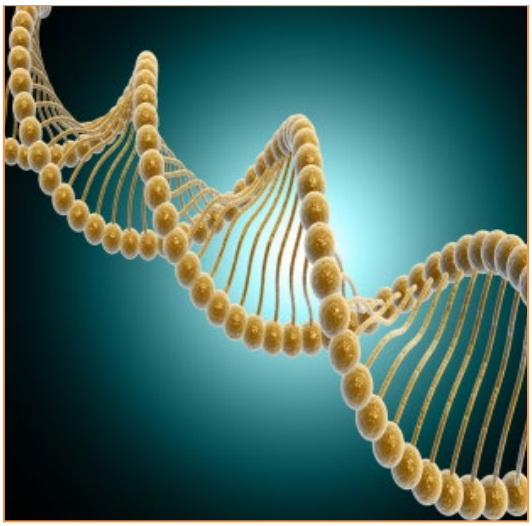
Molecular Allergy Diagnostics

Recombinant or native Allergens in Type I Allergy Diagnostics



Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Tel.: +49 2131 29840 - Fax: +49 2131 2984184 - Email: information@fooke-labs.de

For the measurement of specific IgE in Type I Allergy Diagnostics allergen extracts that contain relevant major and minor allergens as well as non-allergenic components are used. These extracts are suitable for a first screening of patients and are now complemented by recombinant or highly purified native allergens.

What is Molecular in-vitro Allergy Diagnostics or CRD (Component Resolved Diagnostics)?

Molecular allergy diagnostics allows to detect the allergen sensitization of a patient on a molecular level. Therefor native, highly purified and recombinant allergens are used instead of whole allergen extracts.

Components are known since a long time (milk components (Casein, Lactoglobulin, Lactalbumin), insect venoms (Phospholipase A2, Mellitin))

Allergy in-vitro diagnostics comprises the measurement of allergen-specific IgE antibodies in blood

- allergen-specific means: IgE-antibodies, which are directed against a specific allergen component
- molecular allergy diagnostic means: use of allergen molecules in serological tests

The quantitative measurement of specific IgE (sIgE) against allergen component-specific IgE describes the fraction of total IgE in serum which binds specifically to the correspondent allergen component.

sIgE shows a sensitization to the correspondent allergen component.

Whether this sensitization is clinically relevant has to be decided by the doctor on the basis of the anamnesis of the patient.
Molecular allergy in-vitro diagnostic can help him making his decision.

What are allergen molecules, allergen components?

Allergen components are allergy-causing parts in allergen extracts.

Independent of the allergen source, allergen components are summarized in protein families, based on structural and biochemical similarities.

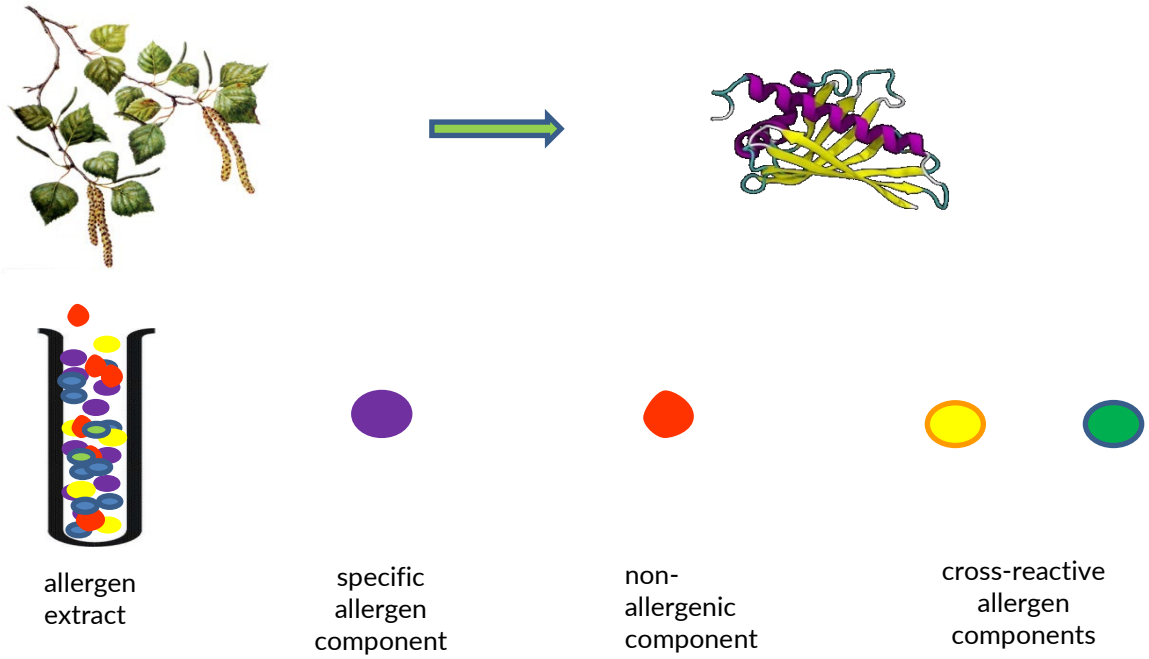
Each allergen source can contain specific and cross-reactive allergen components.

