# Precise diagnosis of allergies by multiplex profiling

Differential allergy diagnostics using molecular components is a powerful tool for pinpointing the precise trigger of an allergy, enabling targeted immunotherapy and comprehensive risk assessment. Multiparameter systems streamline the diagnostic procedure by delivering a comprehensive and detailed patient profile in a single test. The multiplex EUROLINE DPA-Dx (defined partial allergen diagnostics) system provides fast and efficient detection of specific IgE using immunoblot strips containing optimised combinations of relevant allergen components. Each profile is tailored to a specific indication.

by Dr Jacqueline Gosink

## Advanced differential diagnostics

Molecular allergology is a state-of-the-art approach to allergy diagnostics, whereby defined single allergen components are used for detection of specific IgE in place of traditionally used allergen extracts. The molecular components are highly purified proteins, which are either isolated directly from the allergen source or produced recombinantly. They provide a higher level of standardization than allergen extracts and enable highly differentiated diagnostics.

## Improved patient management

Molecular allergy diagnostics greatly facilitate decision-making on whether to implement specific immunotherapy [1].

Before embarking on therapy it is critical to establish the exact trigger of the allergy. However, it is common for patients to show multiple reactions in clinical tests such as skin prick tests and in extract-based antibody assays. These may be due to an actual polysensitization or a monosensitization with cross reactions. The latter involves a reaction against a single source, but the IgE antibodies also bind to structurally related allergens from other sources, inducing an immune response. Analysis of the individual components of the allergen sources and against the most important panallergens enables the precise trigger of the allergy symptoms to be determined quickly and accurately. Specific immunotherapy can then be targeted to the actual allergen source.

Component-resolved diagnostics are also helpful for risk evaluation. Reactions against different components of an allergen can elicit symptoms of varying severity. Molecular profiling can establish if a patient has a low or high risk of severe systemic reactions such as anaphylactic shock. Patients at risk of life-threatening reactions can then be advised on avoidance of the allergen and on appropriate measures to take in an emergency situation.

## Pediatric profile

Sensitizations to peanut, milk and egg are the most common allergies in childhood. Peanut allergy in particular can have serious consequences, often triggering anaphylaxis. Accurate diagnosis of allergies in infants and children is important for assessing the risk of systemic reactions, evaluating the chances of tolerance induction and establishing the necessity of dietary restriction. To enable fast and efficient screening of these sensitisations, the EUROLINE DPA-Dx Pediatrics profile was developed containing the most important allergen components from these three sources (Figure 1).

Egg: In hen's egg allergy, Gal d1 (ovomucoid) is the main allergen and serves as an indicator for the severity of the allergic reaction. Sensitizations to the heat-sensitive components Gal d2 (ovalbumin), Gal d3 (conalbumin) and Gal d4 (lysozyme) are associated only with consumption of raw or slightly cooked eggs. However, ovalbumin is used in vaccines and lysozyme is used as a preserving agent, so patients with these sensitizations may also exhibit reactions to pharmaceutical or food products containing the corresponding component.

