Molecular Allergy Diagnostics Using IgE Singleplex Assays: Methodological and Practical Considerations

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7.1 Introduction

7.1.1 Atopy and Allergen-Specific IgE

The number of atopic diseases, such as allergic rhinoconjunctivitis, allergic bronchial asthma, atopic eczema, and food allergies, has increased worldwide in recent years. Atopy is a genetic predisposition to develop IgE antibodies (IgE), against otherwise harmless, widely distributed environmental allergens. Once sensitized (IgE antibody positive), atopic individuals can develop the aforementioned atopic diseases following subsequent exposure to corresponding allergens.

The present chapter is based on, and modified from, an article by the authors that appeared in 2015 in *Allergo Journal International* (Kleine-Tebbe J, Jakob T: Molecular allergy diagnostics using IgE singleplex determinations: methodological and practical consideration for the use in clinical practice. Allergo J Int 2015;24:185–197. doi: 10.1007/s40629-015-0057-1).

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J. Kleine-Tebbe, T. Jakob (eds.), *Molecular Allergy Diagnostics*,
DOI 10.1007/978-3-319-42499-6_7

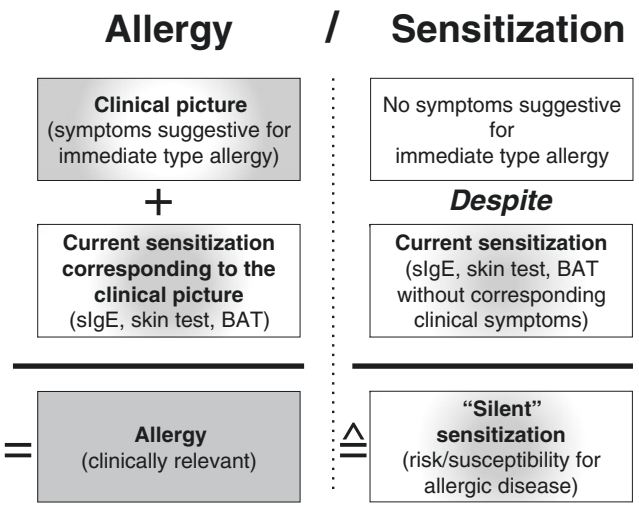

Fig. 7.1 Basic concept in allergy: Relationship between sensitization and clinically relevant allergy. One speaks of a clinically relevant allergy only when specific IgE triggers symptoms in a reproducible manner following allergen exposure. The detection of sIgE in the absence of clinical symptoms is consistent with a silent sensitization, which reveals a risk factor or susceptibility for allergic disease, but one that is not as yet manifested clinically. Abbreviations: BAT: basophil activation test; sIgE: allergen-specific IgE

Recent epidemiological studies have shown that 46.5 % of the adolescent population between the ages of 14 and 17 years (42 % of girls and 51 % of boys; Schmitz et al. 2013) and 48.6 % of the adult population (45 % of women and 52 % of men; Haftenberger et al. 2013) in Germany are sensitized with specific IgE to at least one allergen source (pollen, mites, animal dander, molds, and food).

Diagnostic tests that either directly or indirectly detect IgE antibodies in the context of an increased susceptibility to allergies (*sensitization*) are referred to as sensitization tests. In the presence of allergic symptoms that are consistent with IgE sensitization, one speaks of a *clinically relevant allergy* (⊙ Fig. 7.1). (Kleine-Tebbe and Jakob 2015)

7.1.2 IgE, IgE Receptors, and the Effector Phase of Allergic Reactions: Background Information and Relevance in IgE Antibody Diagnostics

As the least abundant human antibody, IgE was not discovered until 1966 [see Johansson (2011) for a historical summary]. Approximately half of IgE is found as free IgE in the vascular circulation, while the other half is bound by IgE receptors on a variety of cells. The high-affinity IgE receptor (FcεRI) on tissue-bound mast cells and circulating basophils is the most important binding partner (approximately 100,000–250,000 FcεRI/basophilic leukocyte; ⊙ Fig. 7.2) in immediate-type allergic reactions. Although free serum IgE has a half-life of only a few days,

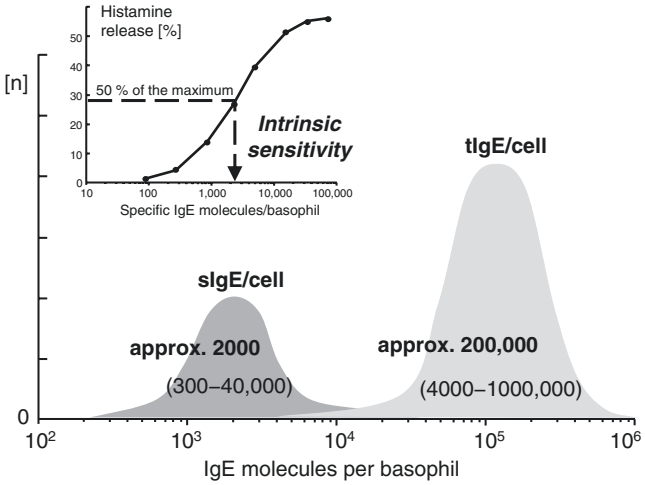

Fig. 7.2 Population-based FcεRI-bound IgE on effector cells. *Light gray area*, total bound IgE/cell (number of FcεRI occupied by IgE with population-based distribution) on basophilic leukocytes. *Dark gray area*, specific IgE/cell required for half-maximal cell activation (intrinsic sensitivity of basophils with population-based distribution). The distribution of both variables is approximately normal and can differ significantly; a fraction (approx. 1 %) of bound total IgE is sufficient for half-maximal allergen-specific activation. For this reason, the specific IgE to total IgE ratio is interesting in the interpretation. *n*: Frequency. *Inset top left*, individual mediator release as a function of cell-bound specific IgE; basis for the population-based normal distributions illustrated in the lower part of the figure

FcεRI-bound IgE persists for approximately 2 weeks due its slow off-rate. It takes approximately 2 months to remove the majority of cell-bound IgE by therapeutic biological drugs such as anti-IgE (omalizumab). Thus, it is not free but rather cell-bound IgE that is essential for the effector phase of the allergic reaction. Upon renewed allergen exposure, specific IgE antibodies are cross-linked, either in pairs or as a large aggregates. It takes an average of 2000 cross-linked IgE molecules to induce a half-maximal cell response (e.g., histamine release; ⊙ Fig. 7.2) only a fraction of total cell-bound IgE (200,000 molecules/basophil). For this reason, basophil tests have an extremely high analytical sensitivity. Phosphorylation of tyrosine kinases (e.g., Syk) activates intracellular signal cascades involving:

- Release of preformed mediators
- De novo synthesis of lipid mediators from the plasma membrane
- Production and release of cytokines

The activation status of effector cells is quantified by the expression of specific surface markers using flow cytometry; basophilic leukocytes from fresh blood are generally used, since they are easier to isolate (basophil activation test, BAT). (for review: Hoffmann et al. 2015).

It was possible to elucidate the effect of the individual IgE repertoire on the effector phase (basophil activation) using polyclonal synthetic Der p 2-specific IgE

antibodies of varying epitope specificity and affinity (Christensen et al. 2008). The following variables have a significant effect on the dose-dependent activation of basophilic leukocytes:

- The total amount of cell-bound IgE.
- The ratio of specific IgE to total IgE (as little as 1 % is sufficient for half-maximal activation of effector cells, see above).
- The number of epitope-specific antibodies capable of binding to a defined allergen (clonality).
- The binding strength between individual IgE antibodies and the allergen (affinity).
- The total number of multivalent specific IgE binding sites that bind strongly to the allergen (avidity).
- The ratio of low- to high-affinity IgE antibodies directed to a defined allergen.

IgE stabilizes the continuously newly synthesized FcεRI receptors at the cell surface (MacGlashan et al. 2001). In this way, the level of total IgE passively regulates the number of its receptors and thus also the amount of cell-bound IgE (MacGlashan 2005). These relationships, which have been studied using basophils since the end of the 1990s, similarly apply to tissue-bound mast cells. The latter, with their cutaneous population (skin mast cells), form the basis for sensitization testing on skin (skin prick test, intradermal skin test). The complex variables involved (Kleine-Tebbe et al. 2006), besides the allergen-specific IgE level, explain why various sensitization tests (specific IgE, titrated skin test, dose-dependent BAT) correspond well qualitatively (concordance between positive or negative results) but not quantitatively (Purohit et al. 2005).

7.1.3 The IgE Repertoire: A Phenomenon with Complex Variables

The IgE synthesized by plasma cells is directed against (glyco)protein surface structures. The more alike and abundant the common binding sites (epitopes) are, the likelier it is that specific IgE will cross to allergens of similar structure—this is the basis of *cross-reactivity* or *cross-sensitization*.

Polyclonally produced IgE antibodies differ in terms of their binding strength (avidity/affinity) and recognition of specific epitopes (Lund et al. 2012). The resulting IgE repertoire, e.g., against *one* allergen molecule, is therefore made up of a multitude of antibodies with differing epitope specificity and binding strength. In the course of the immune response to an allergen, the repertoire can expand and the binding strength will increase through the recognition of further epitopes. To date, it has only been possible to investigate the variables described (epitope specificity, avidity, polyclonality) under experimental conditions, not in routine tests (Christensen et al. 2008). Thus, even modern quantitative singleplex tests for specific IgE determination using individual allergen molecules can recognize only the *total quantity of the polyclonal IgE response* (“the scale of the iceberg”) in the best case, while additional parameters of the allergen-specific repertoire (“the number and height of the various tips of the iceberg”) continue to remain hidden to routine diagnostics (Kleine-Tebbe 2012).

7.1.4 Techniques to Detect Sensitization in Routine Diagnostics

In routine diagnostics, sensitization tests serve to detect IgE either (⊙ Fig. 7.3):

- Directly
- Indirectly

The following methods are available to detect sensitization in IgE-mediated reactions and diseases (Matricardi et al. 2016):

1. Skin tests (skin prick testing, in selected cases intradermal testing; Bousquet et al. 2012; Worm et al. 2015)
2. Serum IgE determination (allergen-specific IgE, total IgE; Matricardi et al. 2016; Hamilton et al. 2016)

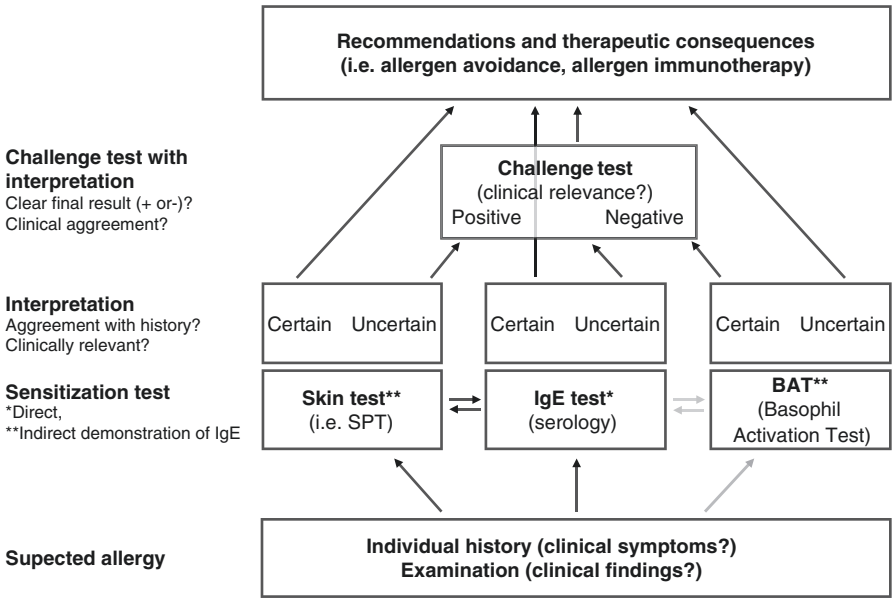

Fig. 7.3 Diagnostic algorithm in IgE-mediated allergies. Following collection of an allergy history and performance of an examination of the patient, their susceptibility risk for allergy (IgE sensitization) to potential allergen sources (e.g., pollen, mites, animal components, mold spores, food, insect venom) is evaluated. To this end, skin prick testing, followed by targeted allergen-specific IgE testing, is usually performed. In rare cases (*gray arrows*, e.g., negative skin prick test and specific IgE results in the case of low total IgE despite strong suspicion of an IgE-mediated reaction), cellular tests with basophil leukocytes, due to their extremely high analytical sensitivity (Kleine-Tebbe et al. 2006), represent an additional option to detect indirectly IgE sensitization. In the ideal case, sensitization tests agree well qualitatively (susceptibility to allergy: yes or no), but poorly on the quantitative level (how strongly positive?) due to additional cellular variables in skin and basophil tests for the indirect detection of IgE. Results are interpreted with reference to the previous clinical history and, in the case of good agreement with the patient history (reliable interpretation), form the basis for further treatment measures. In cases of doubt, challenge testing can help to determine the clinical relevance of IgE sensitizations