The sensitization to specific components can indicate a primary sensitization and has a greater meaning because species-specific IgE is detected.

The membership of allergens to a certain protein family and their characteristics has consequences for the development of allergic symptoms.

The structures have common IgE binding epitopes, which are the basis for cross-reactivity.

Composition of an allergen extract – example birch pollen



Which components are included in the extract?

- Bet v 1, Major allergen, specific
- Bet v 2, Minor allergen, cross-reactive
- Bet v 4, Minor allergen, cross-reactive

.....

- other, non-allergenic substances

Recombinant allergens are genetically engineered.
The extraction of allergens is done from natural raw material followed by chromatographically enrichment (time-consuming, lossy and expensive).

When is an allergen component a main-/ major allergen?

If more than 50% of the affected patients react to this allergen.

Reactions to allergen specific components indicate a primary sensitization: species-specific IgE antibodies.

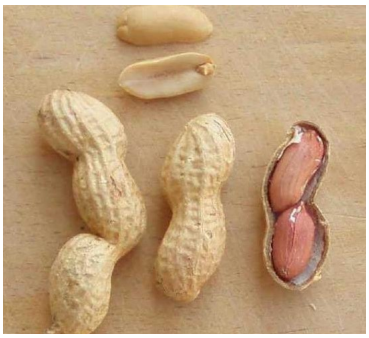
Timothy grass

Phl p 1
Phl p 5

Bee

Api m 1

Peanut



Ara h 2

Reactions to cross-reactive allergen components can indicate sensitizations to different sources, e.g.:

Peach

Pru p 3
(LTP)

The structures have common IgE binding epitopes, which are the basis for cross-reactivity.

Why should allergen components be tested? With which consequences for the patients?

1. To identify primary sensitizations and to give therapy recommendations

The results of testing complete allergen extracts of poly-sensitized patients can lead to further questions and other tests. Through the detection of cross reactive allergens a primary sensitization can be identified and a selection of suitable patients for specific immunotherapy can be made. This is important for an optimal and cost efficient patient management.

Example:

Allergen extract T 3 Birch: positive

Which is the relevant allergen?

With which consequences for the patient?

Components in the birch allergen extract:

Bet v1	specific major allergen, belongs to the Protein family PR-10 (Bet v1-Homologues), family specific marker allergen
Bet v2	minor allergen, belongs to the family of Profilins
Bet v4	minor allergen, belongs to the family of Polcalcins cross-reactive with grass allergens

Possible result 1:

Bet v 1, specific major allergen:	positive	Allergy to birch pollen probable, immunotherapy recommended
Bet v 2, minor allergen, cross-reactive:	negative	
Bet v 4, minor allergen, cross-reactive:	negative	

Possible result 2:

Bet v 1, specific major allergen:	positive	Birch pollen allergy?
Bet v 2, minor allergen, cross-reactive:	positive	primary sensitization to other cross-reactive allergens of the PR-10-Family? consider risks of Immunotherapy?
Bet v 4, minor allergen, cross-reactive:	negative	

Possible result 3:

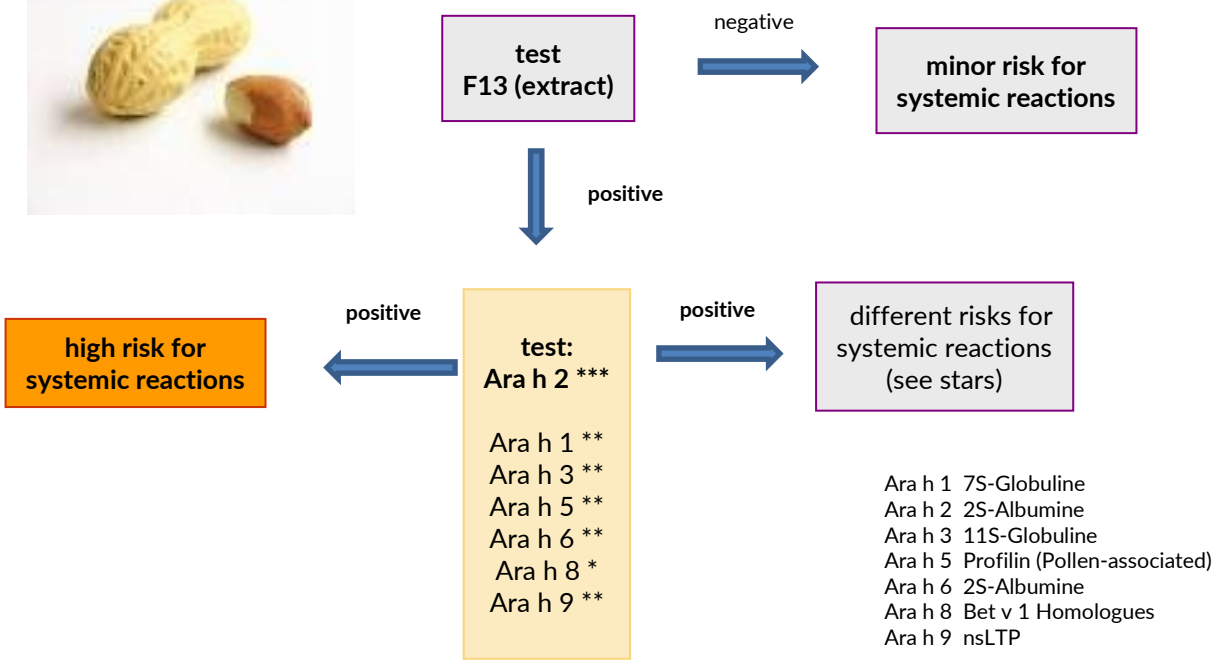
Bet v 1, specific major allergen:	positive	Birch pollen allergy
Bet v 2, minor allergen, cross-reactive:	negative	
Bet v 4, minor allergen, cross-reactive:	positive	other possible cross-reactions with grass pollen

2. To identify risk markers

In selected cases a differentiation of risks for severe reactions to risks for light local reactions by food allergies is possible.

Risk of an anaphylactic shock ⟶ Protection of the patient
Allergen components to identify risk markers

Example peanut allergen



Example Bee / Wasp

Bee I 1

Extract result **positive**

Wasp I 3

Extract result **positive**

Question: Double sensitization?
Which is the relevant allergen?
Or CCD?

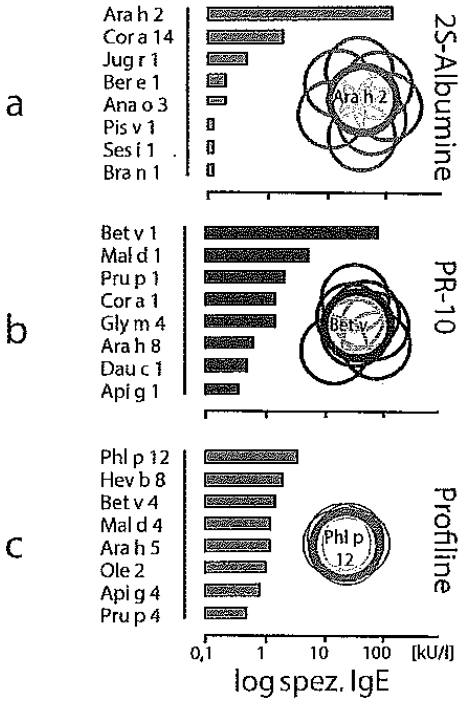
Allergen component of bees:
Api m 1, major allergen, specific
Api m 2, major allergen,