Milk: A reaction to Bos d8 (casein) indicates a strong allergy to milk and milk products. Casein is frequently used as an additive, thus a sensitization also causes intolerance to a wide variety of foods such as meats, chocolates or potato chips. The components Bos d (lactoferrin), Bos d4, Bos d5 and Bos d6 are heat sensitive, and sensitizations to them are
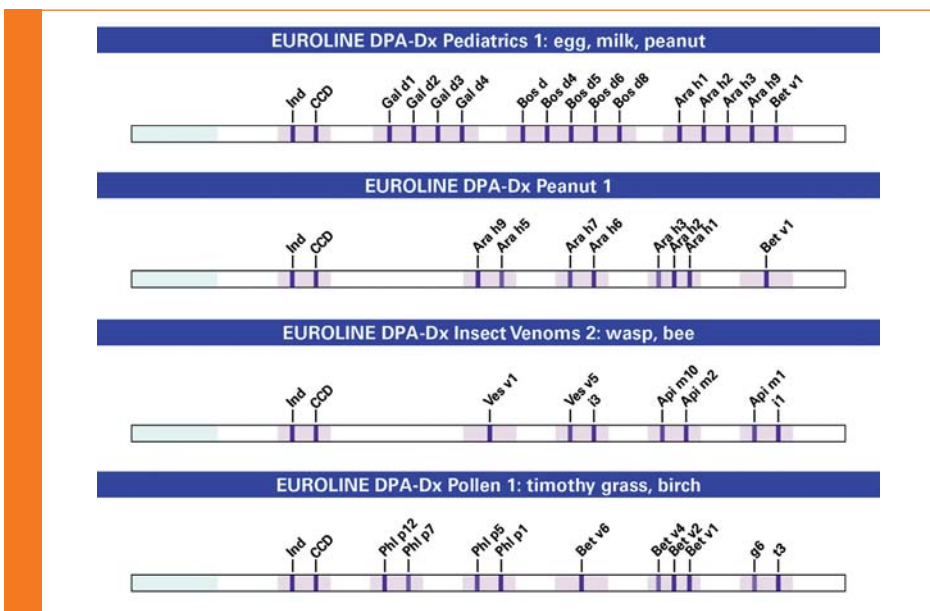


*Figure 1. EUROLINE DPA-Dx profiles*

mainly associated with reactions to fresh milk. Antibodies against Bos d6 (bovine serum albumin) may in addition cause a reaction to beef.

Peanut: The peanut section of the pediatric profile can distinguish a primary peanut sensitization from a cross reaction with birch pollen and establish the risk for the patient. IgE antibodies against the seed storage proteins Ara h1, Ara h2 and Ara h3 and the lipid transfer protein Ara h9 carry a high risk of a systemic reaction. The severity of the allergy is, moreover, greater when multiple high-risk components are involved. Patients at high risk must strictly avoid peanuts, even in trace amounts, and should carry an emergency set. A reaction with the birch pollen component Bet v1 can establish if symptoms are due rather to a cross reaction from a birch pollen allergy. Many foods, including peanut, possess Bet v1 homologues, known as PR10 proteins, which are a frequent cause of cross reactivity. The Bet v1 homologue in peanut is Ara h8. If the peanut allergy symptoms are found to be due to a pollen-associated cross reaction, the risk of anaphylaxis is extremely low. Specific therapy directed at the birch pollen allergy will in this case also alleviate the peanut symptoms.

Thus, the EUROLINE DPA-Dx pediatric profile indicates the presence and extent of milk, egg and peanut sensitizations in children. This provides a basis for decisions on immunotherapy and on which food or food components the child should avoid.

## Peanut profile

The EUROLINE DPA-Dx Peanut 1 profile (Figure 1) provides component-resolved peanut allergy diagnostics at a new level of detail and is the first dedicated

multiparameter test for peanut worldwide. In addition to the high-risk components Ara h1, Ara h2, Ara h3, Ara h9 and the cross-reactive Ara h8 homologue Bet v1 described above, the profile also contains the new components Ara h5, Ara h6 and Ara h7.

Ara h6 is a seed storage protein which is related to Ara h2 and is classified as

a high-risk allergen. IgE antibodies against Ara h6 provoke a strong immune response. Ara h7 is also an Ara h2-related storage protein and reactions against it are likewise considered high risk. Reactions to the pollen-associated allergen Ara h5 on the other hand may be associated with milder symptoms and a lower risk of a severe response. Studies are underway to further unravel the

clinical relevance of reactions against these components.

The EUROLINE DPA-Dx Peanut 1 profile provides the most comprehensive analysis of IgE reactions against peanut. Ara h5 and Ara h7 in particular are exclusive to this test system. The detailed serodiagnostic results assist with fast decision making on therapy and risk minimization.

