

# MULTIPLEX DETECTION OF SPECIFIC IgE

## Comprehensive allergy diagnostics for a global epidemic

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**F**rom pollen to peanuts and pets, allergies are on the increase worldwide. Around 40 per cent of the population in industrialised countries now suffers from at least one allergic ailment. Children, in particular, are frequently affected by atopic eczema, hay fever and allergic asthma. Moreover, many individuals are polysensitised, making diagnosis challenging. In vitro tests for specific IgE antibodies play an essential role in allergy diagnostics, identifying the causative substance with a simple blood test. Cutting-edge allergy tests based on individual allergen components (proteins, also called defined partial allergens) provide the most in-depth analysis and allow primary sensitisations to be differentiated from cross reactions. This accurate diagnosis is crucial for selecting the most suitable specific immunotherapy and for assessing the risk of severe allergic reactions. In the case of food allergies, precise identification of the allergy trigger can prevent patients from needlessly restricting their diet.

### Triggers of Allergic Sensitisation

In a type I hypersensitivity, specific IgE antibodies against a normally innocuous substance such as a pollen, food or insect venom are formed. Once an individual is sensitised, symptoms can manifest shortly after the next contact with the allergen. If a systemic allergic reaction occurs, serious or even life-threatening complications such as anaphylactic shock can develop. Atopy describes a hereditary disposition to developing allergic reactions such as allergic asthma, rhinitis or dermatitis, including atopic eczema.

Inhalation allergies can be triggered by seasonal allergens, such as pollens from trees, grasses and weeds, or by all-year-round indoor allergens, such as house dust mites, domestic animals and mould spores. Symptoms of inhalation allergy include rhinitis, conjunctivitis and allergic asthma. Allergic rhinitis is becoming more frequent worldwide, with a prevalence of 10 to 40 per cent in different regions.

Food allergies manifest with symptoms such as burning or itching in the oral cavity, nausea, gastrointestinal spasms, diarrhoea and skin rashes. Severe attacks can also lead to asthma, breathlessness, increased heart rate, panic attacks, confusion and in rare cases anaphylaxis. The most common foods causing allergic reactions are peanuts, soy, wheat, shellfish, fish, milk, eggs and tree nuts. Allergy to sesame is an emerging concern. Primary food sensitisations have a prevalence of 5 to 10 per cent in babies and infants and 1 to 5 per cent in adults.

Insect venom reactions can occur minutes to hours after the insect sting. Systemic reactions include urticaria, swelling, itching and redness at sites other than the puncture, swelling of the throat or tongue, difficulty breathing, nausea, gastrointestinal cramps, neurological deficiencies with confusion, dizziness and gait disorder, as well as raised pulse and fall in blood pressure. Insect venom reactions can also lead to anaphylactic shock and thus be life-threatening. The prevalence

of insect venom allergy is estimated to be 1 to 7 per cent, for example, in central Europe.

### In vitro Diagnostics

In vitro tests complement conventional diagnostic methods such as the skin-prick test and are essential for optimal patient management and therapy decision-making.

Measurement of total IgE serves as a general screening test to indicate if an allergic reaction may occur. This test aids the differentiation of allergic and intrinsic asthma, allergic and vasomotor rhinitis, or atopic and seborrheic dermatitis. Total IgE can be determined quickly and reliably, in patient serum using ELISA.

Determination of specific IgE against particular allergens helps to identify the triggers of an allergic reaction. Specific IgE can be measured by in vitro assays based on whole extracts of the allergen source or individual proteins thereof. Extract-based assays provide a screening for IgE antibodies against the respective substance. However, cross reactions may occur due to structural similarities between the proteins present in different sources. For example, cross reactions can occur between different pollens, between pollens and foods, or between different insect venoms.

Assays based on purified partial allergens identify the precise allergy-causing proteins, enabling discrimination of primary sensitisations from cross reactions. Moreover, they allow differentiation of reactions to high- and low-risk components, aiding assessment of the patient's risk of severe allergic reactions. These allergen component-resolved diagnostics are essential for decision-making on specific immunotherapy and the necessity of equipping patients with an emergency set.

### Multiparameter IgE Detection by Immunoblot

Immunoblots are highly suited to detection of specific IgE, as they enable many different allergens to be investigated simultaneously. For example, the EUROLINE system provides immunoblots with up to 54 allergens on one test strip. The use of individual membrane chips means that allergens with different properties can be combined on one test strip and profiles compiled according to the exact application.

The EUROLINE tests are simple to

perform, with a choice of incubation protocols for different requirements. For example, the time-optimised protocol yield results in less than three hours, while the volume-optimised protocol requires only small serum volumes, e.g. 100 µl, making it ideal for use in paediatrics. An indicator band on each strip verifies correct performance of the test. Results are evaluated and archived automatically using the EUROLineScan software and issued in standardised EAST classes.

### Cross-reactive Carbohydrate Determinants (CCDs)

Many allergens are glycoproteins, containing oligosaccharide side chains attached to the protein framework. Some patients develop specific IgE antibodies against these carbohydrate structures, known as cross reactive carbohydrate determinants (CCDs). These reactions generally have no clinical relevance but can complicate the interpretation of results in in vitro diagnostics. For this reason, it is helpful to investigate IgE antibodies against CCDs as part of allergy diagnostics to provide additional information on the patient's sensitisation profile. All EUROLINE profiles are equipped with a band of CCD to aid interpretation of positive results. If anti-CCD IgE antibodies are present in patient serum, they can be removed using a special anti-CCD absorbent and the analysis repeated. Anti-CCD antibody reactions are, however, only possible in diagnostics based on particular allergen extracts and native components; they do not occur with recombinantly produced components, as these proteins do not undergo posttranslational glycosylation.