Allergen component of wasps:
Ves v 5, major allergen, specific

3. For the discrimination between sensitizations

With allergen components it can be discriminated between a true sensitization compared to a cross-sensitization in case of poly-sensitized patients. Thus the knowledge about sensitizing allergens and which allergen sources have to be respected can be improved.

Cross-reaction within allergen families



variable cross-reactions within
2S-Albumines

variable cross-reactions within
Bet v 1 – homologues food allergens

distinctive cross-reactions through
similar structures of profilines in pollen,
latex and food

Taken from: J. Kleine-Tebbe, Th. Jakob, Allergo J Int 2015; 24:185

Important Protein Families

Protein family	Occurrence	Sensitivity to heat & proteases	Clinical symptoms
PR-10 (Pathogenesis related protein family number 10); Bet v 1-Homologue	widely spread plant protein in tree pollen, fruits, vegetables and nuts	mostly sensitive (exception: hazelnut, celeriac, soy)	mostly OAS, often tolerance of cooked food; cross-reactivity varies between the species of this family
Profilins	widely spread protein with distinctive cross-reactions, minor allergen in pollen and vegetable food	sensitive	rare appearance of clinical symptoms; OAS by citrus fruits, melon, banana and tomato; can cause reactions in less patients
Storage protein	2S Albumin, 7S/11S-Globulin; can be found in seed and nuts; basic material for the growth of a new plant	resistant	beside OAS often severe and systemic reactions, also to cooked food; little cross-reactivity between the species of the family
nsLTP (non-specific Lipid Transfer Protein)	Can be found in fruits, vegetables, nuts and pollen; representative Pru p 3 as pan allergen	resistant	often beside OAS severe and systemic reactions; reactions also to cooked food; cross-reactivity varies between the species of this family
Parvalbumin	major allergen in fish; marker for cross-reactivity between different fish species	resistant	severe and systemic reactions; also reactions to cooked food possible
Tropomyosin	actin-binding protein; marker for cross-reactivity between crustacean, mites and cockroach	resistant	beside OAS often severe and systemic reactions; also reactions to cooked food possible
Polcalcins	calcium-binding Protein; cross-reactivity between different pollen species; not in vegetable food		hardly clinical relevant
Lipocaline	allergens in animals with fur	resistant	cross-reactivity between the different animal species varies
Serum albumins	allergens in animals; can be found in different tissues and biological liquids	sensitive	cross-reactions between different mammals; e.g. cat and dog, cat and pig

CCD

Cross-Reactive Carbohydrate Determinants

Glycoprotein, which can be found in plants, vegetable foods and invertebrates (e.g. insects), they are highly immunogenic and can lead to antibody formation, e.g. IgE	if a lot of positive results are detected in one test the examination of CCD is important	rare clinical symptoms; importance of CCD is under discussion a negative skin prick test and a positive specific IgE result can indicate CCD IgE antibodies

Interpretation concerning the question of clinical relevance

- Negative test results with allergen molecules exclude a sensitization
- Positive test results indicate a sensitization or allergy disposition
- By the use of species-specific allergens primary sensitizations can be identified
- By the use of marker allergens possible cross-reactivity's can be identified
- On the basis of the clinical symptoms the doctor decides about the clinical relevance

Summary

- The testing with complete allergen extracts is best suited for a first screening
- The testing of allergen components helps:
  - to verify the primary sensitization
  - to identify cross-reactions to further sensitizing allergens
  - to estimate risks, to protect patients
  - to decide for or against immunotherapy
- Substitution for difficult allergen extracts