3. Basophil function tests (BAT and cellular antigen stimulation test, CAST) only in selected indications (Uyttebroek et al. 2014)

While serum IgE determination directly measures free IgE, the skin prick test and BAT yield indirect information on mast cell- and basophil-bound IgE. As such, they are comparable in terms of the diagnostic (qualitative) information they yield, even though there can be significant quantitative variation between results, particularly in the case of different allergen sources and due to the variables mentioned above (Purohit et al. 2005).

For historical reasons, skin prick testing with allergen extracts has become established as a common and robust screening method in routine practice (Bousquet et al. 2012; Worm et al. 2015). However, allergen molecules are easier to apply to laboratory assays than to in vivo testing (e.g., skin tests), since they are classified as a drug when used directly on humans and need to meet the stringent requirements associated with gaining market authorization for in vivo diagnostic agents of this kind. For this reason, allergen molecules are currently—and will probably remain—predominantly used in serological in vitro diagnostics.

7.2 Technological Basis of IgE Determination

Solid-phase immunoassays for the routine diagnosis of specific IgE have been available since the early 1970s. Initially, radioimmunological methods (radioallergosorbent test, RAST) consisted of coupling allergen extracts to activated paper disks in order to bind specific IgE from the sera of allergy sufferers. Today, measurements are no longer red by means of a radioactive tag, but rather by means of enzyme labeling or fluorimetry; moreover, allergenic molecules are also increasingly used in this context (⊙ Table 7.1 provides a selection of single allergens available for singleplex IgE analysis).

7.2.1 Test Design and Test Components

Modern immunoassays to determine allergen-specific IgE antibodies (overview in ⊙ Table 7.2) comprise the following components (Hamilton et al. 2016; Matricardi et al. 2016):

(a) Reaction vessel: plastic (polyethylene) or glass test tubes, plastic microtiter plate with wells, plastic rods or pellets, polyethylene cap with spongelike matrix
(b) Allergen-containing reagent: allergen adsorbed to a solid phase or labeled allergen in the liquid phase
(c) Anti-IgE-Fc antibody (detection antibody specific to the constant Fc fragment of IgE)
(d) Calibration system: e.g., reference serum with defined IgE volume in order to generate a total IgE calibration curve

Table 7.1 Single allergens available for allergen-specific IgE determination using a singleplex assay

Allergen source	Allergen[a]	Protein family/function	Relevance in clinical diagnostics
Tree pollen (see ▶ Chap. 10 for more details)			
Birch	**rBet v 1**	PR-10	Major allergen, marker for sensitization to birch pollen and the Fagales group (alder, hazel, beech, oak), varying cross-reactivity with other members of the PR-10 proteins, e.g., in birch pollen-related food allergy
Birch	**rBet v 2**	Profilin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with other profilins in tree, grass, or weed pollen or plant-based foods
Birch	**rBet v 4**	Polcalcin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with other polcalcins in tree, grass, or weed pollen
Plane (maple-leaved)	**rPla a 1**	Invertase inhibitor	Major allergen, marker for sensitization to plane pollen
Olive tree	**rOle e 1**	Oleaceae group 1	Major allergen, marker for sensitization to olive and ash pollen and other members of the Lamiales (lilac, privet)
Cypress (Arizona)	**nCup a 1**	Pectate lyase	Major allergen, marker for sensitization to cypress, cedar, and juniper; IgE reactivity can be partially based on reactivity to the CCD component
Grass pollen (see ▶ Chap. 10 for more details)			
Meadow foxtail	**nCyn d 1**	Grass group 1	Major allergen, marker for sensitization to meadow foxtail pollen and other grass pollens of the Chlorideae subfamily; IgE reactivity can be partially based on reactivity to the CCD component
Timothy grass	**rPhl p 1**	Grass group 1	Major allergen, marker for sensitization to timothy grass pollen and all other grass pollens (Poaceae)
Timothy grass	**rPhl p 2**	Grass group 2	Minor allergen, marker for sensitization to timothy grass pollen and other grass pollens of the Pooideae subfamily (e.g., rye, smooth meadow grass, rye grass)
Timothy grass	**rPhl p 4**	Berberine bridge enzyme	Minor allergen, marker for sensitization to timothy pollen and other grass pollens; IgE reactivity can be partially based on reactivity to the CCD component
Timothy grass	**rPhl p 5**	Unknown	Major allergen, marker for sensitization to timothy pollen and other grass pollens of the Pooideae subfamily (e.g., rye, smooth meadow grass, rye grass)

(continued)

Table 7.1 (continued)

Allergen source	Allergen[a]	Protein family/function	Relevance in clinical diagnostics
Timothy grass	**rPhl p 6**	Unknown	Minor allergen, marker for sensitization to timothy grass and other grass pollens of the Pooideae subfamily (e.g., rye, smooth meadow grass, rye grass)
Timothy grass	**rPhl p 7**	Polcalcin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with other polcalcins in tree, grass, or weed pollen
Timothy grass	**rPhl p 12**	Profilin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with other profilins in tree, grass, or weed pollen or plant-based foods
Weed pollen (see ▶ Chap. 11 for more details)			
Mugwort	**nArt v 1**	Defensin-like protein	Major allergen, marker for sensitization to mugwort pollen
Mugwort	**nArt v 3**	nsLTP	Major allergen, marker for sensitization to mugwort pollen, Cross-reactive allergen, indicator of potential cross-reactivity with other members of the LTP family, e.g., in mugwort pollen-associated food allergy
Wall pellitory	**rPar j 2**	nsLTP	Major allergen, marker for sensitization to wall pellitory pollen, Cross-reactive allergen, indicator of potential cross-reactivity with other members of the LTP family
Tumbleweed	**nSal k 1**	Pectin methyl esterase	Major allergen, marker allergen for sensitization to tumbleweed pollen
Ribwort	**rPla l 1**	Trypsin inhibitor	Major allergen, marker allergen for sensitization to ribwort pollen
Ragweed (*Ambrosia artemisiifolia*)	**nAmb a 1**	Pectate lyase	Major allergen, marker allergen for sensitization to ragweed pollen (*Ambrosia*)
Legume allergens (see ▶ Chap. 12 for more details)			
Peanut	**rAra h 1**	7S globulin (vicilin)	Marker allergen, indicator of high-risk sensitization
Peanut	**rAra h 2**	2S albumin (conglutin)	Marker allergen, indicator of high-risk sensitization
Peanut	**rAra h 3**	11S globulin (glycinin)	Marker allergen, indicator of high-risk sensitization
Peanut	rAra h 6	2S albumin (conglutin)	Marker allergen, indicator of high-risk sensitization
Peanut	**rAra h 8**	PR-10 protein (Bet v 1 homolog)	Cross-reactive allergen, cross-reactivity generally caused by Bet v 1 sensitization, no high-risk sensitization
Peanut	**rAra h 9**	nsLTP	Cross-reactive allergen, indicator of cross-reactivity with other nsLTPs
Soybean	**rGly m 4**	PR-10 protein (Bet v 1 homolog)	Cross-reactive allergen, cross-reactivity generally caused by Bet v 1 sensitization, local oropharyngeal reactions common, severe local or systemic reactions in isolated cases

Soybean	**Gly m 5**	7S globulin	Marker allergen, indicator of high-risk sensitization
Soybean	**Gly m 6**	11S globulin	Marker allergen, indicator of high-risk sensitization
Soybean	**rGly m 8** (from 2016)	2S albumin	Marker allergen, indicator of high-risk sensitization
Nut allergens (see ▶ Chap. 13 for more details)			
Hazelnut	**rCor a 1.0401**	PR-10 protein (Bet v 1 homolog)	Cross-reactive allergen, cross-reactivity generally caused by Bet v 1 sensitization, no high-risk sensitization, severe clinical reactions uncommon
Hazelnut	**rCor a 8**	nsLTP	Cross-reactive allergen, moderate indicator of cross-reactivity with other nsLTPs
Hazelnut	**Cor a 9**	11S globulin	Marker allergen, indicator of high-risk sensitization
Hazelnut	**rCor a 14**	2S albumin	Marker allergen, indicator of high-risk sensitization
Cashew nut	rAna o 2	11S globulin	Marker allergen, indicator of high-risk sensitization
Brazil nut	**rBer e 1**	2S albumin	Marker allergen, indicator of high-risk sensitization
Walnut	**Jug r 1**	2S albumin	Marker allergen, indicator of high-risk sensitization
Walnut	Jug r 2	7S globulin	Marker allergen, indicator of high-risk sensitization
Walnut	**Jug r 3**	nsLTP	Cross-reactive allergen, indicator of cross-reactivity with other nsLTPs
Fruit and vegetable allergens (see ▶ Chap. 14 for more details)			
Apple	**rMal d 1**	PR-10 protein	Cross-reactive allergen, varying cross-reactivity with other members of the PR-10 proteins, e.g., in birch pollen-associated food allergy
Apple	**rMal d 3**	nsLTP	Marker allergen, cross-reactivity with other nsLTPs, indicator of high-risk sensitization
Carrot	Dau c 1	PR-10 protein	Cross-reactive allergen, varying cross-reactivity with other members of the PR-10 proteins, e.g., in birch pollen-associated food allergy, also potential high-risk sensitization
Carrot	Dau c 4	Profilin	Cross-reactive allergen, high cross-reactivity with other profilins
Carrot	Dau c 5	Isoflavone reductase	
Celery	**Api g 1**	PR-10 protein	Cross-reactive allergen, varying cross-reactivity with other members of the PR-10 proteins, e.g., in birch pollen-associated food allergy, also potential high-risk sensitization
Celery	Api g 10	nsLTP	Marker allergen, cross-reactivity with other nsLTPs, indicator of high-risk sensitization

(continued)

Table 7.1 (continued)

