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Molecular allergy diagnosis: status anno 2015

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Abstract

IgE antibodies play a key role in type I allergic reactions. Today, different in vitro immunoassays for allergen-specific IgE antibodies are available. However, some major issues should be taken into account for correct interpretation of specific IgE (sIgE) antibody results, as these assays do not demonstrate absolute positive and negative predictive values. Therefore, additional diagnostic tests are needed to make the correct diagnosis. During the last two decades significant progress in biochemistry and molecular biology enabled the detection and quantification of sIgE antibodies to allergen protein components and epitope-emulating peptides, also called molecular allergy diagnosis or component resolved diagnosis (CRD). In contrast to conventional sIgE antibody assays, molecular allergy diagnosis makes it possible to discriminate between genuine allergy and merely sensitisation, to establish personalized sensitization patterns and to assess the individual risk of severity of an allergic reaction and finally it helps us to predict the natural course. In this review the use of CRD in inhalant, food, latex and hymenoptera venom allergy will be discussed. The primary focus will be on the most relevant clinical applications of CRD rather than to describe all the currently available allergen components and epitopes. Appropriate experience of our own research group is provided.

Keywords: allergy, component resolved diagnosis, inhalant, food, latex, hymenoptera

List of abbreviations

slgE	specific immunoglobulin E
CRD	component resolved diagnosis
RAST	radioallergosorbent test
FEIA	fluorescent immunoassay
OAS	oral allergy syndrome
Ns-LTP	non-specific lipid transfer protein
YJV	yellow jacket venom

For definitions of abbreviations of components see text and legends of the figures.

Background

IgE antibodies were discovered almost half a century ago as the “reagins” responsible for so-called type I (anaphylactic) reactions [1, 2]. Almost half a decade later, the first *in vitro* solid phase assay for allergen-specific IgE antibodies, called the radioallergosorbent test (RAST) became commercially available. The original RAST was configured as a cyanogen-bromide activated paper disc on which various crude allergen extracts were covalently bound. In this RAST assay, specific IgE (sIgE) antibody that complexes with the coupled allergen was quantified with radio-iodinated polyclonal antihuman IgE antibodies using a γ -counter [3]. Today, however, the traditional RAST technique has been completely abandoned and replaced by a fluorescent immunoassay (FEIA) based on the same cyanogen-bromide activated cellulose which is technically optimized and automated displaying an excellent analytic sensitivity, precision, reproducibility (intra- and inter-assay) and linearity. . Nevertheless, none of the sIgE assays that are hitherto available demonstrates absolute positive and negative predictive values. Negative sIgE results, by no means, exclude an IgE-mediated condition. Vice versa, a positive sIgE result is not an absolute proof for allergy.

As a matter of fact, specific IgE evaluation is based upon the detection and quantification of IgE antibodies directed against crude natural allergen extracts, which can contain genuine but also cross-reactive allergen components. The inherent complexity, variability and instability of natural allergens and the variation between individual sensitization patterns complicate the correct interpretation of sIgE results to crude allergen extracts. Consequently, a positive sIgE against crude extracts should always be interpreted with extreme care as it might merely reflect (cross)sensitization rather than a genuine allergy. For example, it has repeatedly been shown that ubiquitous structures such as cross-reactive

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carbohydrate determinants (CCD) present on glycoproteins of plants and hymenoptera venom, homologues of the major allergen Bet v 1 from birch (*Betula verrucosa*) pollen, profilins and non-specific lipid transfer proteins can elicit a significant number of positive sIgE results without clinical significance [4-6]. This clinically irrelevant serology involves a broad spectrum of cross-reactivity as sIgE antibodies can bind to structural similar or identical epitopes and components present in allergens originating from taxonomically related and distant sources including pollen, plant-derived food, *Hevea* latex and hymenoptera venom. Needless to say that *in vitro* diagnosis of IgE-mediated allergy should benefit from the advent of better standardized extracts and well-characterized allergen components.

