Molecular allergy diagnostics

Targeted identification of allergy triggers

- **Supports you in selecting a suitable specific immunotherapy for each patient**
- **Software-based interpretation of results**

The challenge of allergy diagnostics

Nowadays, almost 40% of the population in industrialised countries suffers from an allergy. Patients often present with unspecific symptoms (e.g. gastro-intestinal problems or respiratory complaints). The doctor then has to begin the search for the cause. Modern allergy diagnostics offer various possibilites to identify the triggering allergen. Conventional tests (e.g. skin-prick tests) ware often supplemented by in vitro test systems for the detection of specific IgE antibodies (sIgE) in the blood of the patient. With multiparameter tests antibodies against many different allergens are analysed simultaneously, yielding fast and comprehensive diagnostic results. Thus, the allergen source can often be identified in the initial step.
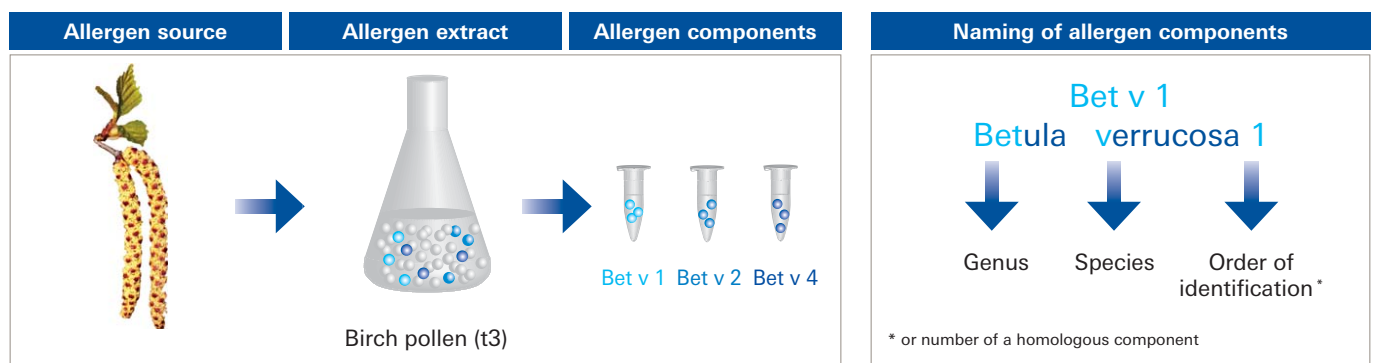
Essential information for assessing the risk of severe allergic reactions and for selecting a specific therapy is obtained from defined partial allergen diagnostics.

From extracts to proteins – defined partial allergen diagnostics (DPA-Dx)

Up to now, IgE antibodies were detected using allergen extracts, which contain a mixture of allergy-triggering proteins (components) from the corresponding allergen source. However, these extracts are not standardised – therefore results of different manufacturers' test systems cannot be compared.

In contrast, defined partial allergen diagnostics (DPA-Dx), also called molecular allergy diagnostics, utilise precisely defined allergen components, which are either isolated directly from the allergen source or produced recombinantly. This enables high standardisation as well as differential diagnostics.

Allergen source	Allergen extract	Allergen components
	Birch pollen (t3)	Bet v 1 Bet v 2 Bet v 4

Naming of allergen components
Bet v 1
Betula verrucosa 1
Genus   Species   Order of identification *
* or number of a homologous component

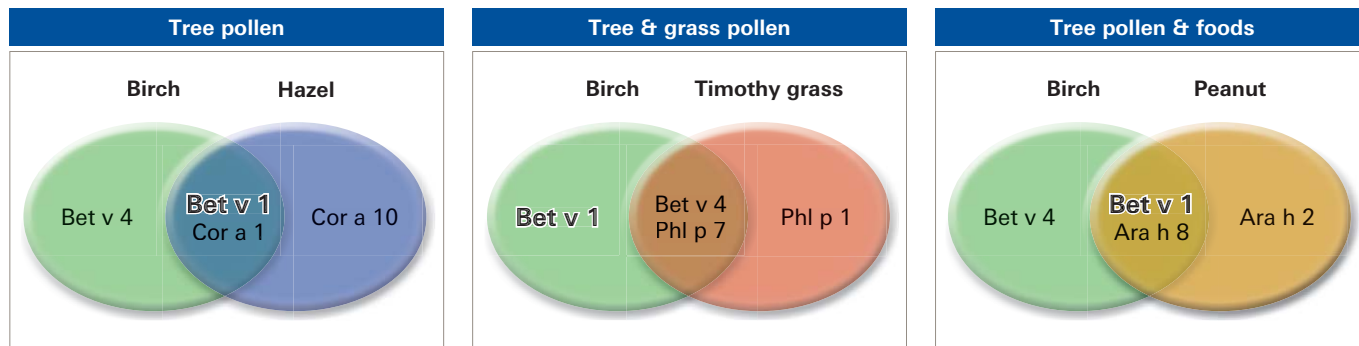# What are the advantages of defined partial allergen diagnostics?