Allergen source	Allergen[a]	Protein family/function	Relevance in clinical diagnostics
Kiwi	Act d 1	Actinidin (cysteine protease)	Marker allergen for primary kiwi sensitization, indicator of high-risk sensitization
Kiwi	Act d 2	Thaumatin-like protein	Minor allergen, potential systemic reactions due to stability to digestion
Kiwi	Act d 5	Kiwellin	Major allergen, marker allergen for primary kiwi sensitization, indicator of high-risk sensitization
Kiwi	**Act d 8**	PR-10 protein	Cross-reactive allergen, varying cross-reactivity with other members of the PR-10 proteins, e.g., in birch pollen-associated food allergy
Peach	**Pru p 1**	PR-10 protein	Cross-reactive allergen, varying cross-reactivity with other members of the PR-10 proteins, e.g., in birch pollen-associated food allergy
Peach	**Pru p 3**	nsLTP	Marker allergen, cross-reactivity with other nsLTPs, indicator of high-risk sensitization, particularly in the Mediterranean region
Peach	**Pru p 4**	Profilin	Cross-reactive allergen, high cross-reactivity with other profilins
Wheat allergens and other food allergens in FDEIA (see ▶ Chap. 15 for more details)			
Wheat	**rTri a 14**	LTP	Minor allergen, Cross-reactive allergen with other LPTs
Wheat	**rTri a 19**	ω-5-Gliadin	Major allergen, marker allergen for WDEIA
Wheat	nTri a 21	α/β-Gliadin	Major allergen, marker allergen for WDEIA
Wheat	nTri a 26	HMW glutenin	Major allergen, marker allergen for WDEIA
Wheat	rTri a 36	LMW glutenin GluB3-23	Major allergen, marker allergen for WDEIA
Wheat	nTri a γ-Gliadin	γ-Gliadin	Major allergen, marker allergen for WDEIA
Wheat	**nGliadin**	α/βγ/ω-Gliadin	Major allergens, marker allergens for WDEIA
Shrimp	**rPen a 1**	Tropomyosin	Major allergen, marker allergen for sensitization to crustaceans, N.B. high cross-reactivity with tropomyosin from other sources
Soy	**rGly m 5**	β-Conglycinin	Marker allergen for genuine soy sensitization
Peach	**rPru p 3**	nsLTP	Marker allergen for peach sensitization, cross-reactivity with various other nsLTPs
Meat (red), innards	**α-Gal**	Galactose-α-1,3-galactose	Marker allergen for delayed meat allergy

Insect venom (see ▶ Chap. 16 for more details)			
Honey bee	**rApi m 1**	Phospholipase A_2	Major allergen, marker for bee venom sensitization
Honey bee	**rApi m 2**	Hyaluronidase	Minor allergen, marker for bee venom sensitization, cross-reactivity with hyaluronidase of wasp venom (Ves v 2) possible
Honey bee	**rApi m 3** (from 2016)	Acid phosphatase	Major allergen, marker for bee venom sensitization
Honey bee	nApi m 4	Melittin	Minor allergen, marker for bee venom sensitization
Honey bee	**rApi m 5** (from 2016)	Vitellogenin	Major allergen, Cross-reactive allergen, cross-reactivity with vitellogenin of wasp venom Ves v 3
Honey bee	**rApi m 10** (from 2015)	Icarapin	Major allergen, marker for bee venom sensitization
Wasp	**rVes v 1**	Phospholipase A_1	Major allergen, marker for wasp venom sensitization
Wasp	rVes v 2	Hyaluronidase	Minor allergen, Cross-reactive allergen, most cross-reactivity with bee venom hyaluronidase (Api m 2)
Wasp	rVes v 3	Vitellogenin	Minor allergen, Cross-reactive allergen, cross-reactivity with bee venom vitellogenin (Api m 5)
Wasp	**rVes v 5**	Antigen 5	Major allergen, marker for wasp venom sensitization
Paper wasp	**rPol d 5**	Antigen 5	Major allergen, marker for paper wasp sensitization, high cross-reactivity with other antigen-5 allergens such as Ves v 5
Animal dander (see ▶ Chap. 17 for more details)			
Cat	**rFel d 1**	Uteroglobin	Major allergen, marker for sensitization to cat dander
Cat	**nFel d 2**	Serum albumin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with other animal danders
Cat	**rFel d 4**	Lipocalin	Minor allergen, Cross-reactive allergen, cross-reactivity with other lipocalins (e.g., Can f 6 or Equ c 1)
Dog	**rCan f 1**	Lipocalin	Marker for sensitization to dog dander
Dog	**rCan f 2**	Lipocalin	Marker for sensitization to dog dander
Dog	**nCan f 3**	Serum albumin	Cross-reactive allergen, indicator of cross-reactivity with other animal danders and components

(continued)

Table 7.1 (continued)

Allergen source	Allergen[a]	Protein family/function	Relevance in clinical diagnostics
Dog	**rCan f 5**	Arginine esterase	Marker for sensitization to dog dander
Horse	**rEqu c 1**	Lipocalin	Major allergen, marker for sensitization to horse dander, cross-reactivity with other lipocalins (e.g., Can f 6 or Fel d 4)
Horse	rEqu c 3	Serum albumin	Minor allergen, Cross-reactive allergen, cross-reactivity with other lipocalins (e.g., Can f 6 or Equ c 1)
Animal food allergens			
Hen's egg	**Gal d 1**	Ovomucoid	Major allergen and marker allergen for chicken egg sensitization, associated with persistent chicken egg allergy
Hen's egg	**Gal d 2**	Ovalbumin	Marker allergen for hen's egg sensitization
Hen's egg	**Gal d 3**	Conalbumin/ovotransferrin	Marker allergen for hen's egg sensitization
Hen's egg	Gal d 5	Livetin/serum albumin	Allergen in egg yolk and chicken meat, indicator of bird egg syndrome
Cow's milk	**Bos d 4**	α-Lactalbumin	Major allergen (whey protein) in cow's milk
Cow's milk	**Bos d 5**	β-Lactoglobulin	Major allergen (whey protein) in cow's milk
Cow's milk	Bos d 6	Serum albumin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with other albumins in animal dander or (uncooked) meat
Cow's milk	**Bos d 8**	Casein	Major allergen in cow milk and cheese, cross-reactions with sheep and goat milk products
Fish allergens (see ▶ Chap. 18 for more details)			
Cod	**rGad c 1**	Parvalbumin	Major allergen, Cross-reactive allergen, high cross-reactivity with parvalbumins from various fish species
Carp	**rCyp c 1**	Parvalbumin	Major allergen, Cross-reactive allergen, high cross-reactivity with parvalbumins from various fish species
House dust mites (see ▶ Chap. 19 for more details)			
House dust mite	**rDer p 1**	Cysteine protease	Major allergen, marker for sensitization to house dust mites
House dust mite	**rDer p 2**	NPC2 family	Major allergen, marker for sensitization to house dust mites
House dust mite	**rDer p 10**	Tropomyosin	Minor allergen, Cross-reactive allergen, indicator of cross-reactivity with tropomyosin from crustaceans, cockroaches
House dust mite	rDer p 23	Chitin-binding protein	Major allergen, marker for sensitization to house dust mites

Mold (see ▶ Chap. 21 for more details)			
Alternaria alternata	**rAlt a 1**	Unknown function	Marker for sensitization to outside air mold *Alternaria*
Aspergillus fumigatus	**rAsp f 1**	Enolase	Marker for sensitization to *Aspergillus fumigatus*
Aspergillus fumigatus	**rAsp f 2**	Mitogillin	Marker for sensitization to *Aspergillus fumigatus*, evidence of allergic bronchopulmonary aspergillosis (ABPA) in conjunction with positive values for Asp f 2, 4, 6
Aspergillus fumigatus	**rAsp f 3**	Fibrinogen-binding protein	Marker for sensitization to *Aspergillus fumigatus*
Aspergillus fumigatus	**rAsp f 4**	Peroxisomal protein	Marker for sensitization to *Aspergillus fumigatus*, evidence of allergic bronchopulmonary aspergillosis (ABPA) in conjunction with positive values for Asp f 2, 4, 6
Aspergillus fumigatus	**rAsp f 6**	Mn-superoxide dismutase	Marker for sensitization to *Aspergillus fumigatus*, evidence of allergic bronchopulmonary aspergillosis (ABPA) in conjunction with positive values for Asp f 2, 4, 6
Latex (see ▶ Chap. 22 for more details)			
Latex	**rHev b 1**	Rubber elongation factor	Marker for latex sensitization, major allergen for patients with spina bifida
Latex	**rHev b 3**	Small rubber particle protein	Marker for latex sensitization, major allergen for patients with spina bifida
Latex	**rHev b 5**	Unknown	Marker for latex sensitization, major allergen for patients with spina bifida and healthcare personnel
Latex	**rHev b 6.01**	Hevein precursor	Marker for latex sensitization, major allergen for healthcare personnel
Latex	**rHev b 8**	Profilin	Cross-reactive allergen, indicator of cross-reactivity with other profilins in tree, grass, and weed pollen or plant-based foods

[a]**Boldface**: available as singleplex assays for IgE determinations

Table 7.2 Various test principles for determining specific IgE antibodies in a singleplex assay

IgE assay format	Short description	Advantages	Disadvantages
Solid-phase assay	Solid-phase assays for IgE determination have been established for many years. The allergens coupled to a solid phase directly bind all allergen-specific antibodies (e.g., IgE, IgG); washing removes unbound antibodies. The bound specific IgE antibodies are then determined using radiolabeled anti-IgE antibodies. The latter are marked with suitable reagents (fluorescence, chemiluminescence) and substrates that enable quantification of bound specific IgE antibodies	In the case of a large surface area of the solid phase used and surplus allergens/allergen sources, complete binding of all specific IgE antibodies is possible (prerequisite of true quantitation) However, low-affinity IgE antibodies are also bound	In the case of low surface area of the solid phase (e.g., paper disk) and no surplus allergens/allergen sources, true quantitation of specific IgE is not possible and competitive inhibition of the IgE signal by allergen-specific IgG antibodies occurs (particularly in high titers, e.g., after allergen-specific immunotherapy)
Liquid-phase assay	This test format involves the use of liquid and labeled allergens to bind allergen-specific IgE. Following the appropriate washing procedure, the allergen–IgE marker complexes are bound by immobilized reagents (e.g., the biotin–streptavidin system). The use of appropriate substrates likewise enables quantitation of primarily bound specific IgE on the allergens used	Rapid binding kinetics due to the liquid phase	True quantitation of specific IgE not possible in the absence of surplus allergens/allergen sources
Reverse IgE assay	With this test system, all IgE antibodies (e.g., contained in serum) are bound by immobilized anti-IgE antibodies in a first step. Following the removal of unbound antibodies (e.g., IgG), allergen-specific IgE can be identified by adding appropriately labeled liquid allergens. By labeling the allergens, it is possible to quantify specific bound antibodies	No inhibition caused by the high proportion of allergen-specific IgG antibodies	Limited binding capacity particularly in the case of extremely high total IgE (>2000 kU/l)

(e) Reaction buffer: mineral- and protein-containing solutions for constant pH values and constant protein matrix to ensure minimal nonspecific binding
(f) Human serum with specific IgE antibodies and negative serum controls
(g) Data processing system (software or algorithm)

The *allergen-containing reagent* (b) is considered the most complex component of the test materials, irrespective of whether extracts of biological origin or single defined allergen molecules are involved.

The second and equally important component is the *anti-IgE reagent* (c), generated either polyclonally in various animal hosts (rabbit, goat, horse) or as monoclonal mouse antibodies with defined binding to epitopes on the Fc region of human IgE antibodies. Monoclonal and polyclonal antibodies to IgE are often used in combination in order to achieve parallelism and linearity in the test system over a broad concentration range.

The *calibration system* (d) is the third key component of IgE determination. Since there are no internationally accepted standards for allergen-specific IgE tests, a total IgE calibration curve enables the units measured to be expressed as quantitative allergen-specific IgE antibody levels (⊙ Fig. 7.4): kU_A/l (where "A" stands for "allergen specific," thereby distinguishing units from the internationally standardized kU/l = IU/ml for total IgE determination). The randomly assigned "classes" that have evolved over time serve to semiquantitatively and broadly categorize IgE concentrations and, in the authors' view, play only a minor role today. The test systems currently available, as well as their test principles, are shown in ⊙ Table 7.2.