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Component resolved diagnosis (CRD): principles and techniques

During the last 2 decades significant progress in biochemistry and molecular biology enabled the characterization, cloning and recombinant production of relevant allergen protein components and epitope-emulating peptides, allowing detection and quantification of sIgE antibodies to these proteins or sequential epitopes, viz. CRD (figure 1). The first component to be identified and cloned was the major allergen Der p 1 from house dust mite (*Dermatophagoides pteronyssinus*) in the late 1980s [7]. In contrast to conventional sIgE antibody assays, CRD does not rely upon crude extract preparations obtained from native allergens (generally poorly defined mixtures containing both allergenic and non-allergenic components) but on sIgE antibodies directed towards single components purified from natural sources or produced by recombinant techniques. There are several databases on allergen components and protein families, such as the official International Union of Immunological Societies (IUIS) allergen nomenclature database (<http://www.allergen.org>)

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and the Allfam allergen database grouping allergens into protein families and subfamilies (www.meduniwien.ac.at/allergen/allfam). The principles, potential and shortcomings of CRD have been comprehensively reviewed elsewhere [8-11]. As shown in figure 2, CRD involves specific marker components and substructures to study the genuine allergic sensitization of patients to a particular allergen source as well as sensitization to cross-reactive determinants or components that point to cross-sensitization to various, frequently unrelated, allergen sources that are only weakly associated with clinical manifestations.

These so-called “gatekeeper” tests allow an improved discrimination between genuine allergy and merely sensitization [5, 12-14] and furthermore enables us to establish personalized sensitization patterns (with constituent components of a particular allergen that are recognized by the individual patient) [12, 14-23]. Determining the sensitization profile of an individual patient creates the opportunity to assess the individual risk of severity of an allergic reaction and to predict the natural course (outgrow vs. persistence). Moreover, in this context CRD has unveiled that these sensitization profiles might show geographical and age-related differences with clear distinct clinical outcomes [13, 23-26]. Finally, CRD might facilitate selection of patients for allergen-specific immunotherapy and contribute to the monitoring of the immunological effects of therapy [27-29]. However, CRD also demonstrates shortcomings. For example, hitherto, not all relevant allergen components are available. Moreover, we have repeatedly demonstrated CRD with various Bet v 1 homologues such as Mal d 1 from apple (*Malus domestica*) [5] and Cor a 1 from hazelnut (*Corylus avellana*) [13] to be of no added value to discriminate between merely sensitization and genuine allergy. Alternatively, when using these individual components or epitopes for the diagnosis of allergy, the number of tests necessary to enable a correct diagnosis increases significantly since more than one component needs to be included to

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allow identification of the entire repertoire of disease relevant peptides and epitopes. The microarray technique for CRD elegantly enables sIgE antibody testing in a multiplex format and allows the simultaneous quantification of many sIgE antibodies. The major advantage of this multiplex technique lies in its potential to study significant numbers of components in parallel, detecting sIgE antibody abundance, functionality, and interaction concerning numerous allergenic determinants using only minute amounts of patients' serum which is particularly important in infants and children. For a description of molecular allergy diagnosis by microarray see [10, 30]. However, this "at random testing" carries the risk to generate a significant amount of complex information that is not per se clinically relevant. Therefore, educational initiatives on the appropriate application and correct interpretation of CRD, particularly multiplexed CRD, have recently been started [11].

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Component resolved diagnosis (CRD): clinical indications and experience

This review primarily focuses on "when to apply" molecular allergy diagnosis rather than to describe all the currently available allergen components and epitopes.

Inhalant allergies

In most cases of traditional inhalant allergies (e.g. house dust mite, cat, dog and pollen) correct diagnosis can readily be established upon thorough history taking supplemented by conventional diagnostics, i.e. conventional singleplexed sIgE antibody tests and skin tests using natural allergen extracts. In our opinion, the added value of CRD mainly lies in the correct identification of patients who are not sensitized to major pollen allergens and in whom prescription of allergen-specific immunotherapy is anticipated, as patients sensitized to so-called minor allergens alone are unlikely to receive sufficient amounts of responsible allergen to achieve a successful outcome from their therapy. For example, in our regions not

all patients with a birch pollen allergy are sensitized to the major birch pollen allergen Bet v 1 [4], and will therefore not benefit from pollen immunotherapy that contains mainly Bet v 1. A similar situation could occur in southern Spain where a considerable number of patients is not sensitized to the major allergen Ole e 1 from olive tree (*Olea europaea*) but is sensitized to the minor allergens Ole e 7 and Ole e 9 [20] and are therefore highly unlikely to benefit from an Ole e 1-based hyposensitization. Another scenario in which CRD could add to correct selection of patients for pollen-specific immunotherapy is pollen polysensitization. As a matter of fact, most of our patients with pollen allergy are not monosensitized but polysensitized to different pollen species from trees, grasses and/or mugwort [31]. In such cases additional information on the individual sensitization profile could help to orient allergen-specific immunotherapy, particularly when there is overlap between the different flowering seasons and when CRD would indicate not all sensitizations to be clinically relevant but some merely to represent an *in vitro* cross-reactivity phenomenon. The impact of molecular diagnosis on the allergen selection for pollen immunotherapy has been demonstrated on several occasions [27, 28, 32]. From these studies it emerges that CRD results alter initial prescription of allergen-specific immunotherapy in up to 50% of the patients, both in children as well as adults. Obviously, molecular allergy diagnosis is a prerequisite for future pollen component-resolved specific immunotherapy due to the high heterogeneity of geographically-related sensitization profiles [33, 34]. Finally, molecular diagnosis in inhalant allergy might also help to elucidate on cross-reactivity between aeroallergens and food allergens (see below).