1) Identification of cross reactions

The identification of the primary allergy trigger is decisive for therapy. However, patient serum often reacts with several antigen extracts in multiparameter tests. This can be due to different reasons:

- The patient is sensitised to several allergens (multiple sensitisation).
- The patient is primarily sensitised to allergen A and therefore has only formed sIgE to this allergen. But further allergens (B, C, D...) are so structurally similar to A that the IgE antibodies against allergen A also react with allergens B, C and D (cross reactions).

Allergen components that are unique to a particular allergen source are species specific. However, they may have high structural similarity (homology) to components of other, remote allergen sources. Because of this homology, an IgE antibody that is actually specific may recognise several allergen components, resulting in cross reactions.

For example, a pollen-allergic patient who has a confirmed sensitisation to Bet v 1 from birch pollen will probably also react to the homologue Cor a 1 from hazel pollen. But a Bet v 1-induced cross reaction with grasses is unlikely, since there is no Bet v 1 homologue in grass pollens. If a birch-pollen allergic patient nevertheless reacts to grasses, this could in turn be because birch and grass pollens also contain ubiquitous allergens (pan allergens) which can also lead to cross reactions. Bet v 1 homologues are, moreover, found not just in tree pollen but also in foods. Thus, Bet v 1-specific antibodies can also cross react with Ara h 8 from peanut. If this causes symptoms it is known as a birch pollen-associated food allergy.

| Tree pollen	Tree & grass pollen	Tree pollen & foods
Birch **Hazel**	**Birch** **Timothy grass**	**Birch** **Peanut**
Bet v 4 — Bet v 1 / Cor a 1 — Cor a 10	Bet v 1 — Bet v 4 / Phl p 7 — Phl p 1	Bet v 4 — Bet v 1 / Ara h 8 — Ara h 2

The identification of the exact allergen components enables clarification of possible cross reactions with allergen sources that have not been taken into consideration.

2) Risk analysis and risk management

Allergen components belong to various protein families, which indicate the risk of severe allergic reactions. If a patient is sensitised to, for example, allergens from the family of profilins, then mild symptoms are to be expected. In contrast, patients who are sensitised to allergens belonging to the family of storage proteins have a high risk of systemic reactions and should therefore carry an emergency set.

Furthermore, families of proteins differ in their heat stability, which plays an important role in food allergies. Heat-labile allergen components (profilins, PR10 proteins) in foods are generally denatured by cooking processes, thus reducing the risk of a reaction.

Risk of severe reactions

Risk

Storage proteins

LTP

PR10

Profilins

Heat stability
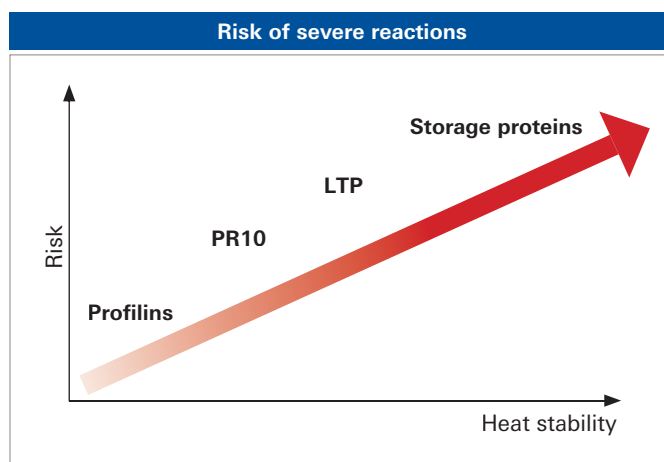
3) Selection of suitable therapy

Depending on the trigger, allergies can be treated by avoidance of the allergen or by specific immunotherapy (SIT). SIT has a high chance of success when the patient is primarily sensitised to the main components of the allergen extract, the so-called major allergens (Schmid-Grendelmeier P, Hautarzt 2010, 61: 946 – 953).