7.2.2 Detection Thresholds in sIgE Determination

The lower detection threshold limit for specific IgE was formerly 0.35 kU_A/l. The analytical sensitivity of IgE assays is now enhanced thanks to more sensitive calibration and improved resolution of low IgE values. Thus, modern specific IgE antibody immunoassays now produce values below 0.35 down to 0.1 kU_A/l (⊙ Fig. 7.4). This range is particularly informative and relevant when total IgE is extremely low (<20 kU/l). The upper detection limit is 100 kU_A/l for most specific

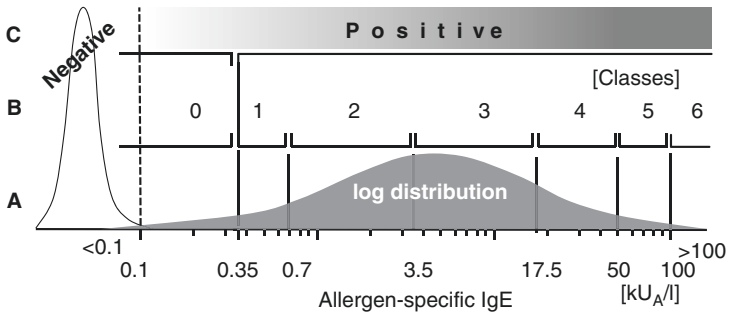

Fig. 7.4 Options for the evaluation of logarithmically distributed allergen-specific IgE levels. *A* quantitative, *B* semiquantitative (in the German Medical Association guidelines, this term is no longer used; specific IgE levels given in classes are considered as qualitative evaluations), *C* qualitative. Allergen-specific IgE levels expressed as units of specific IgE, kU_A/l (*A* stands for allergen specific), using WHO standards for total IgE determination (heterologous calibration). *white area under the curve*, population of serum samples with no allergen-specific IgE (levels fall below the detection limit of 0.1 kU_A/l). *Dark gray area*, population of positive serum samples with logarithmic (hypothetically normal) distribution of allergen-specific IgE levels above the detection limit of 0.1 kU_A/l

IgE detection methods. Therefore, sera with higher specific IgE levels should be measured in a diluted form (1:10) in order to determine the actual value after multiplying ×10.

7.2.3 Specific IgE–Total IgE Ratio

A number of modern assays have shown that the unit for total IgE (kU/l) corresponds to the heterologously calibrated units for allergen-specific IgE (kU_A/l) (Kober and Perborn 2006). Working on this assumption, both variables, specific and total IgE, can be directly compared and used to improve interpretation (Hamilton et al. 2010) The ratio of specific IgE to total IgE (also referred to as *antibody-specific activity*; Hamilton et al. 2010) is given particular significance in the case of:

- Extremely low total IgE levels (e.g., <20 kU/l), for instance, in:
  - Some atopic patients with unusually low total serum IgE levels
  - Non-atopic patients with IgE sensitization to particular allergens, e.g., insect venom or occupational allergens
- Extremely high total IgE levels, for instance, in:
  - Atopic patients with currently or previously manifest atopic dermatitis
  - Patients with other causes of extremely high total IgE such as allergic bronchopulmonary aspergillosis (Renz et al. 2010)

It is important to bear in mind that normal distribution of IgE is not linear, but rather logarithmic, and thus it needs to be represented using a logarithmic scale (⊙ Fig. 7.4).

The ratio of specific IgE to total IgE in serum is found in the same way on effector cells (mast cells, basophils). If specific IgE is given relative (e.g., in percent) to total IgE (see ⊙ Fig. 7.5 for a more detailed explanation) (Hamilton et al. 2010), the values relating to individual total IgE levels are normalized: By taking this step, one can expect better concordance between the relative specific IgE proportion (in percent) and the quantitative analysis of other sensitization tests (skin prick test, BAT).

7.2.4 Isoforms: Natural Variants of Allergen Molecules

Points of criticism on the use of allergen molecules relate to their origin or production:

When derived from natural sources, even defined allergens are variable mixtures with multiple molecule variants (isoforms), which bind IgE with varying strength depending on the individual IgE repertoire. Mixing isoforms potentially has the advantage of covering all IgE specificities; however, complex mixtures of this kind are challenging to purify and standardize.

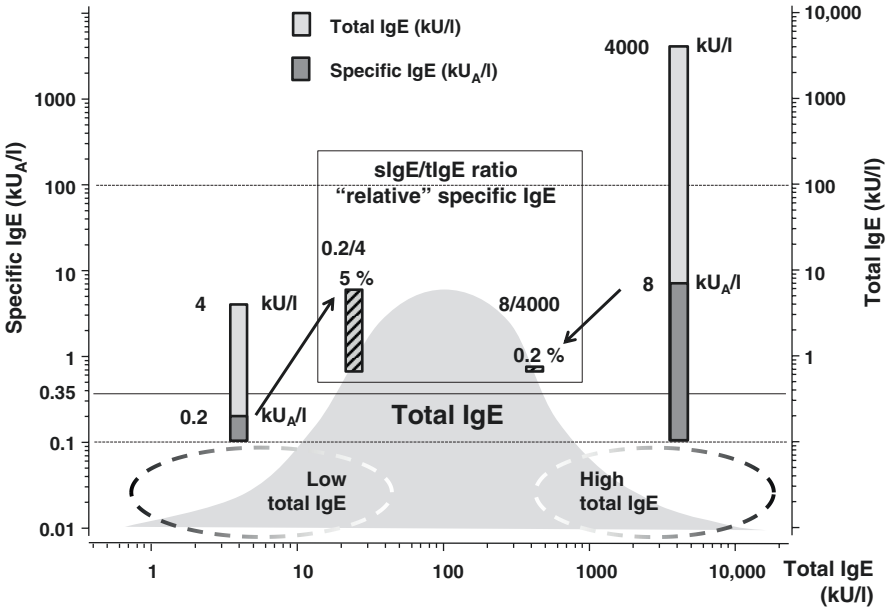

Fig. 7.5 Significance of the total and specific immunoglobulin E ratio. Due to the variability of total IgE levels, logarithmically distributed specific IgE (*dark gray bars*) can also be expressed as a relative quantity of total IgE (*light gray bars*) (Hamilton et al. 2010). This process "normalizes" specific IgE to total IgE on a percentage basis (*hatched bars*). Primarily the borderline cases (see numerical examples) with particularly low (normal distribution curve, far left) or extremely high total IgE (normal distribution curve, far right) make it clear that specific IgE can only be correctly interpreted once total IgE is known. This ratio of specific to total IgE is also found on the surface of effector cells (mast cells, basophil granulocytes), thereby providing the basis for diagnostic ex vivo (basophil activation test, BAT) and in vivo tests (skin prick test, provocation test)

Therefore, allergen molecules are predominantly used in recombinant form for molecular IgE diagnostics (Matricardi et al. 2016). This presupposes the selection of a representative isoform that determines as many (ideally all) specific IgE antibodies to the allergen in question. A further condition is correct protein folding that corresponds to the natural allergen. If both these prerequisites are fulfilled, the quality of this type of reagent can be considerably better controlled by means of process-integrated standardization.

7.3 Possible Applications for Allergen Molecules in IgE Diagnostics

Serological in vitro diagnosis can be modified in different ways using single allergens (⊙ Fig. 7.6):

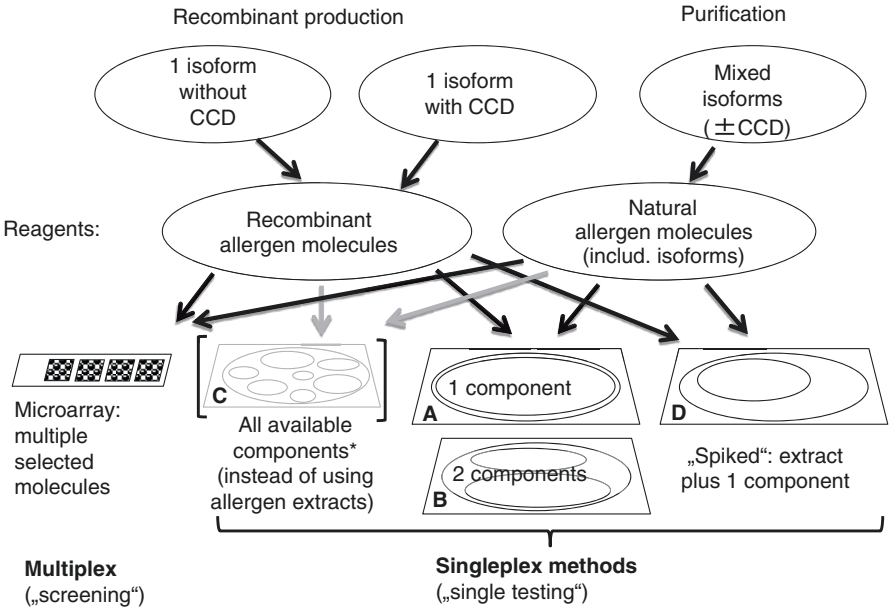

Fig. 7.6 Selection and application of allergen molecules for diagnostic purposes. *A*, *B*, *C*, and *D*: Variants of the test system for IgE singleplex assays (singleplex) using purified or recombinantly produced single allergens.

(a) Allergen molecules are used individually as reagents for specific IgE determination (currently the most frequent application).
(b) Selected allergens are combined as reagents for specific IgE determination (combination of important marker allergens, such as Ph1 p 1 and Ph1 p 5, or cross-reactive allergens such as Phl p 7 and Phl p 12).
(c) All available individual components of an allergen source can be used as a mix instead of a complex allergen extract (theoretically possible, but not yet implemented due to complexity, costs, and questionable use).
(d) Individual components can be added to allergen extracts ("spiking") to increase assay sensitivity (e.g., in the case of underrepresented components) (▸ Chap. 8).

Variant (a) enables the targeted and precise differentiation of sensitizations by means of single allergens. This procedure has also known as component-specific or component-resolved diagnostics (CRD) (Valenta et al. 1999) and currently plays the

largest role in molecular allergy diagnostics (typical case studies in Kleine-Tebbe and Jappe 2014).

7.3.1 Distinction Between Purified and Recombinantly Produced Components

An important crossroads for the manufacturers of diagnostic products is the decision whether to use purified natural single allergens with all their variants (isoforms) or whether to select a single, recombinantly produced protein.

The latter should be representative and have the major IgE binding sites in order to be able to cover, as far as possible, all allergy sufferers sensitized to this allergen molecule.

This problem does not apply when natural components are used, since these generally contain all molecule variants occurring in natural allergen sources. It is only important to ensure here that the preparations do not contain any impurities with other allergens. This is particularly challenging if the allergen to be purified is available in very small quantities in the allergen source, while other allergens are present in high concentrations. A typical example would be allergens in bee venom (Api m 3, Api m 5, and Api m 10), which are present at less than 1 % of the venom dry weight, while Api m 4 (melittin), with more than 40 % of the venom dry weight, render clean purification of the abovementioned allergens virtually impossible.

Another problem with purified natural allergens is encountered when glycoproteins with N-glycan sugar side chains are involved, which are recognized as cross-reactive carbohydrate determinants (CCD) by CCD-specific IgE, thereby falsifying results (▸ Chap. 6).

In contrast to the purification of allergens from natural sources, the recombinant production of allergens by selecting the appropriate expression system enables one to circumvent the problem of cross-reactive carbohydrate side chains (CCD). Thus, expressing allergens in *Escherichia coli* bacteria permits their production without CCD, while production in yeast cells or certain insect cells makes allergens with normal or modified carbohydrate side chains possible.