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Food allergy

Food constitutes an increasing cause of sensitization and genuine allergy in both children and adults. Exposure to food allergens predominantly occurs via the gut where various (non) immunological mechanisms prevent food allergens to trigger the immune system. However, some food allergens may escape from these surveillance mechanisms and finally lead to so-called primary IgE-mediated food allergies. Alternatively, IgE-mediated food allergy, particularly to plant food, can also be acquired secondarily via cross-reactivity to structurally similar epitopes present in allergens from non-related sources such as pollen, *Hevea* latex and marihuana (*Cannabis sativa*) (see below). During the last decade it has increasingly been recognized that food allergy displays clear geographic and age-related variations regarding the severity of symptoms depending on the sensitization profile of the individual patient that can partially be predicted using CRD. This review will focus on some particular food allergy syndromes, most of them being related to a sensitization to one of the most relevant superfamilies of plant- or animal-derived food allergens as displayed in figure 3.

Primary plant-derived food allergies

At present, CRD has mainly been applied in allergy to peanut, hazelnut, kiwi, apple and soy. From studies, it has emerged that molecular diagnosis may constitute an asset in the discrimination between genuine allergy and merely sensitization, enable an individual risk assessment of severity which could lead to strict dietary recommendations (or *vice versa* avoidance of unnecessary dietary measures). Different studies have shown that molecular diagnosis of peanut allergy is more accurate to predict clinical outcomes than measuring sIgE to crude peanut (*Arachis hypogaea*) extract (for review: [35]). Ara h 2, a member of the prolamin superfamily, is considered to be the major allergen of peanut and has been

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described as the most important component for accurate discrimination between peanut allergic patients and peanut tolerant individuals. Other clinically important peanut components are Ara h 1 and Ara h 3, members of the cupin superfamily and Ara h 6 displaying high sequence homology with Ara h 2. A primary peanut allergy due to sensitization to these components is more frequently seen in children and is often associated with severe allergic reactions upon ingestion of even small amounts of peanut (traces). Finally, Ara h 9 appears to be an important peanut component in the Mediterranean area [24, 36]. Sensitization to Ara h 9 makes part of the peach-induced LTP syndrome that is dominated by a sensitization to Pru p 3, the LTP in peach (*Prunus persica*) and may also lead to severe peanut allergy [37]. Alternatively, in northern Europe, patients frequently present with a mild oral allergy syndrome (OAS) resulting from a cross-reactivity between Ara h 8 from peanut and Bet v 1, the major allergen component from birch (*Betula verrucosa*) pollen [38].

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Another food allergy that has already extensively been subjected to molecular diagnosis is hazelnut (*Corylus avellana*) allergy. In our region, it has been observed that over 90% of children is sensitized to Cor a 9 and/or 14. Sensitization to these components is unrelated to birch pollen allergy and is predominantly associated with more severe symptoms on consumption of raw or processed nuts [13, 23, 39]. Moreover, sensitization to these components was demonstrated already to occur in infants suffering from atopic dermatitis [23, 40]. In contrast, adults generally suffer from a secondary hazelnut allergy and display local discomfort of the oropharyngeal cavity, i.e. an OAS, due to sensitization to the Bet v 1 homologue Cor a 1.04. Alternatively, adults from a Mediterranean non-birch endemic region are rarely sensitized to Cor a 1.04 but predominantly react against the lipid transfer protein

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Cor a 8 which is frequently related to more severe symptoms [26]. Similarly to the sensitization to Ara h 9 from peanut, sensitization to Cor a 8 is governed by its homologue Pru p 3 [41].

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Recently it was shown that also kiwi (*Actinidia deliciosa*) allergy displays geographical differences in sensitization profiles [25]. Patients from Iceland were mainly sensitized to Act d 1 (actinidin) and are at elevated risk for severe reactions. Patients from western, central and eastern Europe were mainly sensitized to the Bet v 1 homologue Act d 8, and those from southern Europe to Act d 9 (profilin) and Act d 10 (LTP).