Only the results from defined partial allergen diagnostics can deliver this information. The doctor can then specifically select the optimal treatment, and the patient is spared the stress of unnecessary allergen avoidance or ineffective SIT.

Apportionment of allergens

Major allergens: > 50 %   **Minor allergens:** < 50 %

...of patients who are sensitised to a particular allergen source react to these allergen components.
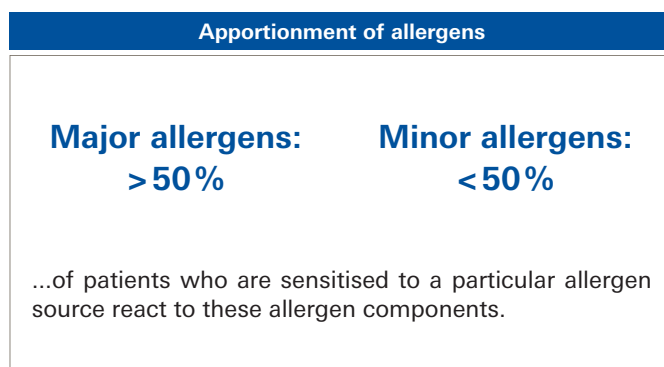
Case example pollen allergy

Christine presents to an allergologist with unspecific symptoms. She suffers from recurring rhinoconjunctivitis, which begins in spring. After detailed anamnesis the doctor decides to perform a multiparameter test containing numerous inhalation allergens. This test is positive for birch (t3) and timothy grass (g6).

In order to find out if the patient's results are due to a cross reaction or a multiple sensitisation, the reactions to allergen components from birch and timothy grass are analysed (DPA-Dx Pollen 1).

Defined partial allergen diagnostics with the DPA-Dx Pollen 1

Christine — DPA-Dx Pollen 1
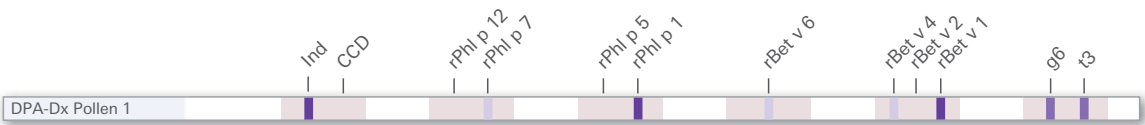
	Allergen	Concentration	Class	0	1	2	3	4	5	6
Extracts	Birch (t3)	60 kU/l	5							
	Timothy grass (g6)	60 kU/l	5							
Major allergen	rBet v1 (birch) (t215)	79 kU/l	5							
	rBet v2 (birch) (t216)	<0.35 kU/l	0							
Minor allergens	rBet v4 (birch) (t220)	0.47 kU/l	1							
	rBet v6 (birch) (t225)	0.47 kU/l	1							
Major allergens	rPhl p1 (timothy gr.) (g205)	73 kU/l	5							
	rPhl p5 (timothy gr.) (g215)	<0.35 kU/l	0							
Minor allergens	rPhl p7 (timonthy gr.) (g210)	0.47 kU/l	1							
	rPhl p12 (timothy gr.) (g212)	<0.35 kU/l	0							
	CCD (CCD)	<0.35 kU/l	0							

Result:
Positive reaction to the species-specific major allergens of birch and timothy grass, negative for the minor allergens