Due to patent protection, some manufacturers are not permitted to use recombinant allergen molecules, meaning that they are only able to offer purified allergens for molecular allergy diagnostics (⊙ Table 7.3 lists a selection of single allergens, commonly available test platforms, and manufacturers of diagnostic agents).

Table 7.3 A (by no means exhaustive) selection of single allergens for specific IgE diagnostics

Allergen source	Species	Allergen component	Suppliers in Germany				
			Euroimmun	Dr. Fooke Laboratorien	Omega Diagnostics	Siemens Healthcare	Thermo Fisher Phadia
			Test systems				
			Euroline	Allergo-o-liq	Allergozyme IgE	Immulite 2000	(a) ImmunoCAP[5] (b) ImmunoCAP ISAC112[6]
			Test principle and Internet information				
			Enzyme allergo sorbent strip test[1]	Reversed enzyme allergo sorbent test[2]	Enzyme allergo sorbent test[3]	Chemiluminescence enzyme immunoassay with allergens in fluid phase[4]	(a) Fluorescence enzyme allergo sorbent test[5] (b) Multiple fluorescence enzyme allergo sorbent test[6]
Tree pollen	Birch	Bet v 1 (major allergen)	r (DPA-Dx[a])	r (RT301)	n (x901)	n (A89)	r (t215)
		Bet v 2 (profilin)	r (DPA-Dx)	r (RT302)	n (x907)	r (A127)	r (t216)
	Ash/olive	Ole e 1 (major allergen)	–	r (RT901)	–	n (A482)	r (t224)
Grass pollen	Timothy grass	Phl p 1 (major allergen)	r (DPA-Dx)	r (RG601)	n (x903)	–	r (g205)
		Phl p 5 (major allergen)	r (DPA-Dx)	r (RG605)	n (x902)	–	r (g215)
		Phl p 7 (polcalcin)	r (DPA-Dx)	r (RG607)	–	–	r (g210)
		Phl p 12 (profilin)	r (DPA-Dx)	r (RG612)	–	–	r (g212) among others

Weed pollen	Mugwort	Art v 1 (major allergen)	–	r (RW601)	–	n (A753)	n (w231)
	Ragweed (*Ambrosia*)	Amb a 1 (major allergen)	–	n (NW101)	–	–	n (w230)
Rosaceae fruits	Apple	Mal d 1 (Bet v 1H)	–	r (RF491)	–	r (A464L2)	r (f434)
		Mal d 4 (profilin)	–	–	–	r (A796L2)	–
	Peach	Pru p 3 (LTP)	–	r (RF533)	–	n (A603L2)	r (f420)
	Cherry	Pru av 1 (Bet v 1H)	–	–	–	r (A597L2)	–
		Pru av 3 (LTP)	–	–	–	r (A599L2)	–
		Pru av 4 (profilin)	–	–	–	r (A600L2)	–
Tree nuts	Hazelnut	Cor a 1 (Bet v 1H)	–	r (RF171)	–	–	r (f428)
		Cor a 9 (11S legumin)	–	–	–	–	r (f440)
		Cor a 14 (2S albumin)	–	–	–	–	r (f439)
Pulses	Peanut	Ara h 1 (7S globulin)	r (f422) DPA-Dx[b]	n (NF131)	–		r (f422)
		Ara h 2 (2S albumin)	r (f423) DPA-Dx[b]	n (NF132)	–	–	r (f432)
		Ara h 3 (11S globulin)	r (f424) DPA-Dx[b]	–	–	–	r (f424)
		Ara h 9 (LTP)	r (f427) DPA-Dx[b]	r (RF139)	–	–	r (f427)

(continued)

Table 7.3 (continued)

Insect venom	Bee venom	Api m 1	r (i208) DPA-Dx[c]	r (RI101)	–	r (A45)	r(i208)
		Api m 2	r (i213) DPA-Dx[c]	r (RI102)	–	r (A46)	–
		Api m 10	r (i216) DPA-Dx[c]		–	–	r(i217)
	Wasp venom	Ves v 1	r (i211) DPA-Dx[c]	–	–	r (A668)	r(i211)
		Ves v 5	r (i209) DPA-Dx[c]	r (RI305)	–	r (A670)	r(i209)

r recombinant component, *n* natural component, purified from extracts, *in parentheses* company-specific laboratory codes
Bet v 1H Bet v 1 homologous PR-10 protein, *2S albumin* storage protein, *11S legumin* storage protein
[a]DPA-Dx: Defined partial allergen diagnostics, panel strip test (DP 3210-1601-1 E) with Bet v 4 and Bet v 6, as well as birch pollen and timothy grass extract
[b]Panel strip test for peanut sensitizations (DPA-Dx Peanut 1) with rAra h 1, 2, 3, 5, 6, 7, 9, rBet v 1 and carbohydrate side chain reagent (CCD marker)
[c]Panel strip test for insect venom sensitizations (DP 3850-1601-2 E) with Api m 1, 2, and 10 and Ves v 1 and 5, bee venom and wasp venom extracts, and CCD marker
Internet information on test principles
[1]http://www.euroimmun.com/produkte/indikationen/allergologie/molekulare-allergologie.html
[2]http://www.fooke-labs.com/grobritannien-uk/downloadbereich/index.php
[3]http://www.omegadiagnostics.de/products/by-area/allergozyme-specific-ige/ (only in Germany)
[4]http://healthcare.siemens.com/clinical-specialities/allergy/laboratorian-information
[5]http://www.phadia.com/en-US/Products/Products/ImmunoCAP-Assays/ImmunoCAP-Specific-IgE/Test-Principle-ImmunoCAP-Specific-IgE/
[6]http://www.phadia.com/en-US/Products/Products/ImmunoCAP-ISAC/Test-Principle-ImmunoCAP-ISAC/

7.3.2 Laboratory-Scale Evaluation: Assay Sensitivity and Analytical Specificity (Selectivity)

Test method efficacy is investigated on an international basis using the variables "sensitivity and specificity". As part of this process, a distinction is made between two pairs of definitions: analytical and diagnostic sensitivity and specificity.

Definition of Terms Used to Measure Test Method Efficacy

Analytical sensitivity is defined as the slope of an (immuno)assay's calibration curve. The *actual sensitivity* (*lower detection limit*) of a test, on the other hand, is determined and expressed today using the following variables (Armbruster and Pry 2008):

- Limit of blank (LoB)
- Limit of detection (LoD)
- Limit of quantitation (LoQ)

LoB: LoB is defined as the highest test signal obtained from repeated blank measurements (serum sample without IgE): $\text{LoB} = \text{Mean}_{\text{Blank}} + 1.645\ (\text{SD}_{\text{blank}})$.

LoD: LoD refers to the weakest signal or lowest concentration of specific IgE antibodies reliably determined from the test: $\text{LoD} = \text{LoB} + 1.645\ (\text{SD}_{\text{lowest concentration sample}})$.

LoQ: LoQ is the lowest concentration of specific IgE antibodies that can be reliably detected within a predefined range. LoQ may be equivalent to or higher than LoD.

These definitions have been introduced in international laboratory guidelines (Hamilton et al. 2016) for IgE determination methods and are particularly important when single allergens are used (Matricardi et al. 2016).

Assay sensitivity is often enhanced (i.e., LoQ is lower) when using allergen molecules, particularly if these allergens are underrepresented in the natural extract or are entirely absent due to their instability. Greater assay sensitivity (lower LoQ) is thus an important argument in support of the use of allergen molecules for the diagnosis of specific IgE (⊙ Fig. 7.7 and ⊙ Table 7.4).

Example

Sensitization to wheat extract is found in only 20–30 % of patients with wheat-dependent exercise-induced anaphylaxis (WDEIA), while sIgE to Tri a 19 (ω-5 gliadin) is detected in 80–90 % of cases. Since the gliadins responsible for WDEIA are not water soluble, they are not present in sufficient quantities in

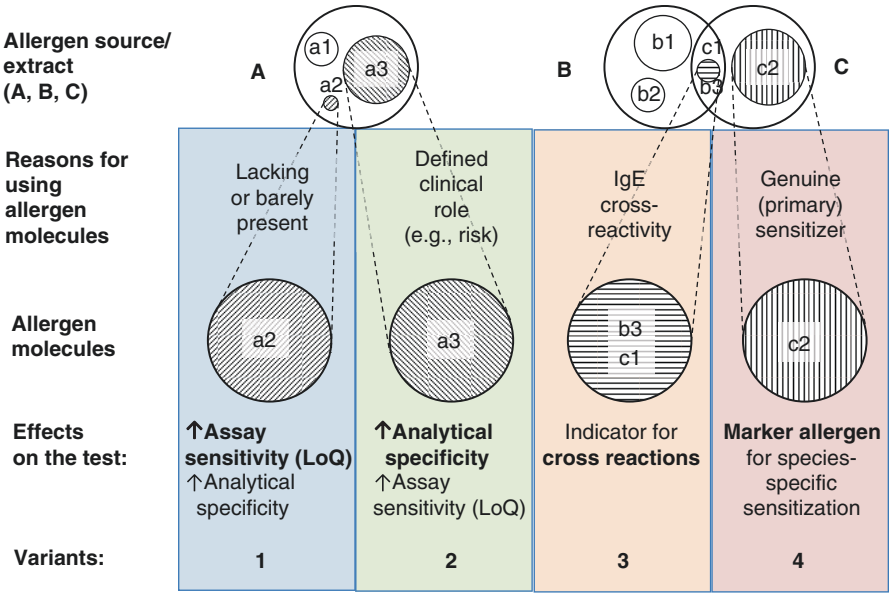

Fig. 7.7 Methodological rationales for molecular allergy diagnostics. The variants *1–4* shown in the figure (see also ⊙ Table 7.4) reflect universal arguments for the methodological use of allergen molecules. They move (virtually) exclusively on the test (sensitization) level, irrespective of the patient's clinical status. Thus, they improve only the sensitization test per se, without affecting clinical test findings/interpretation, which must always be performed by the treating physician (or person requesting the test) based on clinical information provided by the patient (history/provocation) on a case-by-case basis (Adapted from Hamilton et al. 2016)

Table 7.4 Improved test characteristics due to using defined allergen molecules for specific immunoglobulin E (IgE) determination in a singleplex assay (see also ⊙ Fig. 7.7 for variants)

Variants	1	2	3	4
Examples (allergen source, allergen carrier)	Greater assay sensitivity due to lower limit of quantitation (LoQ)	Improved analytical specificity (selectivity)	Cross-reactive allergens	Species/ family-specific marker allergens
Cat		Fel d 2	Fel d 2	Fel d 1
Hazelnut	Cor a 1 (Bet v 1 homolog)	Cor a 14 (2S albumin) Cor a 9 (11S globulin) Cor a 8 (LTP, Mediterranean region)		
Kiwi	Act d 8 (Bet v 1 homolog)		Act d 8 (Bet v 1 homolog)	
Peach	Pru p 1 (Bet v 1 homolog)	Pru p 3 (LTP, marker, Mediterranean region)	Pru p 1 (Bet v 1 homolog) Pru p 4 (profilin)	

Table 7.4 (continued)