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Similarly, sensitization to apple (*Malus domestica*) was observed to exhibit geographically different sensitization profiles with different clinical outcomes. In the Netherlands, Austria, and Italy apple allergy generally is mild and predominantly presents as isolated oral symptoms related to birch pollinosis and sensitization to Bet v 1 and its apple homologue, Mal d 1. In Spain, apple allergy is more severe with systemic reactions in one-third of the patients and is related to peach allergy and sensitization to Mal d 3, the ns-LTP from apple [42]. These sensitization profiles were recently confirmed by Gomez *et al* [43]. Alternatively, as already addressed above, as traditional sIgE to crude apple extract [44], CRD with Mal d 1 does not discriminate between birch pollen allergic patients with or without apple allergy [5].

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CRD may also be useful in diagnosis of soy (*Glycine max*) allergy. A primary soy allergy is often based on sensitization to soy β -conglycinin (Gly m 5) and soy glycinin (Gly m 6) and related to severe symptoms. However, in contrast to most Bet v 1 associated allergies, sensitization to the Bet v 1 homologue Gly m 4 has also been linked with severe soy allergy. [45, 46].

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Finally, determination of allergen sensitization profiles might also be useful in diagnosing particular food allergies such as exercise-induced wheat allergy from Ω -5 (Tri a 19 from *Triticum aestivum*) [47] or $\alpha\beta\gamma$ gliadins sensitization [48].

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Secondary plant-derived food allergies

Pollen allergy constitutes an important health problem. Traditionally patients mainly suffer from seasonal rhinoconjunctivitis and/or asthma. However, more than half of the patients with a pollen allergy will also present a so-called pollen-related food allergy. The best known representative of the pollen-related food allergies is the “birch-fruit-vegetable” syndrome that results from an extensive cross-reactivity between the major birch pollen allergen Bet v 1 and its labile homologues in fruits, vegetables and nuts (see figure 3). These Bet v 1 homologues such as Mal d 1 from apple, Cor a 1.04 from hazelnut, Ara h 8 from peanut are considered biomarkers of mild allergic reactions mostly characterized by an OAS, although more generalized reactions are not excluded, particularly with Gly m 4 from soy (*Glycine max*) [46, 49]. As already exemplified higher, CRD with Bet v 1 homologues such as Mal d 1 [5] and Cor a 1 [13] appears to be of no value to discriminate between sensitization and genuine allergy. Actually, diagnosis of an OAS is generally easily established on clinical grounds and documentation of a (birch) pollen allergy. Another well-known example of secondary fruit and/or vegetable allergy is the “latex-fruit” syndrome. As described below in the section about latex allergy, patients suffering from latex allergy can display distinct sensitization profiles. A large majority of latex allergic patients is sensitized to so-called defence and/or structural proteins such as Hev b 2, 5 and 6. These proteins are quite ubiquitously distributed in plant kingdom and might explain the occurrence of a latex-fruit syndrome mainly involving tropical fruits such as banana, avocado and chestnut [50]. In

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contrast, other patients, mainly children with spina bifida, are sensitized to the rubber particle associated proteins Hev b 1 and 3, that by definition are confined to rubber synthesising plants and will not display a latex-fruit syndrome. However, at present there are no comprehensive risk assessment studies evaluating sensitization patterns and food allergy in latex allergy. Today, CRD in the context of latex allergy has mainly to be seen in the discrimination between clinically relevant and irrelevant IgE serology (see below).

Finally, we recently described a potential association between sensitization to *Cannabis sativa* (that, by using multiplexed CRD, appeared to result from a sensitization to the non-commercially available Cannabis 3, the ns-LTP from cannabis) and other foods containing ns-LTPs. This “cannabis-food” connection seems frequently to extend beyond fruits and vegetables and might also involve alcoholic beverages, tobacco and *Hevea latex* [21, 51].

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Animal-derived food allergies

In contrast to sIgE to crude plant-derived food allergens, quantification of sIgE to crude animal-derived food allergens seems considerably more reliable to establish correct diagnosis. Therefore the additional value of CRD in animal-derived food allergy has mainly to be sought in the identification of prognostic biomarkers enabling to predict long-term prognosis (tolerance vs. persistence) and the outcome of controlled challenges with raw and/or processed (baked or cooked) food. For example, some studies have disclosed that sensitization to ovomucoid (Gal d 1), the major (thermo-resistant) allergen from hen’s egg white (chicken is *Galus domesticus*), is associated with a poor prognosis with respect to clinical severity and outgrow [52-54]. In one of these studies it was shown that children demonstrating an ovomucoid sIgE < 1 kUA/L had 97% chance to tolerate baked egg, whereas those showing an ovomucoid sIgE > 10.2 kUA/L had 88% chance to react to baked egg [53].