Interpretation:
Multiple sensitisation to birch and timothy grass

Therapy recommendation:
Double therapy (SIT) against birch and grass pollens with good prognosis, since the patient is sensitised to the major allergens
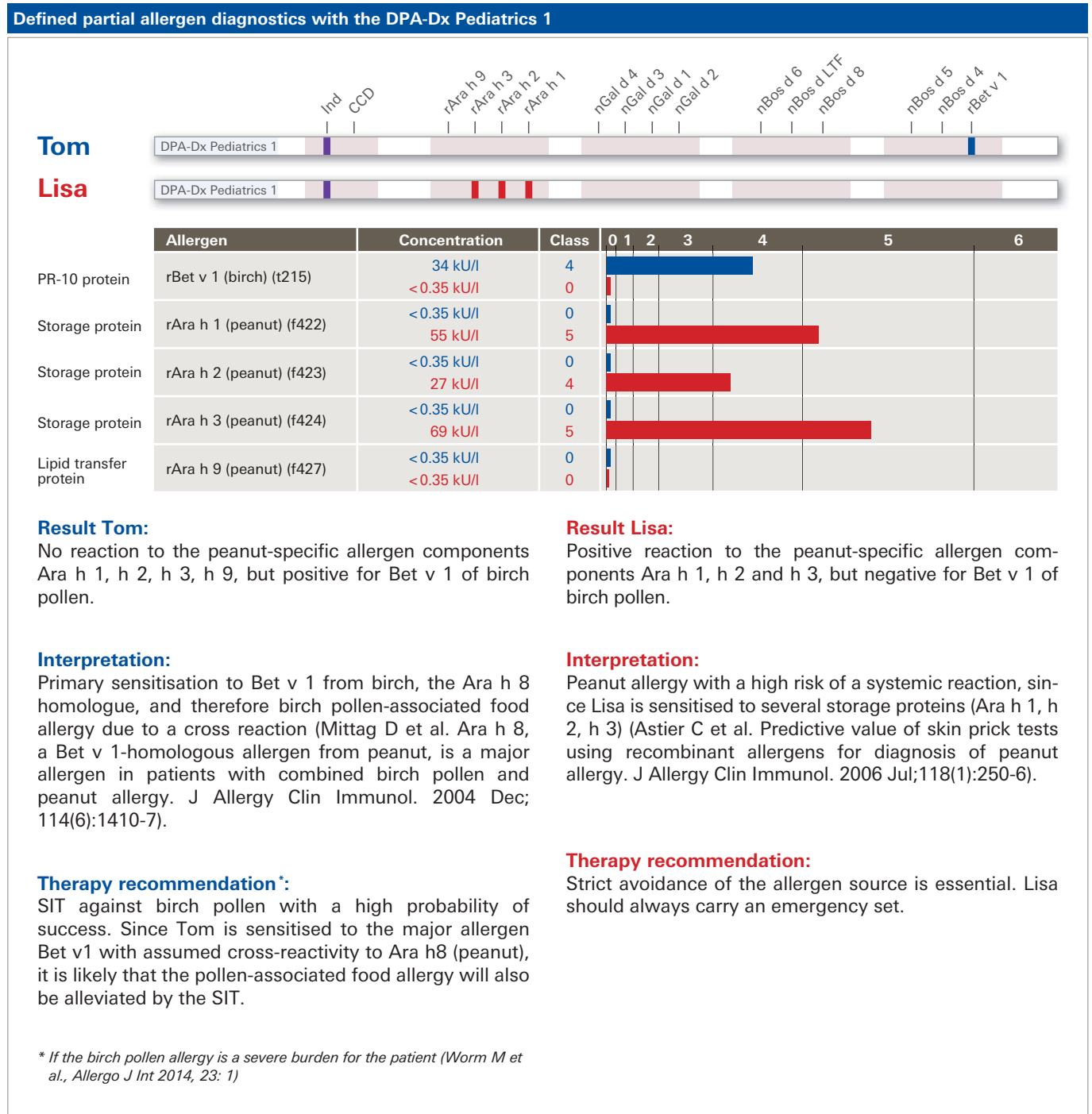