Available recombinant (R) and highly purified native (N) allergens

REF	Allergen	Allergen source	Significance	Further attributes
Mites				
ND 11	Der p 1	*D. pteronyssinus*	Major allergen	Cysteine protease; cross-reactive with Der f 1
ND 12	Der p 2	D. pteronyssinus	Major allergen	NPC2 family; cross-reactive with Der f 2
RD 110	Der p 10	D. pteronyssinus	Minor allergen	Tropomyosin; cross-reactive with Tropomyosin's of crustacean / mites / insects
RD 123	Der p 23	D. pteronyssinus	Major allergen	Peritrophin-protein; mite specific allergen component
ND 21	Der f 1	D. farinae	Major allergen	Cysteine protease; cross-reactive with Der p 1
ND 22	Der f 2	D. farinae	Major allergen	NPC2 family; cross-reactive with Der p 2
Animals				
RE 11	Fel d 1	Cat	Major allergen	Uteroglobin; highly specific allergen component
Foods				
RF 144	Gly m 4	Soy	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v 1 homologues, underrepresented in allergen extracts
RF 180	Cyp c 1	Carp	Major allergen	Parvalbumin; cross-reactive to various fish species
NF 24	Tropomyosin	Shrimp	Major allergen	Tropomyosin; cross-reactive with Tropomyosin's of crustacean / mites / insects
N gal 1	α-Gal	Thyroglobulin	Marker	Marker for "red-meat-allergy"
RF 311	Dau c 1	Carrot	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v 1 homologues
RF 491	Mal d 1	Apple	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v 1 homologues
RF 493	Mal d 3	Apple	Minor allergen	nsLTP (non-specific Lipid-Transfer-Protein); cross-reactive to various Lipid-Transfer-Proteins (LTP) like Pru p 3
RF 441	Fra a 1	Strawberry	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v 1 homologues
RF 443	Fra a 3	Strawberry	Minor allergen	nsLTP (non-specific Lipid-Transfer-Protein); cross-reactive to various Lipid-Transfer-Proteins (LTP) like Pru p 3
NF 131	Ara h 1	Peanut	Major allergen	Storage protein (7S Globulin); Risk marker for systemic reactions
NF 132	Ara h 2	Peanut	Major allergen	Storage protein (2S-Albumin); Risk marker for severe systemic reactions
NF 133	Ara h 3	Peanut	Major allergen	Storage protein (11S Globulin); Risk marker for systemic reactions
NF 136	Ara h 6	Peanut	Major allergen	Storage protein (2S-Albumin); Risk marker for systemic reactions
RF 138	Ara h 8	Peanut	Major allergen	PR-10-Protein, Bet v 1 Homologue
RF 139	Ara h 9	Peanut	Minor allergen	nsLTP (non-specific Lipid-Transfer-Protein); cross-reactive to various Lipid-Transfer-Proteins (LTP) like Pru p 3
RF 171	Cor a 1	Hazelnut	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v1 homologues
RF 178	Cor a 8	Hazelnut	Pan allergen	nsLTP (non-specific Lipid-Transfer-Protein); Risk marker for systemic reactions, potential marker for sensitizations to LTP
NF 179	Cor a 9	Hazelnut	Major allergen	Storage protein (11S Globulin); Risk marker for systemic reactions
RF1714	Cor a 14	Hazelnut	Minor allergen	2s Albumin
RF 531	Pru p 1	Peach	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v1 homologues
RF 533	Pru p 3	Peach	Pan allergen	nsLTP (non-specific Lipid-Transfer-Protein); Risk marker for systemic reactions, marker for sensitizations against LTP (Lipid Transfer Protein) in fruits
RF 534	Pru p 4	Peach	Minor allergen	Profilin; cross-reactive with grasses / herbs / trees / several fruits
Hen´s Egg and Milk Allergens				
F 67	Gal d 2	Hen´s Egg	Major allergen	Ovalbumin; cross-reactive with Ovalbumin´s other bird egg´s
F 68	Gal d 1	Hen´s Egg	Major allergen	Ovomucoid; indication for reactions to egg´s in all ways of preparation (fresh and cooked)
NF 103	Gal d 3	Hen´s Egg	Major allergen	Indication for reactions to raw or just light cooked egg; instable to heat
F 76	Bos d 4	Cow´s milk	Major allergen	Alpha-Lactalbumin; instable to heat
F 77	Bos d 5	Cow´s milk	Major allergen	Beta-Lactoglobulin; instable to heat
F 78	Bos d 8/9/10	Cow´s milk	Major allergen	Casein; indication for reactions to cow´s milk in all ways of preparation (fresh as well as cooked and in all products made out of it like e.g. cheese); cross-reactive with caseins of milk of sheeps / buffaloes / goats