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More recently, Tan et al [55] demonstrated that a skin prick test result for ovomucoid exceeding 11 mm was very likely to predict a reaction to baked egg. In these children, deferring a challenge with egg (white) would be appropriate. Others, however, showed ovomucoid sIgE not to be superior to egg white prick tests or sIgE in predicting outcome of baked egg challenge [56].

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With respect to cows' milk allergy it appears that molecular diagnosis has to go beyond CRD. It has been demonstrated that sensitization to thermo-resistant linear epitopes from α -lactalbumin (Bos d 4, *Bos domesticus* is cow), β -lactoglobulin (Bos d 5) and casein (Bos d 8) is associated with a poorer prognosis than a sensitization to the thermolabile conformational epitopes from these components [57-59]. However, today, the use of peptide microarray assays is very expensive and only available for research purposes. Hitherto, cow's milk-specific IgE still appears to be the best prognostic marker for cow's milk allergy [60].

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There are also invertebrate components available such as recombinant parvalbumin (Gad c 1) from codfish (*Gadus callaria*) and recombinant tropomyosins Pen a 1 from shrimp (*Penaeus aztecus*) and Der p 10 from house dust mite (*Dermatophagoides pteronyssinus*). However diagnosis of fish and crustaceans allergy can generally readily be established using conventional sIgE assays. Moreover, we demonstrated that patients erroneously designated to be allergic to "scampi" (*Nephrops norvegicus*) and that had actually reacted to the giant fresh water prawn (*Macrobrachium rosenbergii*) demonstrated sensitization to tropomyosin in only one quarter of the cases [61].

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Finally, molecular diagnosis can help to elucidate on the cause of mammalian meat allergy. Actually, molecular diagnosis can help to discriminate between meat allergy as a result of an underlying milk allergy, cat allergy or sensitization to a mammalian carbohydrate moiety. In

meat allergy resulting from milk allergy (meat-milk syndrome) molecular diagnosis will point to sensitization to bovine serum albumin (Bos d 6) [62, 63] and/or bovine IgG (Bos d 7) [64].

The cat-pork syndrome is based on cross-reactivity between cat serum albumin (Fel d 2, *Felis domesticus* is cat) [65] and pork serum albumin (Sus s 6, *Sus scrofa domestica* is pig). Finally,

the latter meat allergy is induced by tick bites and is due to a sensitization to the mammalian oligosaccharide galactose-1,3- α -galactose (α -gal) [66]. Discrimination between these different kinds is of relevance, mainly because a sensitization to α -gal will usually present as delayed reactions and can be associated with a sensitization to gelatin [22].

Moreover, in these patients administration of monoclonal antibodies produced in non-primate mammals might trigger anaphylaxis, even upon first exposure [67].

Latex allergy

Correct identification of latex-sensitized patients with genuine *Hevea* latex allergy is of paramount importance as these patients require potentially lifesaving preventing measures. On the other hand, identification of clinically irrelevant latex-sensitization due to sensitization to cross-reactive components such as profilin and CCD epitopes might prevent unnecessary and generally expensive latex avoidance measures. Today, several components from *Hevea brasiliensis* have been identified and successfully cloned and became available for molecular diagnosis of latex allergy. As a matter of fact, the commercially available component-specific IgE assays for natural latex are non-glycosylated recombinant Hev b 1, 3, 5, 6.01, 6.02, 8, 9 and 11. Particularly rHev b 5 and 6 and in a lesser extent also rHev b 1 and 3 (both rubber particle-associated proteins) have repeatedly been shown to be the most important biomarkers to diagnose genuine latex allergy [12, 68-72]. Sensitization to Hev b 5 and 6 is primarily found in adult health care workers and to a lesser extent also in children

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suffering from spina bifida. In contrast, sensitization to *Hevea* profilin Hev b 8 (latex profilin) generally, but certainly not always, points to a clinical irrelevant cross-reactivity. For example, in our own series, in all patients diagnosis of latex allergy could be established by the combination rHev b 1, 3, 5 and 6.02. Over three-quarters of our patients were sensitized to rHev b 5 and/or 6.02. Some also displayed sIgE reactivity against rHev b 1 and/or b 3. In contrast, none of the individuals showing a clinically irrelevant sensitization to natural rubber latex demonstrated IgE reactivity to one of these components but three-quarters of them displayed a positive microarray result for rHev b 8 [12]. Recently, however, we identified some patients with an overt latex allergy apparently related to monosensitization to Hev b 12, the non-specific lipid transfer protein of latex that is currently not commercially available [73]. Obviously, as all available latex components are non-glycosylated proteins, they constitute a helpful instrument to depict clinically irrelevant positive sIgE latex result resulting from a sensitization to plant-derived and invertebrate CCD.