Conclusion: The DPA-Dx Pollen 1 profile can identify the exact allergy triggers (birch and timothy grass) and provide an important fundament for therapy decisions and prognosis of SIT.
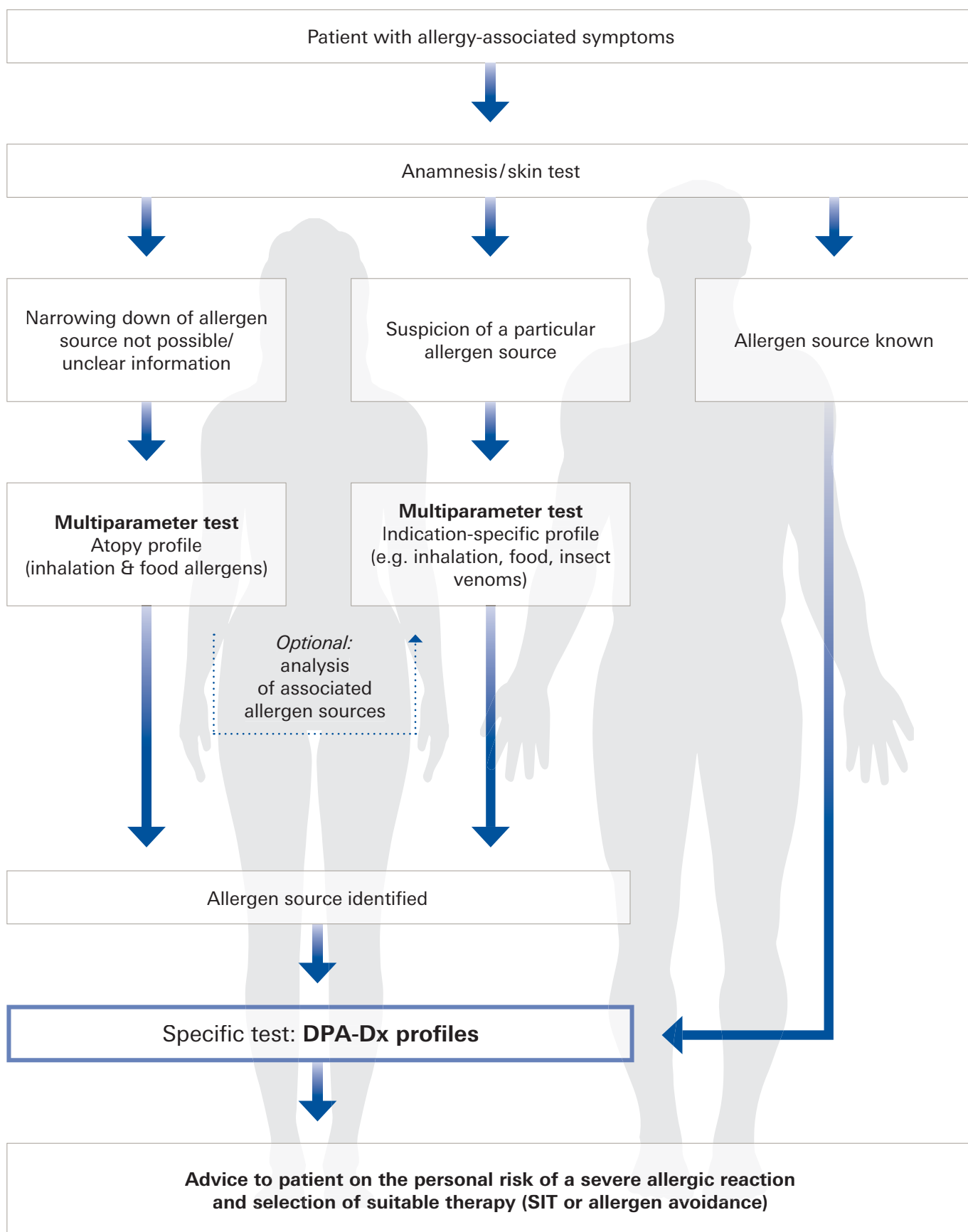
Case example peanut allergy