## Insect venoms profile

Allergies against insect venoms can elicit severe reactions, especially in adults. However, patients with insect venom allergy often react with both bee and wasp venom in clinical tests due to shared epitopes. Molecular testing can establish if the patient has a true double sensitization or a cross reaction between the insect venoms.

The new EUROLINE DPA-Dx Insect Venoms Profile 2 (Figure 1) provides the specific components Ves v1 and Ves v5 from wasp (Vespula vulgaris) venom and Api m1, Api m2 and Api m10 from bee (Apis mellifera) venom, together with native extracts of both venoms for a complete analysis. Inclusion of the Api m10 component increases the sensitivity of the test, since this component is underrepresented in native allergen extracts.

Differentiation and characterization of insect venom sensitizations using this profile can help discern the need for single or double immunotherapy and the likelihood of therapy success. In particular, patients who are sensitized to Api m10 may not benefit from immunotherapy since the bee venom therapeutic extract does not contain large amounts of this component.

## Pollen profile

Pollen allergy can be difficult to diagnose, even with seasonal collation and clinical tests. In skin prick tests patients often react with multiple airborne allergens. Analysis by molecular methods can establish if the symptoms are due to true polysensitization or arise from cross reactions with panallergens such as polcalcins or profilins. Polcalcins are calcium-binding proteins which are found in many types of pollen, while profilins are actin-binding proteins which are present in pollen and in many plant foods. Both produce a high sensitization rate in pollen-allergic patients.

In Europe birch and sweet-grass pollen allergies are the most common pollen sensitizations. These two allergies can be accurately diagnosed and differentiated using the EUROLINE DPA-Dx Pollen 1 profile (Figure 1). This assay combines whole extracts, specific marker molecules and cross reactive components from birch and timothy grass, yielding a detailed patient profile. Reactions with the species-specific major allergens Bet v1 from birch and Phl p1 and/or Phl p5 from timothy grass are evidence of a primary sensitization and an indication for specific immunotherapy. Reactions with the polcalcins Bet v4 and Phl p7, the profilins Bet v2 and Phl p12 and the isoflavone reductase component Bet v6 indicate cross reactions. Diagnosis using this profile can guide decision-making on the suitability of immunotherapy for the patient and predict its chances of success.

## Comprehensive system

The DPA-Dx profiles described here are part of the established EUROLINE allergy system, which provides efficient multiparameter analysis of IgE antibodies against up to 54 allergens in parallel. The system encompasses over 400 different allergens and allergen components, which are combined into indication-oriented, country-specific or region-specific profiles. Indication areas encompass inhalation, food, atopy, insect venoms, cross reactions and pediatric allergies. All profiles include an integrated CCD (cross-reactive carbohydrate determinant), unique to this system, to aid the interpretation of cross reactions.

The EUROLINE test procedure is fast and easy and requires only a small amount of serum (100 µl to 400 µl), making it ideal for pediatric diagnosis. The immunoblots can be processed manually or fully automatically using the EUROBlotOne device. This compact table-top device (Figure 2) automates the entire immunoblot procedure from sample entry to report release. Results are evaluated, interpreted and archived by the user-friendly EUROLineScan software.

## Perspectives

Allergies are a major health and socioenonomic burden worldwide, but especially in industrialized nations. More than 40% of the population in Europe now suffers from at least one form of allergy. Children are frequency affected by atopic dermatitis, allergic rhinitis and allergic asthma. Molecular allergology systems represent the future of allergy diagnostics as they can pinpoint the precise trigger of the allergy, thus facilitating therapy decisions and risk assessment. The EUROLINE DPA-Dx test systems described here focus on some of the most common and potentially most severe allergies, including peanut, milk, egg, insect venoms, and birch and grass pollen. Further profiles for other clinically important allergies are in development.

## Reference

1. Matricardi P. and Hofmaier S. Allergy diagnostics – Targeted therapy through molecular multiparameter tests. Forum Sanitas (2015) 3: 23-25

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Figure 2. EUROBlotOne device