Hymenoptera venom allergy

For patients presenting an IgE-mediated reaction to hymenoptera stings a re-sting may cause life-threatening reactions. In such patients correct diagnosis is mandatory for effective management, i.e. venom-specific immunotherapy. Generally, identification of the culprit implies a detailed history along with the quantification of venom-specific IgE antibodies and intradermal venom skin tests. Unfortunately, due to uncertainties associated with these tests, correct diagnosis is not always straightforward. First, it should be kept in mind that both tests can yield false negative results. For example, in a recent series of 308 patients with yellow jacket venom (YJV) allergy (i.e. wasp allergy), titrated skin testing correctly identified 282 of 308 (93.7%) of the cases, whereas the conventional YJV sIgE assay, that is

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not supplemented with recombinant Ves v 5 (the major allergen Antigen 5 from *Vespula vulgaris*), yielded a sensitivity of 83.4% [74]. Similarly, in our series sensitivity of the conventional YJV sIgE assay yielded a sensitivity of 83.4% [14]. Second, sIgE-binding tests frequently display a poor specificity that is related to the nature of the applied venom allergen extracts. Actually, conventional and rVes v 5-spiked YJV sIgE assays have been using whole-venom extract preparations that harbour highly cross-reactive structures that can significantly hamper diagnosis by generating clinically irrelevant results. Although the vast majority of patients are allergic to only a single venom, up to two-thirds of the patients can display double-positive sIgE results to both YJ and honeybee venom [14, 75]. The major cause for these double-positive sIgE results relates to the presence of structurally similar N-linked CCD rather than to homologous peptide epitopes [76, 77]. Among a Flemish population, sensitization to these highly deceptive CCD was detected in about one-quarter of the patients with grass pollen allergy and one-fifth of patients with genuine hymenoptera venom sensitization, particularly sensitization to honeybee venom [4]. The strategies that can be adopted to detect and circumvent this CCD issue, are the use of glycan biomarkers, basophil activation tests as well as molecular diagnosis applying non-glycosylated species-specific allergen components. These have recently been reviewed elsewhere [78]. From this review it is clear that CRD with recombinant Ves v 5 and Ves v 1 from *Vespula vulgaris* significantly improves diagnosis of YJV allergy, whereas rApi m 1 from *Apis mellifera* does clearly not cover the entire IgE reactivity profile of honeybee venom and that additional molecules, particularly Api m 3, 4 or 10 are needed to enable correct diagnosis of honeybee venom allergy [79]. It also emerged that CRD can be helpful to discriminate between *Vespula vulgaris* and *Polistes dominula* allergy, provided that the Antigen 5 (Ves v 5, Pol d 5) and phospholipases (Ves v 1, Pol d 1) of both vespid species are used [80]. Despite the

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identification and expression of various venom allergen compounds of the fire ant (*Solenopsis invicta*), jack jumper ant (*Myrmecia pilosula*), and the Chinese needle ant (*Pachycondyla chinensis*) [81], to our knowledge, no readily available component-specific assays have been developed hitherto.

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Conclusion

Diagnosis of IgE mediated allergies is often not straightforward as traditional in vitro immunoassays for allergen-specific IgE antibodies do not demonstrate absolute positive and negative predictive values. Therefore, additional diagnostic tests are needed to make the correct diagnosis. During the last two decades significant progress in biochemistry and molecular biology enabled the detection and quantification of sIgE antibodies to allergen protein components and epitope-emulating peptides, also called molecular allergy diagnosis or component resolved diagnosis (CRD). In contrast to conventional sIgE antibody assays, CRD allows to discriminate between genuine allergy and merely sensitisation, to assess the individual risk of severity of an allergic reaction, to establish personalized sensitization patterns, to predict the natural course and finally to facilitate the selection of patients for allergen-specific immunotherapy and to monitor the immunological effects of treatment. CRD can be valuable in the diagnosis of inhalant, food, latex and hymenoptera allergy. Anno 2015, the main application of CRD are food and venom allergy. However, additional studies are mandatory to allow entrance of CRD in mainstream use.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author contributions

VGA, ME, FM, SV, BC and ED wrote the paper.