Tom and Lisa attend an allergologist on the same day with unspecific symptoms (prickling in the mouth, eczema, nausea, rhinoconjunctivitis). After detailed anamnesis the doctor decides to perform a multiparameter test for IgE antibodies against food allergens. A sensitisation to peanut is diagnosed in both patients.

In order to assess the risk of a severe systemic reaction and an anaphylactic shock, defined partial allergen diagnostics are used for the next step (DPA-Dx Pediatrics 1).

Defined partial allergen diagnostics with the DPA-Dx Pediatrics 1

Tom
DPA-Dx Pediatrics 1

Lisa
DPA-Dx Pediatrics 1

Labels across strip: Ind, CCD, rAra h 9, rAra h 3, rAra h 2, rAra h 1, nGal d 4, nGal d 3, nGal d 1, nGal d 2, nBos d 6, nBos d LTF, nBos d 8, nBos d 5, nBos d 4, rBet v 1

	Allergen	Concentration	Class	0	1	2	3	4	5	6
PR-10 protein	rBet v 1 (birch) (t215)	34 kU/l	4							
		< 0.35 kU/l	0							
Storage protein	rAra h 1 (peanut) (f422)	< 0.35 kU/l	0							
		55 kU/l	5							
Storage protein	rAra h 2 (peanut) (f423)	< 0.35 kU/l	0							
		27 kU/l	4							
Storage protein	rAra h 3 (peanut) (f424)	< 0.35 kU/l	0							
		69 kU/l	5							
Lipid transfer protein	rAra h 9 (peanut) (f427)	< 0.35 kU/l	0							
		< 0.35 kU/l	0							

Result Tom:
No reaction to the peanut-specific allergen components Ara h 1, h 2, h 3, h 9, but positive for Bet v 1 of birch pollen.

Interpretation:
Primary sensitisation to Bet v 1 from birch, the Ara h 8 homologue, and therefore birch pollen-associated food allergy due to a cross reaction (Mittag D et al. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. J Allergy Clin Immunol. 2004 Dec; 114(6):1410-7).

Therapy recommendation[*]:
SIT against birch pollen with a high probability of success. Since Tom is sensitised to the major allergen Bet v1 with assumed cross-reactivity to Ara h8 (peanut), it is likely that the pollen-associated food allergy will also be alleviated by the SIT.

[*] If the birch pollen allergy is a severe burden for the patient (Worm M et al., Allergo J Int 2014, 23: 1)

Result Lisa:
Positive reaction to the peanut-specific allergen components Ara h 1, h 2 and h 3, but negative for Bet v 1 of birch pollen.

Interpretation:
Peanut allergy with a high risk of a systemic reaction, since Lisa is sensitised to several storage proteins (Ara h 1, h 2, h 3) (Astier C et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol. 2006 Jul;118(1):250-6).

Therapy recommendation:
Strict avoidance of the allergen source is essential. Lisa should always carry an emergency set.

Conclusion: Only defined partial allergen diagnostics provide information about the risk for a severe reaction. Tom does not have a true peanut allergy – he does not have a high risk of life-threatening reactions, and a strict peanut-free diet is not absolutely necessary (Asarnoj A et al. Peanut component Ara h 8 sensitization and tolerance to peanut. J Allergy Clin Immunol. 2012 Aug;130(2):468-72). For Lisa, however, even traces of peanut could trigger a severe systemic reaction.

Strategies for efficient allergy diagnostics

Patient with allergy-associated symptoms

Anamnesis / skin test

| Narrowing down of allergen source not possible/ unclear information | Suspicion of a particular allergen source | Allergen source known |

Multiparameter test
Atopy profile
(inhalation & food allergens)

Multiparameter test
Indication-specific profile
(e.g. inhalation, food, insect venoms)

Optional:
analysis
of associated
allergen sources

Allergen source identified

Specific test: **DPA-Dx profiles**

**Advice to patient on the personal risk of a severe allergic reaction
and selection of suitable therapy (SIT or allergen avoidance)**

Efficient multiplex tests – the key to optimal therapy

- Differentiation between cross reactions and multiple sensitisation
- Assessment of risk of severe allergic reactions
- Targeted selection of patients suitable for specific immunotherapy (SIT)
- Improved prognosis of therapy success and potential tolerance induction
- Prevention of unnecessary burden to patients due to lifestyle changes (avoidance) or multiple therapies