Variants	1	2	3	4
Examples (allergen source, allergen carrier)	Greater assay sensitivity due to lower limit of quantitation (LoQ)	Improved analytical specificity (selectivity)	Cross-reactive allergens	Species/ family-specific marker allergens
Peanut	Ara h 10 Ara h 11 (oleosins)	Ara h 1 (7S globulin) Ara h 2 (2S albumin) Ara h 3 (11S globulin) Ara h 6/7 (2S albumin) Ara h 9 (LTP, Mediterranean region)	Ara h 8 (Bet v 1 homolog) Ara h 5[a]	
Soy	Gly m 4 (Bet v 1 homolog)	Gly m 5 Gly m 6		
Wheat	Tri a 19 (ω-5 gliadin)			
Meat	α-GAL	α-GAL		
Bee venom	Api m 3 Api m 4 Api m 10	Api m 1 Api m 3 Api m 4 Api m 10		Api m 1 Api m 3 Api m 4 Api m 10
Wasp venom	Ves v 5	Ves v 1 Ves v 5		Ves v 1 Ves v 5
Birch (hazel, alder, birch pollen) and beech family (beech, oak pollen)		Bet v 1	Bet v 2[a] Bet v 4[b]	Bet v 1
Oleaceae (ash, olive pollen)		Ole e 1	Ole e 2[a] Ole e 3[b]	Ole e 1
Poaceae (sweet grass pollen)		Phl p 1 Phl p 5	Phl p 12[a] Phl p 7[b]	Phl p 1 Phl p 5
Mugwort pollen		Art v 1	Art v 4[a] Art v 5[b]	Art v 1
Ragweed pollen		Amb a 1	Amb a 8[a] Amb a 10[b]	Amb a 1

Adapted from Hamilton et al. (2016)

The benefit of allergen molecules as diagnostic reagents from different allergen sources/extracts (left column), the rationales, and potentially improved test characteristics (top line) vary and depend on the individual diagnostic question and the specific allergen used

[a]Profilin family members: widespread, highly conserved, and extremely cross-reactive panallergens in pollen and plant-based foods

[b]Polcalcin family members (Ca^{++} – binding proteins): widespread, highly conserved, and extremely cross-reactive panallergens in pollen

aqueous wheat extracts. This problem can be avoided by using recombinantly produced Tri a 19 in the test system.

The *analytical specificity* of an IgE test method can relate to the specificity of the antibody class on the one hand, whereby the test effectively determines IgE and no antibodies of other classes, such as IgA, IgD, IgG, or IgM (Hamilton et al. 2016).

On the other hand, analytical specificity can relate to a more targeted, more "selective" IgE determination of particular allergen molecules: Whereas an allergen extract, as a complex protein mixture, determines the total IgE repertoire to an allergen source, only a proportion of specific antibodies are determined when using allergen molecules, thereby increasing analytical specificity (selectivity) (Matricardi et al. 2016).

This permits more targeted (more analytically specific) detection or exclusion of sensitization particularly in the case of allergen molecules with special characteristics—such as high stability and a relatively high proportion of total protein (e.g., Ara h 2 or Cor a 14) and thus an increased risk for severe reactions to food (peanut or hazelnut).

Example

More than 10 % of German children and adolescents show specific IgE to peanut extract—caused primarily by pollen-associated cross-reactions. Diagnosis using the stable and risk-related peanut storage protein, Ara h 2, yields elevated values in only a fraction of patients (approx. maximum 0.4 %, Kirsten Beyer, personal communication), thereby affording greater analytical specificity (selectivity) compared with peanut extract.

7.3.3 Universal Arguments for the Use of Molecular Allergens in IgE Diagnostics

Four arguments generally provide plausible support for the use of single allergens (⊙ Table 7.5). In this context, particularly the improved assay sensitivity (LoQ) and the increased analytical specificity mentioned above help to justify the use of allergen molecules (⊙ Fig. 7.7 and examples in ⊙ Table 7.4) (Hamilton et al. 2016; Matricardi et al. 2016):

1. Provided that allergen molecules (e.g., when present in insufficient proportions or absent in the extract) increase the assay sensitivity (LoQ) of IgE determination, their use is both useful and important.
2. Provided that allergen molecules permit improved analytical specificity (selectivity) by binding a partial amount of the specific IgE repertoire, as well as additional clinical findings (e.g., increased burden of risk, degree of clinical severity, other associated clinical characteristics), their use is, once again, useful and recommended from a diagnostic perspective.
3. Certain allergen molecules, by binding cross-reactive IgE antibodies, serve as an indicator for cross-sensitizations. In the case of positive results, they indirectly

Table 7.5 General criteria for optimizing tests and universal arguments to support the use of allergen molecules in specific immunoglobulin E (IgE) determination

	Analytical criteria (for possible test optimization)		Clinical criteria (potential clinical advantages)
1	**Assay sensitivity ↑** **Limit of quantitation (LoQ) ↓**	I	*Diagnostic sensitivity ↑*
2	**Analytical specificity ↑**	II	*Diagnostic specificity ↑*
3	**Indicator for serological cross-reactivity**	III	*Indicator for clinical cross-reactivity*
4	**Marker for primary/genuine sensitizations**	IV	*Prediction of clinically relevant reactions (PPV, NPV)*

Diagnostic methods in allergology can be evaluated analytically, i.e., on the test level (left column) and clinically (right column). The use of allergen molecules for IgE determination primarily improves the analytical criteria (1–4). Using single allergens frequently alters several criteria/variables

To what extent single allergens can optimize diagnostic/clinical criteria (right column, I–IV) depends on the cohort investigated, the single allergens in question, and the study endpoints selected. In general, clinical criteria are based on the individual interpretation of test results on the basis of clinical history and, where appropriate, reproducible symptoms in the affected allergy sufferer. Thus, they go beyond the actual results of allergen-specific IgE tests (sensitization, yes or no). Diagnostic/clinical criteria (right column) are therefore:

Less suited to the evaluation of sensitization tests (hence the italic font)
Often not at all necessary to demonstrate the benefits of single allergens
Fraught with unsatisfactory results due to their limited ability to predict clinical results

illustrate the lack of analytical specificity of IgE tests against allergen extracts (in affected individuals with potential cross-reactions).

4. Depending on findings, particular allergen molecules are suitable as protein family or species-specific IgE-binding marker allergens to detect or exclude genuine ("primary") sensitization.

It should be noted here that all the abovementioned arguments relate primarily to sensitization and do not take the clinical status of the patient into consideration. Examples of and indications for the detection of specific sensitization using single allergens are listed in ⊙ Table 7.6.

7.4 Clinical Evaluation: Diagnostic Sensitivity and Specificity

Diagnostic sensitivity and specificity relate to the symptoms of the affected allergy sufferer. A precondition to assessing and calculating these is unequivocal clinical information from the patient or, in case of doubt, additional provocation tests to confirm the clinical diagnosis (⊙ Table 7.5, right column).

However, allergen-specific IgE diagnostics only cover sensitization (susceptibility to allergy) and cannot per se predict the clinical reaction (Hamilton et al. 2016; Matricardi et al. 2016; Renz et al. 2010). Therefore, concordant results (positive history and positive specific IgE), for instance, are often referred to as *clinically relevant* (instead of correctly positive). The same applies to concordant negative results that

Table 7.6 Examples for the use of single allergens to detect sensitization: typical clinical questions in the case of suspected inhalant, food, and insect venom allergy. Potential advantages of using allergen molecules for IgE singleplex determinations and the significance of results

			Diagnostic advantages compared with extracts					Significance of results		
Indication	Diagnostic question	Allergen	Assay sensitivity	Analytical specificity	Indicator for cross-reactivity	Marker for primary sensitization	Allergen source	Positive	Negative	Comments
Suspected inhalant allergy (aeroallergens)										
Tree pollen sensitization?	Suspicion/ exclusion of sensitization to birch, hazel, alder, birch, and oak	Bet v 1	?	↑	(+)	+	Birch pollen extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, other tree pollen responsible, e.g., ash?	Cross-reactivity to all Fagales pollen and potentially to Bet v 1 cross-reactive foods (e.g., pome and stone fruits, nuts, carrots, celery, and soy) present
Ash or Olive pollen sensitization?	Suspicion/ exclusion of sensitization to ash and olive tree pollen	Ole e 1	?	↑	(+)	(+)	Olive pollen extract	Suspicion of olive/ash pollen sensitization confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, other tree pollens responsible, e.g., birch, oak, beech?	Cross-reactivity to pollen in the Oleaceae family (olive pollen, ash pollen) present

Grass pollen sensitization?	Suspicion/ exclusion of sensitization to grass pollen	Phl p 1 and Phl p 5	↑	↑	(+)	+	Timothy grass pollen extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, other seasonal allergens responsible, e.g., *Alternaria* mold or simultaneously flowering weed pollen?	Cross-reactivity to all sweet grasses present
Mugwort pollen sensitization?	Suspicion/ exclusion of sensitization to mugwort pollen	Art v 1	?	↑	(+)	+	Mugwort pollen extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, other seasonal allergen sources responsible, e.g., *Alternaria*?	Cross-reactivity possible to foods with Art v 1 homologous allergens
Ragweed pollen sensitization?	Suspicion/ exclusion of sensitization to ragweed pollen	Amb a 1	?	↑	–	+	Ragweed pollen extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, other weed pollens responsible, e.g., mugwort?	

(continued)

Table 7.6 (continued)

Indication	Diagnostic question	Allergen	Diagnostic advantages compared with extracts				Allergen source	Significance of results		Comments
			Assay sensitivity	Analytical specificity	Indicator for cross-reactivity	Marker for primary sensitization		Positive	Negative	
Sensitization to pollen panallergen profilin	Suspicion/ exclusion of sensitization to universal pollen and food allergen profilin (e.g., in the case of numerous reactions to pollen extracts in skin tests)?	Phl p 12 or Bet v 2	↑	↑	++	–	Grass pollen or birch pollen	Suspicion confirmed; profilin sensitization evidently possible cause of multiple reactions to pollen extracts: essential to ask about oropharyngeal symptoms elicited by profilin-containing foods	Suspicion excluded	Cross-reactivity to all pollens (tree, grass, weed) and numerous plant-based food allergens present
Sensitization to pollen panallergen polcalcin	Suspicion/ exclusion of sensitization to universal pollen allergen polcalcin (e.g., in the case of numerous reactions to pollen extracts in skin tests)?	Phl p 7 or Bet v 4	↑	↑	++	–	Grass pollen or birch pollen	Suspicion confirmed; polcalcin sensitization evidently possible cause of multiple reactions to pollen extracts	Suspicion excluded	Cross-reactivity to all pollens (tree, grass, weed) present

Alternaria sensitization?	Suspicion/ exclusion of sensitization to seasonal mold *Alternaria*	Alt a 1	?	↑	–	+	*Alternaria* extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, other seasonal allergen responsible, e.g., grass pollen or simultaneously flowering weed pollen (mugwort, ribwort, goosefoot)?	Cross-reactivity to all sweet grasses present
House dust mite sensitization?	Suspicion/ exclusion of sensitization to house dust mites *Dermatophagoides pter./far.*	Der p 1 and Der p 2 or Der f 1 and Der f 2	?	↑	(+)	+	House dust mite extract from *Dermatophagoides pter.* or *Dermatophagoides far.*	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion of house dust mite sensitization largely excluded; in rare cases, other major allergens, e.g., Der p 23, are responsible for sensitization	No confirmed advantages over extract diagnostics; distinction between *D. pter* and *D. far.* not possible
Cat sensitization?	Suspicion/ exclusion of sensitization to cats	Fel d 1	?	↑	–	+	Cat fur extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, are other perennial allergens relevant?	