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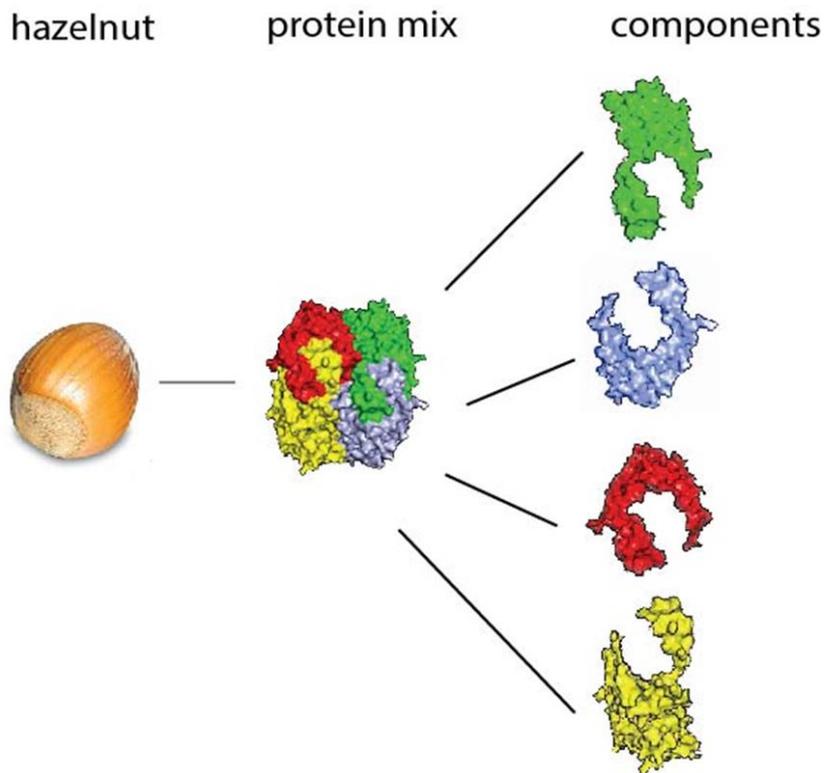
Gewijzigde veldcode

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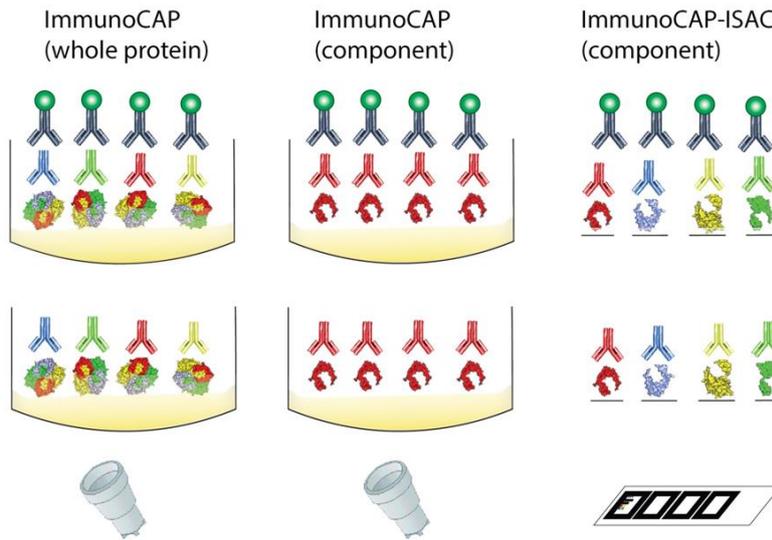
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Figure 1: Composition of an allergen (e.g. hazelnut)



An allergen (e.g. hazelnut) is composed out of a mixture of different allergenic and non-allergenic components (i.e. proteins). These components can be characterized, cloned and produced as recombinant proteins. When expressed in bacteria, these components are deviated from highly cross-reactive plant carbohydrate determinants that severely undermine specificity of conventional sIgE assays (see text).