### Comprehensive and Targeted Profiles

Whole allergen extracts are available for an enormous range of allergen sources and enable fast and comprehensive screening in allergy diagnostics. The EUROLINE range includes many indication-oriented profiles (e.g. food, inhalation, atopy, insect venoms or paediatrics), as well as region-specific profiles, which consider local allergen exposure. For example, various profiles focus on food or inhalation allergens in the Middle East region, the Gulf states, the Maghreb, Iran, Lebanon, the Mediterranean region, Turkey, India, China or South East Asia.

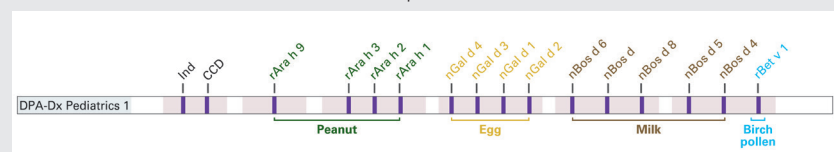
The EUROLINE range also includes assays for defined partial allergen diagnostics (DPA-Dx). These combine species-specific marker molecules with cross-reactive panallergens for highly differentiated diagnostics. Comprehensive profiles are available for peanut, insect venoms, paediatric food allergies, pollens, and Mediterranean-specific inhalation allergens.

In particular applications, extracts and defined partial allergens are combined in one profile. This enables parallel investigation of sensitisations to different whole allergens and precise characterisation of important allergic reactions in one test.

### Example: Paediatric Food Allergies

The component-resolved profile EUROLINE DPA-Dx Paediatrics 1 provides detailed diagnostics of the most common food allergies in childhood, by combining key partial allergens from milk, egg and peanut in one multiplex test (Figure 1). ►

▼ FIGURE 1. EUROLINE DPA-Dx Paediatrics profile



▼ FIGURE 2. EUROLINE Food Lebanon 3 profile



Reactions to the heat-stable protein Bos d 8 (casein) from cow's milk indicate a persistent sensitisation encompassing raw and cooked milk, milk products as well as casein-containing products such as chocolates and potato chips. Patients with reactions to the heat-sensitive milk proteins Bos d, Bos d 4, Bos d 5 and Bos d 6, on the other hand, may tolerate cooked milk, as well as yogurt, cheese and bakery products.

Similarly, high levels of IgE antibodies against the heat-stable protein Gal d 1 from egg are associated with a persistent sensitisation, and patients should avoid both raw and cooked eggs. Patients with reactions against the heat-sensitive egg proteins Gal d 2, Gal d 3 and Gal d 4 may tolerate cooked eggs.

In peanut allergy, reactions against the seed storage proteins Ara h 1, Ara h 2, and Ara h 3 and the lipid transfer protein Ara h 9 carry a high risk of a severe reaction. The severity of the reaction is, moreover greater when multiple high-risk components are involved. Patients at high risk must strictly avoid peanuts, even in trace amounts, and


always carry an emergency kit. A reaction with the birch pollen component Bet v 1, which is homologous to the peanut component Ara h 8, indicates that the peanut-allergy symptoms are due to a pollen-associated cross reaction. In this case, the risk of anaphylaxis is extremely low, and a strict peanut-free diet is not absolutely necessary. If specific immunotherapy against the pollen allergy is undertaken, this would most likely additionally alleviate the peanut symptoms.

### Example: Food Allergies in the Middle East Region

The region-specific profile EUROLINE Food Lebanon 3 focuses on foods which commonly cause allergies in this region (Figure 2). The profile contains extracts from 34 different foods, encompassing fish, shellfish, egg, cow's milk, flour, yeast, fruits, vegetables, nuts and meats. In addition, five components from cow's milk are also included, namely Bos d, Bos d 4, Bos d 5, Bos d 6 and Bos d 8. The profile thus enables a comprehensive analysis of sensitisations to an extremely diverse range of foods, and, at the same

time, an in-depth characterisation of allergic reactions to cow's milk.

### Perspectives

The burden of allergic diseases worldwide is anticipated to increase significantly in the future, from one billion people today to four billion by the 2050s. In vitro tests for specific IgE are non-invasive and risk-free for the patient and provide important support in the diagnosis of allergies. These simple blood tests are especially useful in countries with limited healthcare resources for more labour-intensive and skilled tests such as the double-blind placebo-controlled food challenge. The advent of component-resolved diagnostics has ushered in a new era of allergy diagnostics, enabling targeted risk assessment, therapy decision-making and patient monitoring. The identification of proteins targeted by specific IgE is, moreover, an ongoing process. Thus, these state-of-the-art allergy diagnostics will continue to play an increasingly prominent role in the future, as more allergies become characterised at the molecular level. 

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## DPA-Dx – a new dimension in allergy diagnostics

Identification of the allergy-triggering components using defined partial allergen diagnostics (DPA-Dx):

- Risk assessment for the severity of allergic reactions
- Selection of the most suitable specific immunotherapy
- Discrimination of (CCD-dependent) cross reactions from multiple sensitisations

Insect venoms	Food allergies (pediatrics)	Peanut	Pollen	Pollen Southern Europe
				 <b>NEW</b>

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