Grasses and Corn Pollen				
RG 601	Phl p 1	Timothy Grass	Major allergen	Beta-expansin; decision support for immunotherapy, cross-reactive with other grasses
RG 605	Phl p 5	Timothy Grass	Major allergen	Decision support for immunotherapy, cross-reactive with other grasses
RG 607	Phl p 7	Timothy Grass	Minor allergen	Polcalcin; cross-reactive with various pollen species (grasses / herbs / trees)
RG 612	Phl p 12	Timothy Grass	Minor allergen	Profilin; Pan allergen, cross-reactive with various pollen species (grasses / herbs / trees / latex / fruits)
Insect venoms				
RI 101	Api m 1	Honey bee venom	Major allergen	Phospholipase A2; decision support for immunotherapy, cross-reactive with allergen components of wasp and bumble bee
RI 102	Api m 2	Honey bee venom	Major allergen	Hyaluronidase; cross-reactive to various insect venoms (wasp / hornet)
RI 110	Api m 10	Honey bee venom	Major allergen	Indicates Bee venom Allergy, under representated in therapeutic extracts
RI 301	Ves v 1	Wasp venom	Major allergen	Phospholipase A1, cross-reactive with various insect venoms (wasp / hornet)
RI 305	Ves v 5	Wasp venom	Major allergen	Antigen 5; decision support for immunotherapy, cross-reactive with various insect venoms (bee / hornet)
Latex				
RK 821	Hev b 1	Latex	Major allergen	Latex elongation factor; specific allergen component, common by patients with Spina bifida
RK 825	Hev b 5	Latex	Major allergen	Specific allergen component
RK 826	Hev b 6	Latex	Major allergen	Chitin-binding Protein; specific allergen component, cross-reactive with vegetable food (fruits / vegetables)
RK 828	Hev b 8	Latex	Pan allergen	Profilin; cross-reactive with various pollen species (grass/herbs/trees/fruits)
Weeds, Flowers, Molds				
NW 101	Amb a 1	Common ragweed	Major allergen	Pectate lyase; specific allergen component
RM 601	Alt a 1	Alternaria alternata	Major allergen	Cross reactions with other molds unknown
RW 601	Art v 1	Mugwort	Major allergen	Defensin-like protein; specific allergen component
Trees				
RT 301	Bet v 1	Birch	Major allergen	PR-10 Protein; decision support for immunotherapy, cross-reactive with Bet v 1 homologues (in fruits etc.)
RT 302	Bet v 2	Birch	Minor allergen	Profilin; Pan allergen, cross-reactive with grasses / trees / herbs / various fruits (e.g. banana / apple)
RT 304	Bet v 4	Birch	Minor allergen	Polcalcin; cross-reactive with various pollen species (grasses / herbs / trees)
RT 401	Cor a 1	Hazel	Major allergen	PR-10-Protein; Bet v 1 homologue, cross-reactive with various Bet v1 homologues
RT 901	Ole e 1	Olive	Major allergen	Common olive group 1; specific allergen component; also marker for sensitizations to ash, lilac and privet; decision support for immunotherapy
CCD				
NF 253	CCD	Horseradish	Marker	cross-reactivity based on CCD
Allergen mixtures				
RG 620	Phl p 1/ Phl p 5	Timothy Grass	Major allergens	Mixture of major allergens, decision support for immunotherapy
RG 621	Phl p 7/ Phl p12	Timothy Grass	Minor allergens	Mixture of minor allergens, decision support for immunotherapy

Dr. Fooke-Achterrath Laboratorien GmbH
Habichtweg 16
41468 Neuss
Germany
Tel.: +49 2131 2984 0
Fax: +49 2131 2984 184
Email: information@fooke-labs.de
www.fooke-labs.de