(continued)

Table 7.6 (continued)

Indication	Diagnostic question	Allergen	Diagnostic advantages compared with extracts				Allergen source	Significance of results		Comments
			Assay sensitivity	Analytical specificity	Indicator for cross-reactivity	Marker for primary sensitization		Positive	Negative	
Dog sensitization	Suspicion/ exclusion of sensitization to dog	Can f 1	?	↑	–	+	Dog hair extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, are other perennial allergens relevant?	
Suspected food allergy (marker/Cross-reactive allergen)										
Birch pollen-associated food cross-reactivity?	Suspicion/ exclusion of sensitization to cross-reactive plant-based foods (pome and stone fruits, hazelnuts, carrots, celery, soy)	Bet v 1	↑	↑	+	(+)	Birch pollen extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms to the relevant, potentially cross-reactive plant-based food (▶ Chap. 2)	Suspicion excluded, are other cross-reactive allergens in plant-based foods relevant, e.g., profilin or LTP?	Well-suited marker for Bet v 1-associated cross-reactivity; better than commercial fruit, nut, and vegetable extracts

Profilin-associated food cross-reactivity?	Suspicion/ exclusion of sensitization to cross-reactive plant-based foods (fruit including rosaceae fruits, melon, citrus fruits, berries, nuts, vegetables, pulses)	Bet v 2 or Phl p 12	↑	↑	+	(+)	Birch pollen extract or grass pollen extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms to the relevant, potentially cross-reactive plant-based food (▶ Chap. 3)	Suspicion excluded, are other cross-reactive allergens in plant-based foods relevant, e.g., due to Bet v 1 or LTP sensitization?	Potential marker for profilin-associated cross-reactivity; more suitable than commercial fruit, nut, vegetable, pulse extracts
LTP-related food sensitization/ cross-reactivity?	Suspicion/ exclusion of sensitization to cross-reactive plant-based foods (fruit including rosaceae fruits, grapes, citrus fruits, berries, nuts, vegetables, pulses)	Pru p 3	↑	↑	+	(+)	Peach extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms to the relevant, potentially cross-reactive plant-based food (▶ Chap. 4)	Suspicion excluded, are other cross-reactive allergens in plant-based foods relevant, e.g., Bet v 1 or profilin?	Marker for LTP-related cross-reactivity; more suitable than fruit, nut, vegetable, and pulse extracts

(continued)

Table 7.6 (continued)

Indication	Diagnostic question	Allergen	Diagnostic advantages compared with extracts				Allergen source	Significance of results		Comments
			Assay sensitivity	Analytical specificity	Indicator for cross-reactivity	Marker for primary sensitization		Positive	Negative	
Wheat-dependent exercise-induced anaphylaxis?	Suspicion/ exclusion of sensitization to wheat protein due to anaphylactic reactions to physical exertion?	Tri a 19	↑↑	↑	–	+	Wheat extract	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms to wheat-containing foods in conjunction with physical exertion	Suspicion excluded, other potential wheat allergens (Tria a 14?) or food allergens (LTP?) relevant?	Marker for ω-5-gliadin-specific wheat sensitization
Risk-related peanut sensitization?	Suspicion/ exclusion of sensitization to peanut proteins based on anaphylactic reactions in the previous history?	Ara h 2, (Ara h 6), Ara h 1, Ara h 3	↑	↑	–	+	Peanut	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, are other potential peanut allergens (Ara h 6, oleosins?) or panallergens (LTP?) relevant?	Marker for primary, genuine peanut allergy (assuming IgE levels to other storage proteins are significantly lower); marker for hazardous systemic reactions

Risk-related soy sensitization?	Suspicion/ exclusion of sensitization to soy protein based on anaphylactic reactions in the previous history?	Gly m 5, Gly m 6, Gly m 8 (2017)	↑	↑	–	+	Soybean	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, are other potential soy allergens (Gly m 4? oleosins?) or panallergens (LTP?) relevant?	Marker for primary, genuine soy allergy (assuming IgE levels to other storage proteins are significantly lower); marker for hazardous systemic reactions
Risk-related hazelnut sensitization?	Suspicion/ exclusion of sensitization to hazelnut proteins based on systemic/ anaphylactic reactions in the previous history?	Cor a 14, Cor a 9	↑	↑	–	+	Hazelnut	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, are other potential hazelnut allergens (Cor a 11?) or panallergens (LTP) relevant?	Marker for primary, genuine hazelnut allergy (assuming IgE levels to other storage proteins are significantly lower); marker for hazardous systemic reactions

(continued)

Table 7.6 (continued)

Indication	Diagnostic question	Allergen	Diagnostic advantages compared with extracts				Allergen source	Significance of results		Comments
			Assay sensitivity	Analytical specificity	Indicator for cross-reactivity	Marker for primary sensitization		Positive	Negative	
Risk-related nut sensitization?	Suspicion/ exclusion of sensitization to: (a) Walnut proteins (b) Brazil nut proteins (c) Cashew kernel proteins based on systemic/ anaphylactic reactions in the previous history?	(a) Jug r 1, Jug r 2 (b) Ber e 1 (c) Ana o 2	↑	↑	–	+	(a) Walnut (b) Brazil nut (c) Cashew kernel	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion excluded, are other potential storage proteins or panallergens (LTP) relevant?	Marker for primary, genuine nut allergy (assuming IgE levels to other storage proteins are significantly lower); marker for hazardous systemic reactions
Suspected insect venom allergy (insect venom allergens)										
Bee venom sensitization?	Suspicion/ exclusion of sensitization to bee venom proteins based on systemic/ anaphylactic reactions in the previous history?	Api m 1, Api m 3, Api m 4, Api m 10,	↑	↑	–	+	Bee venom	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Are other been venom proteins (Api m 2, Api m 3, Api m 4, Api m 5, and Api m 10) relevant?	Marker for genuine, primary been venom sensitization

Wasp venom sensitization?	Suspicion/ exclusion of sensitization to wasp venom proteins based on systemic/ anaphylactic reactions in the previous history?	Ves v 1, Ves v 5	↑	↑	–	+	Wasp venom	Suspicion confirmed; clinically relevant only in the presence of corresponding symptoms	Suspicion largely excluded	Marker for genuine, primary wasp venom sensitization

? Effect on the test statistic (e.g., assay sensitivity) not unequivocally elucidated
↑ Increases the relevant performance (e.g., assay sensitivity, analytical specificity)
+ Positive differentiation (e.g., as an indicator for cross-reactivity or marker of primary sensitization)
(+) Limited advantages in terms of differentiation (cross-reactivity or primary sensitization)
– No advantages in terms of differentiation (cross-reactivity or primary sensitization)

exclude allergy, and thereby also an underlying sensitization. A positive IgE finding combined with a negative history is often classified as *clinically irrelevant* (instead of false positive). Declaring clinically irrelevant results as false positive does not go to the core of the matter, since ultimately the test result, i.e., the allergen-specific IgE that is present, can very well be valid and cannot be questioned.

A number of clinical studies have investigated the diagnostic sensitivity and specificity of individual allergens from one allergen source (selection in ⊙ Table 7.7). By increasing assay sensitivity (low LoQ), absent or underrepresented allergens were also able to significantly increase diagnostic sensitivity. However, increased sensitizations were reported parallel to this, even in individuals with no clinically relevant reactions.

Table 7.7 Examples of the successful clinical validation of molecular allergy diagnostics (plant allergen sources) (Kleine-Tebbe and Jappe 2013)

Allergen source	Allergens	Comments	References
Hazelnut	rCor a 1.04 rCor a 2 rCor a 8 nCor a 9 rCor a 11	Clinical evaluation of component-specific diagnostics in hazelnut-allergic individuals from various regions (Denmark, Switzerland, and Spain); diagnosis partially confirmed by controlled oral challenge, additional cohorts with pollen allergy, and non-atopics; overall heterogeneous sensitization profiles depending on the region investigated	Hansen et al. (2009)
Carrot	rDau c 1.0104 rDau c 1.0201 rDau c 4 rDau c IFR 1 rDau c IFR 2 rDau c Cyc	(a) Clinical evaluation of three carrot allergens in carrot-allergic individuals (confirmed as such by oral challenge) compared with birch pollen-allergic individuals with no carrot allergy or non-atopic controls (b) Clinical evaluation of component-specific diagnostics in carrot-allergic individuals from various regions (Denmark, Switzerland, and Spain); diagnosis partially confirmed by controlled oral challenge, additional cohorts with pollen allergy, and non-atopics; overall heterogeneous sensitization profiles depending on the region investigated	Ballmer-Weber et al. (2005, 2012)
Cherry	rPru av 1 rPru av 3 rPru av 4	Clinical evaluation of component-specific diagnostics in cherry-allergic individuals from Central and Southern Europe (Spain); diagnosis partially confirmed by controlled oral challenge, additional cohorts with pollen allergy, and non-atopics; heterogeneous sensitization profiles depending on the region investigated and clear superiority of single allergens compared with extract-based diagnostics (skin prick test; specific IgE with cherry extracts)	Reuter et al. (2006)

Table 7.7 (continued)

Allergen source	Allergens	Comments	References
Celery	rApi g 1.01 rApi g 4 nApi g 5	Clinical evaluation of component-specific diagnostics in celery-allergic individuals; diagnosis confirmed by controlled oral challenge, additional cohorts with pollen allergy, and non-atopics; clear superiority of single allergens compared with extract-based diagnostics; nApi g 5-specific IgE is targeted predominantly against CCD	Ballmer-Weber et al. (2000), Bauermeister et al. (2009)

The interdependence between diagnostic sensitivity and specificity is a fundamental problem in testing and is often represented in "receiver operating characteristic" (ROC) curves (⊙ Fig. 7.8). Better diagnostic sensitivity and specificity for the risk assessment of severe clinical reactions has been described for some single allergens, such as Ara h 2 or other high-risk allergens from the 2S-albumin group of storage proteins (overview in Lange et al. 2014). Moreover, predictive specific IgE decision points for positive or negative oral challenge in children with suspected peanut or hazelnut allergy have been defined with the help of risk-related 2S albumins [Beyer et al. (2015); see also ▶ Chaps. 11 and 12].

A clinical reaction (or absence thereof) can never be predicted in a foolproof manner (to 100 %) using sensitization tests such as IgE determination (Beyer et al. 2015). Therefore, methodological arguments first need to be considered for future assessments of the diagnostic suitability of allergen molecules (⊙ Table 7.5, left column). Even without a complete clinical evaluation (including diagnostic sensitivity and specificity, as well as predictive value, ⊙ Table 7.5, right column, study examples in ⊙ Table 7.7), the analytical test's characteristics of IgE diagnostics using allergen molecules are, in many cases, significantly better compared with allergen extracts (▶ Sect. 7.3.3) (Matricardi et al. 2016). This viewpoint is reflected in the updated international laboratory guidelines on IgE test methods (Hamilton et al. 2016) and should serve to ease and accelerate the evaluation and introduction of allergen molecules for diagnostic purposes in the future.

7.5 Interpretation to Establish Clinical Relevance

Ultimately, the central question relates to the clinical relevance of the specific IgE concentrations obtained:

- The following basic rule still applies: a *positive specific IgE result* is consistent with a sensitization that is only clinically relevant in the presence of corresponding symptoms.
- A *negative specific IgE result* (e.g., to an allergen molecule or a mixture of natural isoforms of a single allergen) largely excludes allergic sensitization to the tested allergen, however, only if:
  - Total IgE is sufficiently high.
  - The allergen is available intact and in adequate quantities.

