1, 6, 7.-Sesame Tri to 19, 20, 21.-Wheat</td>
<td>High number of sensitizations in Spain and worldwide usually initiated in childhood. Present in nuts, seeds, legumes and cereals (gliadins). Sensitization through the digestive tract, although transcutaneous sensitization associated with atopic dermatitis has also been described. Resistant to heat and digestion and in case of food allergy, frequent anaphylactic reactions have been described, although the spectrum is very broad. May cause reactions with both raw and cooked foods. High in vitro cross-reactivity, however in vivo only cross-reactivity within the same botanical family.</td>
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<tr>
<td>Polcalcins (Calcium binding proteins)</td>
<td>Bet v 4.-Birch pollen Phil p 7.- Phleum pratense (grass) pollen</td>
<td>Polcalcins are panallergens that sensitize a minority of pollen-allergic patients, although exact prevalence is not well known. Present exclusively in pollen tissue. Their clinical relevance remains controversial. Extensive in vitro cross-reactivity between pollens.</td>
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<tr>
<td>β-parvoalbumins</td>
<td>Gad c 1.-Cod Cyp c 1.-Carp</td>
<td>High number of sensitizations in Spain and worldwide usually initiated in childhood. Main allergens in fish. May produce symptoms both through the digestive and respiratory routes. Heat and digestion stable proteins can cause allergic symptoms with both raw and cooked foods. High cross-reactivity both in vitro and in vivo between practically all fish. Patients can tolerate fish low in β-parvalbumin such as tuna or swordfish (Xiphias gladius).</td>
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<td>Tropomyosins</td>
<td>Pen a 1.-Prawn Der p 10. Mites (D. pteronyssinus) Bla g 7.-Cockroach (Blatella germanica)</td>
<td>High number of sensitizations in Spain and worldwide usually initiated in childhood. Main allergens in shellfish. May induce symptoms both through the digestive and respiratory routes. Heat and digestion stable proteins can cause allergic symptoms with both raw and cooked foods. Extensive cross-reactivity both in vitro and in vivo between crustaceans, cephalopods and mollusks.</td>
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<td>Animal</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<td>Anisakis simplex</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<td>Mussel</td>
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<td>Clam</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Cuttlefish</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Octopus</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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| Proteins present in different biological and solid fluids, i.e. cow's milk, mammalian meats (beef and pork) and poultry (chicken). Sensitization to serum albumin can cause respiratory symptoms due to sensitization to dander / mammalian epithelium, such as cat pig syndrome or against egg / chicken and feather meats such as bird-egg syndrome, as well as reactions against foods such as meat and milk of mammals such as veal. |

<table>
<thead>
<tr>
<th>Serum albumins</th>
<th>Proteins present in different biological and solid fluids, i.e. cow's milk, mammalian meats (beef and pork) and poultry (chicken). Sensitization to serum albumin can cause respiratory symptoms due to sensitization to dander / mammalian epithelium, such as cat pig syndrome or against egg / chicken and feather meats such as bird-egg syndrome, as well as reactions against foods such as meat and milk of mammals such as veal.</th>
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<tbody>
<tr>
<td>Fel d 2.-Cat</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Can f 3.-Dog</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<td>Bos d 6.-Veal</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Sus s 1.-Pig</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Equ c 3.-Horse</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Gal d 5.-Chicken</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<td>Hen egg</td>
<td>Reported also in vitro cross-reactivity with nematodes and insects</td>
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<tr>
<th>Lipocalins</th>
<th>Mammalian derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Important allergens in pet allergy. Limited cross reactivity between different species.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can f 1, 2, 4,</td>
<td>Mammalian derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Important allergens in pet allergy. Limited cross reactivity between different species.</td>
</tr>
<tr>
<td>6.-Dog</td>
<td>Mammalian derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Important allergens in pet allergy. Limited cross reactivity between different species.</td>
</tr>
<tr>
<td>Fel d 4.-Cat</td>
<td>Mammalian derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Important allergens in pet allergy. Limited cross reactivity between different species.</td>
</tr>
<tr>
<td>Equ c 1.-Horse</td>
<td>Mammalian derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Important allergens in pet allergy. Limited cross reactivity between different species.</td>
</tr>
<tr>
<td>Mus m 1.-Mouse</td>
<td>Mammalian derived major allergens causing respiratory sensitization. Associated with greater severity of respiratory symptoms (asthma). Stable proteins. Important allergens in pet allergy. Limited cross reactivity between different species.</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>CCD and “CCD-Bearing proteins”</th>
<th>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</th>
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<tbody>
<tr>
<td>Ana c 2.-Pineapple</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
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<tr>
<td>MUXF3</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
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<tr>
<td>(fraction Ana c 2 from Pineapple)</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
</tr>
<tr>
<td>Jug r 2.-Walnut</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
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<tr>
<td>Pla a 2.-Plane</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
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<tr>
<td>tree pollen</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
</tr>
<tr>
<td>Cup a 1.-Cupressus</td>
<td>May be present in approximately 30% of polysensitized patients. Marker for sensitization to cross-reactive carbohydrate residues of proteins (pollen, hymenoptera, latex). Rarely associated with clinical symptoms. Associated with multiple positive results, especially in vitro specific IgE to whole extracts. May be detected when using purified native allergens (MUXF3, nJug r 2, nPla a 2, nCup a 1, nCry j 1, nPhl p 4) but not to recombinant allergens without CCDs (rOle e 1 and rPhl p 1) or by use of CCD-inhibitors in the determination.</td>
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<tr>
<td>arizonica pollen</td>
<td>Cry j 1.-</td>
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<td>Cryptomeria japonica</td>
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<td>pollen</td>
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<td>Phl p 1 and 4.-</td>
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<td>Pollen of Phleum</td>
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<td>pratense (grass)</td>
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<td>Cyn d 1.-</td>
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<td>Pollen of Cynodon</td>
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<td>dactylon (grass)</td>
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<td>Ole e 1.-Olive pollen</td>
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Table 2. Comparison of a representative singleplex (ImmunoCAP) and multiplex (ISAC) IgE testing assays

<table>
<thead>
<tr>
<th></th>
<th>Singleplex (ImmunoCAP)</th>
<th>Multiplex (ISAC)</th>
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</thead>
<tbody>
<tr>
<td>Amount of allergen on assay</td>
<td>~1-2µg/determination</td>
<td>~100 pg/spot</td>
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<tr>
<td>Read-out</td>
<td>Quantitative</td>
<td>Semi-quantitative</td>
</tr>
<tr>
<td>Amount of serum needed</td>
<td>~40µl/determination</td>
<td>~30µl/chip (112 allergens)</td>
</tr>
<tr>
<td>Procedure</td>
<td>Automated</td>
<td>Manual</td>
</tr>
<tr>
<td>Result variation coefficient</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Interference with IgG4</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Interference in cases with high total IgE levels</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>IgE detection (affinity)</td>
<td>Low and high affinity IgE</td>
<td>High affinity IgE</td>
</tr>
<tr>
<td>Useful for patient monitoring and follow-up</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>CCD-inhibitor added</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Results units</td>
<td>kUₐ/L</td>
<td>ISU-E</td>
</tr>
<tr>
<td>Global availability</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>