EUROIMMUN products for defined partial allergen diagnostics

DPA-Dx profiles	Allergen components	Allergen extracts	Order number
DPA-Dx Pollen 1	**Birch** (rBet v 1, rBet v 2, rBet v 4, rBet v 6) **Timothy grass** (rPhl p 1, rPhl p 5, rPhl p 7, rPhl p 12)	Birch (t3) Timothy grass (g6)	DP 3210-1601-1 E
DPA-Dx Pollen Southern Europe 1	**Birch** (rBet v 1) **Cypress** (nCup a 1) **Olive** (rOle e 1) **Oak** (rQue a 1) **Hazel** (rCor a 1.0101) **Wall pellitory** (rPar j 2) **Timothy grass** (rPhl p 1, rPhl p 5, rPhl p 7, rPhl p 12) **Alternaria alternata** (rAlt a 1)	Birch (t3) Olive (t9) Cypress (t23) Wall pellitory (w21) Timothy grass (g6) A. alternata (m6)	DP 3211-1601-1 E
DPA-Dx Pediatrics 1	**Milk** (nBos d LTF, nBos d 4, nBos d 5, nBos d 6, nBos d 8) **Egg** (nGal d 1, nGal d 2, nGal d 3, nGal d 4) **Peanut** (rAra h 1, rAra h 2, rAra h 3, rAra h 9)	–	DP 3812-1601-1 E
DPA-Dx Pediatrics 2	**Milk** (nBos d LTF, nBos d 4, nBos d 5, nBos d 6, nBos d 8) **Egg** (nGal d 1, nGal d 2, nGal d 3, nGal d 4) **Peanut** (rAra h 1, rAra h 2, rAra h 3, rAra h 5, rAra h 6, rAra h 7, rAra h 9) **Birch** (rBet v 1)	–	DP 3812-1601-2 E
DPA-Dx Milk 1	**Milk** (nBos d LTF, nBos d 4, nBos d 5, nBos d 6, nBos d 8)	Milk (f2)	DP 3510-1601-1 E
DPA-Dx Peanut 1	**Peanut** (rAra h 1, rAra h 2, rAra h 3, rAra h 5, rAra h 6, rAra h 7, rAra h 9) **Birch** (rBet v 1)	–	DP 3511-1601-1 E
DPA-Dx Insect venoms 3	**Bee venom** (rApi m 1, rApi m 2, rApi m 10) **Wasp venom** (rVes v 1, rVes v 5)	Bee venom (i1) Wasp venom (i3) Hornet venom (i75)	DP 3850-1601-3 E
DPA-Dx Insect venoms Southern Europe 1	**Bee venom** (rApi m 1, rApi m 2, rApi m 10) **Wasp venom** (rVes v 1, rVes v 5) **Paper wasp venom** (rPol d 1, rPol d 5)	Bee venom (i1) Wasp venom (i3) Hornet venom (i75) Paper wasp venom (i77)	DP 3851-1601-1 E
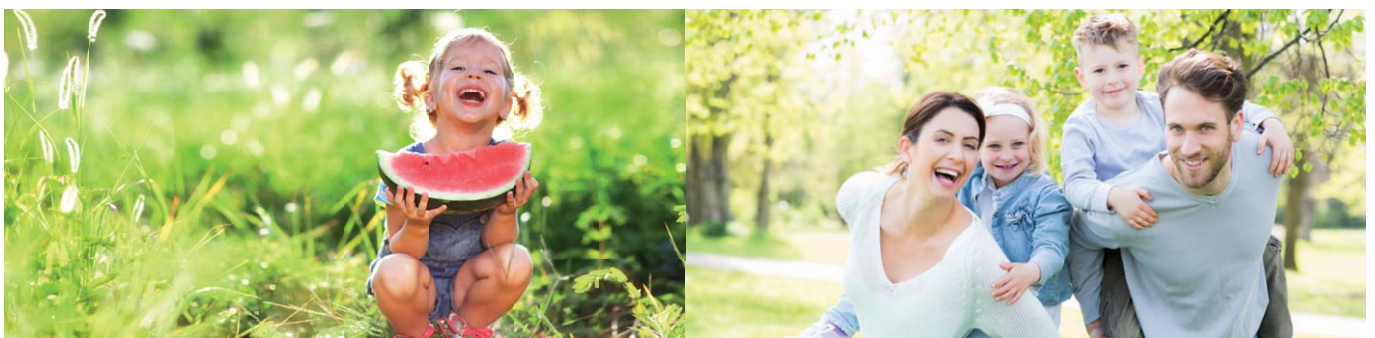
Advantages of EUROIMMUN products for allergy diagnostics

- Indication-specific profiles (multiparameter tests)
- Integrated CCD marker in every test
- Only small sample volumes (100–400 µl) necessary; ideal for paediatric patients
- Highly efficient processing: from sample to result in less than 3.5 hours
- Individual automation options
- Standardised evaluation according to EAST class system with EUROLineScan

EUROIMMUN Medizinische Labordiagnostika AG · Seekamp 31 · 23560 Lübeck (Germany) · Phone: +49 451 20 32-0 · www.euroimmun.com

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