Figure 2: Principle of measurement of specific IgE (sIgE)



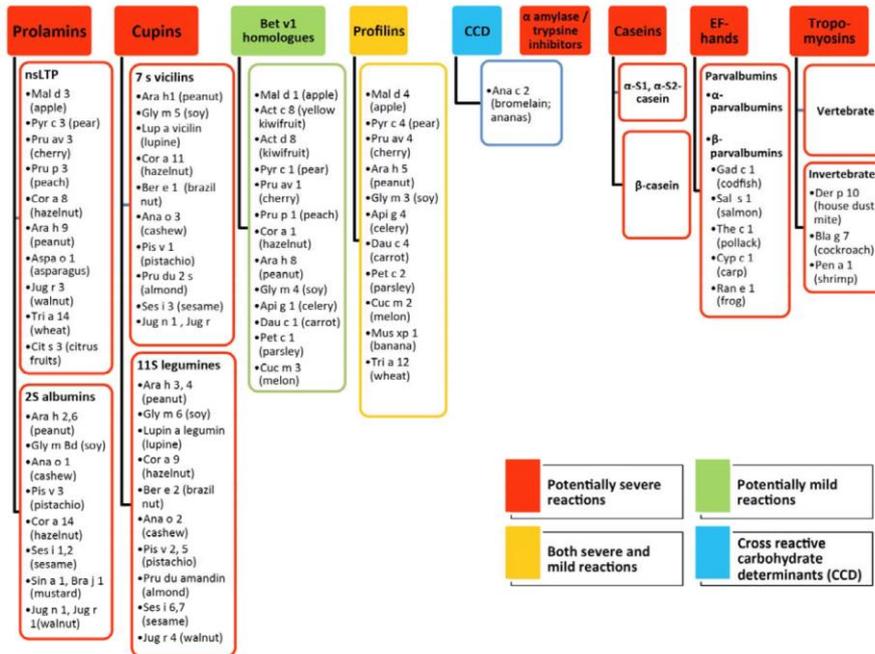
In conventional FEIA ImmunoCAP (left panels), a heterogeneous protein extract containing different components of interest is coupled on a cellulose solid phase. Specific IgE (sIgE) antibodies in the patients' serum recognising the antigen will bind to form a so-called antigen-antibody immune complex. Subsequently, an enzyme-conjugated antihuman-IgE antibody is added. This secondary antibody will bind the antigen-antibody immune complex. Finally, a substrate is added that will be metabolized to produce a quantifiable fluorescence.

In component-resolved diagnosis (CRD) two techniques are available. First in a singleplexed assay single purified or recombinant components (e.g. protein or peptide components) are coupled to a solid phase as described above (centre panels) and detection of sIgE is exactly the same as in the conventional technique. In the multiplexed assay, single native and/or recombinant components are spotted on a polymer coated

slide (right panels), sIgE from the patient blood sample bind to the components and these antibodies are detected with a fluorochrome labelled anti-IgE. Finally, the reaction is scanned in a laser scanner and results are evaluated by image analysis software.

Figure 3: Superfamilies and most representative allergen components of both plant- and animal-derived food allergens (not exhaustive) [82]

Gewijzigde veldcode



Allergens are indexed according to the IUIS nomenclature (www.allergen.org). The first 3 letters and the one after the space define the genus and the species, respectively. The cypher denotes the number of the allergen (frequently but not always in order of description). In brackets are specified the common names. Mal d (*Malus domestica*), Pyr c (*Pyrus communis*), Pru av (*Prunus avium*), Pru p (*Prunus persica*), Cor a (*Corylus avellana*), Ara h (*Arachis hypogaea*), Aspa o (*Asparagus officinalis*), Jug r (*Juglans regia*), Tri a (*Triticum aestivum*), Cit s (*Citrus sinensis*), Gly m (*Glycine max*), Ana o (*Anacardium occidentale*), Pis v (*Pistaciavera*), Ses i (*Sesame indicum*), Lup a (*Lupinus albus*), Ber e (*Bertholletia excelsa*), Pru du (*Prunus dulcis*), Act c (*Actinidia chinensis*), Act d (*Actinidia deliciosa*), Api g (*apium graveolens*), Dau c (*Daucus carota*), Pet c (*Petroselinum crispum*), Cuc m (*Cucumis melo*), Mus sp (*Musa species*), Ana c (*Ananas comosus*), Gad c (*Gadus*

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callarias), Sal s (Salmo salar), The c (Theragra chalcogramma), Cyp c (Cyprinus carpio), Ran e (Rana esculenta), Der p (Dermatophagoides pteronyssinus), Bla g (Blatella germanica), Pen a (Penaeus aztecus). The colors indicate the potential severity of clinical reactions associated with sensitization to a particular component.