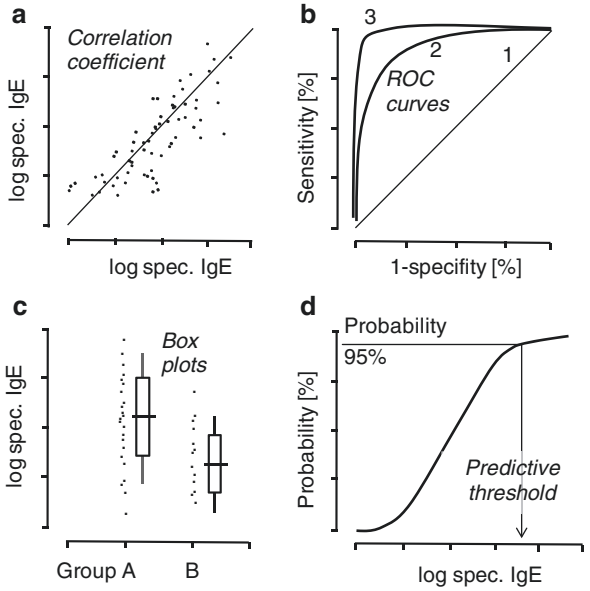

Fig. 7.8 Typical data analysis of IgE laboratory results (e.g., with allergen molecules). (**a**) Concordance between logarithmically distributed specific IgE levels (e.g., to a natural or recombinant allergen molecules). (**b**) Diagnostic efficacy (*ROC* receiver operating curves) when using allergens (allergen molecules). The *line* (*1*) represents a test situation in which diagnostic sensitivity (*y*-axis) and specificity (*x*-axis: 1 specificity) are equally inadequate (corresponding to a pure chance level = "toss of a coin"); the *line* (*2*) and more so the *line* (*3*) represent tests with better diagnostic sensitivity and specificity. A right-angled ROC curve (=100 % diagnostic efficacy) that closely follows the top left corner would be ideal. (**c**) Individual values and median values with 25 and 75 percentiles in the comparison of groups. (**d**) Cut-off values (threshold levels) for the prediction of clinical reactions (e.g., 95 % probability of a positive challenge test)

– The analytical assay sensitivity of the IgE determination method has been optimized and is correspondingly high.

Finally, irrespective of whether allergen extracts or molecules are used for diagnostic purposes, only a physician can determine the clinical relevance of an allergic sensitization, not the test (Kleine-Tebbe and Jakob 2015).

Therefore, all diagnostic findings from sensitization tests—and that applies equally to allergen molecules—need to be evaluated in the clinical context and in conjunction with the individual patient's previous history.

7.6 Potential and Quantitative Concepts of Molecular Allergology

Diagnostic methods using single allergens (Matricardi et al. 2016) open up new opportunities to differentiate the IgE response to certain allergen sources. Some marker allergens are characteristic of certain allergen sources and enable their

unequivocal classification. These triggers of genuine, primary sensitization are also referred to as species-specific allergens and can be used as "markers" for certain allergen sources (⊙ Tables 7.4 and 7.6). Thus, in most parts of Europe, for example, it is possible to reliably detect sensitizations to pollen using marker allergens and to exclude potential cross-reactions.

This is particularly useful in the case of additional sensitizations to panallergens from the polcalcin and profilin families (▶ Chap. 3), in order to reestablish the analytical specificity of exclusively extract-based diagnosis that is otherwise inadequate in this setting. Polcalcins and profilins are present in a wide variety of allergen sources and, due to their high structural similarity, are responsible for marked cross-reactions. Although rarely of clinical relevance, they complicate specific diagnosis when extracts alone are used, since the latter contain both markers and cross-reactive allergens.

As part of the test interpretation, primary sensitization in the case of a series of positive IgE results can be deduced from the level of IgE concentrations:

The primary sensitizing allergen has the most epitopes recognized by specific IgE antibodies. In contrast, the number of cross-reactive epitopes of structurally related, similar protein allergens is often lower or of lower affinity.

The following rule of thumb applies: The highest IgE antibody level to a protein compared with other members of the same protein family likely reveals the primary sensitizer.

7.6.1 The Use of Singleplex IgE Assays in Bet v 1-Related Cross-Reactivity

A classic example of this is the PR-10 protein family, in the case of which primary birch pollen sensitization is evidenced by high Bet v 1-specific IgE levels, while Bet v 1-related secondary pollen or food sensitizations are reflected in low IgE values to the relevant Bet v 1 homologous PR-10 proteins (⊙ Fig. 7.9b). Moreover, the structural relationship between allergens in a family can be indirectly ascertained from the level of specific IgE (⊙ Fig. 7.9).

7.6.2 The Use of Singleplex IgE Assays in Profilin Sensitization

In the case of strong structural similarity and marked cross-reactivity within an allergen family, one can expect comparable specific IgE levels to the individual proteins, as observed with profilins, for example (⊙ Fig. 7.9c). Determining IgE to profilins from different allergen sources is unlikely to bring any benefit here. A single IgE measurement, e.g., to grass pollen profilin Phl p 12 or birch pollen profilin Bet v 2, is sufficient. Other profilin sources could also come into question, e.g., latex or annual mercury (Mer a 1, only in multiplex ImmunoCAP ISAC). It is possible to establish the clinical relevance of IgE sensitization by means of detailed patient interviews, e.g., potential symptoms induced by botanically unrelated pollen plants or reactions to plant-based foods that, in particular, do not belong to the Bet v 1 cluster, e.g., melon and banana, as well as exotic and citrus fruits (Santos and van Ree 2011).

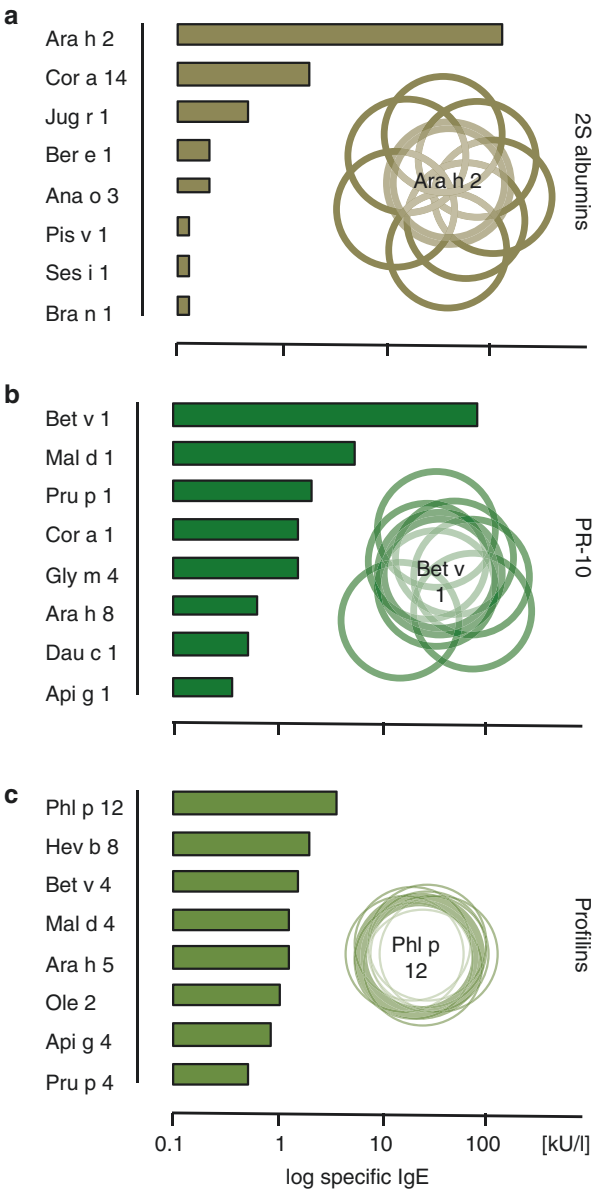

Fig. 7.9 Immunoglobulin E (IgE) levels to allergen molecules depending on structural similarity within an allergen family. (**a**) Variable, limited cross-reactivity between 2S albumins (stable storage proteins in nuts, pulses, and seeds). (**b**) Variable cross-reactivity between Bet v 1 homologous food allergens. (**c**) High cross-reactivity due to the strongly preserved and similar structure of profilins (in pollen, latex, and foods)

7.6.3 The Use of Singleplex IgE Assays Against Storage Proteins

IgE levels against members of the same protein family can vary significantly in the case of low structural similarity and correspondingly low cross-reactivity, as can be seen with the example of storage proteins (⊙ Fig. 7.9a).

Although the typical basic structure of storage proteins, i.e.,:

- 2S albumins
- 7S globulins
- 11S globulins

from different allergen sources—such as legumes (peanut, soybean), tree nuts (hazel and walnut), and seeds—is similar, only partially cross-reactive, potential IgE-binding epitopes are present. As a result, a complex pattern of possible cross-reactivities emerges, depending on the individual IgE repertoire. The IgE response to one storage protein (e.g., Ara h 3 from peanut) does not permit an assessment of IgE reactivity to other members of the 11S globulin family (e.g., Gly m 6 from soybean or Cor a 9 from hazelnut). Thus, strictly speaking, the sensitization pattern to storage proteins can only be determined by using all available proteins from this storage protein family. Unfortunately, not all members of these stable allergens from tree nuts, capsule and stone fruits, as well as seeds are as yet available, meaning that gaps remain in our diagnostic potential for the time being.

As a result, the highest IgE level to a particular storage protein (e.g., Ara h 2 from the 2S albumin group) likely reveals the primary source of sensitization (e.g., peanut). Lower levels, e.g., to corresponding soy (2S albumin Gly m 8) or hazel nut allergens (2S albumin Cor a 14) signal potential IgE cross-reactivity. However, their clinical relevance and the associated risk of reactions following consumption of the respective allergen source cannot be established from the level of specific IgE, but needs instead to be conclusively established by the patient's history or provocation tests.

Higher than expected IgE levels (to a food protein investigated as a secondary allergen source) raise doubts about the suspected primary allergen source and should be carefully investigated for plausibility.

Only when the corresponding proteins from the same protein family yield wholly negative IgE values can one assume that serological cross-reactivity is absent and that no clinical (cross-)reactions are to be expected.

Thus, a negative result is particularly important for the exclusion of an allergic (cross-)reaction.

It is here that the current limitations of molecular allergy diagnostics become apparent, since a structural relationship between allergens, depending on individual IgE repertoires, can determine highly variable cross-reactivities: from completely absent to strong IgE binding of similar epitopes. The various serological and clinical reaction patterns are ultimately based on numerous variables that go beyond the purely structural characteristics of the allergens (Kleine-Tebbe and Jakob 2015):

- Personal IgE repertoires with individual patterns of serological and potential clinical cross-reactions
- Proportion of the allergen relative to the total protein or total weight
- Stability of the relevant allergens, which depends on the processing of the foodstuff
- Volume of the foodstuff consumed
- Cofactors for a systemic or anaphylactic reaction

Against the background of these factors, efforts to make successful clinical predictions on the basis of molecule-specific IgE sensitizations are limited in their scope. It is essential, therefore, to correct overblown expectations of molecular diagnostics. IgE sensitization tests can be optimized using defined allergens and plausible criteria (depending predominantly on the clinical phenotype). The advantages for serological diagnosis, however, lie in testing each allergen separately.

Conclusions

Singleplex determinations of allergen-specific IgE against allergen molecules enable sensitization (i.e., allergic disposition) to be detected or excluded in a targeted manner. The novel opportunities offered by molecular allergology—increased detection sensitivity and heightened analytical specificity, a marker function for primary sensitizations, and an indicator function for serological cross-reactions—improve test characteristics, thereby broadening the opportunities offered hitherto exclusively extract-based diagnostics. Thus, carefully defined allergen molecules serve as a useful complement to the reagents available to date and optimize IgE determinations and the detection of specific sensitization in the context of allergy diagnosis.

Our additional knowledge of molecular relationships enables a more comprehensive and specific interpretation of IgE profiles and sensitization patterns on the basis of singleplex determinations and make counseling easier. A prerequisite of this, however, is that the clinical relevance of these findings continues to be ultimately based on individual symptoms and reactions in the affected patient on a case-by-case basis.

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