Molecular allergology – probing deeper into the triggers of allergies

Molecular allergology is a cutting edge technology that enables the triggers of allergies to be characterized to a new level of detail. Two new component-resolved immunoblot test systems provide in-depth profiling of allergic reactions against birch and grass pollens and against bee and wasp venoms. The molecular tests supplement the established Euroline allergy range, which comprises a comprehensive spectrum of application-oriented profiles designed for use in any diagnostic laboratory.

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Advanced diagnostic approach

Molecular allergology or component-resolved diagnostics is a novel approach to allergy diagnostics, whereby allergen components are used for specific IgE detection in place of the usual whole extracts. Defined partial allergen diagnostics (DPA-Dx) introduce a new dimension to differential allergy diagnostics.

Precise, in-depth profiling

The raw allergen preparations of substances such as pollen that are traditionally used for in vitro allergy diagnostics are generally not well characterized and are thus difficult to standardize. In contrast, the allergenic targets used in molecular allergology tests are defined recombinant proteins, which are capable of delivering precise information about the source of sensitization. The in-depth profiling enables allergologists to:

- Identify disease-causing allergens
- Assess the risk of cross reactions
- Determine patients' suitability for specific immunotherapy

Multiple pollen sensitizations

Pollen allergies are the most frequently occurring inhalation allergies, with sensitizations to birch and grass pollen as the most common ones. Typically, patients with multiple pollen sensitizations suffer from rhinitis, conjunctivitis and allergic asthma. The allergen extract-based determination of specific IgE antibodies encompasses sensitizations against major allergens and cross reacting minor allergens.

The Euroline DPA-Dx Pollen 1 profile (Figure 1) combines the major and minor allergens of birch (Bet v1, Bet v2, Bet v4, Bet v6) and timothy grass (Phl p1, Phl p5, Phl p7, Phl p12), allowing the differentiation of pollen cross reactions from true multiple pollen sensitizations.

The efficacy of the assay has been confirmed by clinical studies. In one study the test successfully confirmed sensitizations to birch or grass pollen in 77 patients with clinically and anamnestically diagnosed allergies (1), and in a further study the test verified allergic reactions in 44 patients with birch and grass pollen double sensitizations (2). Furthermore, the test system correlated well with comparable commercial assays, demonstrating an EAST class correlation of 95-100% for each of the allergen components.

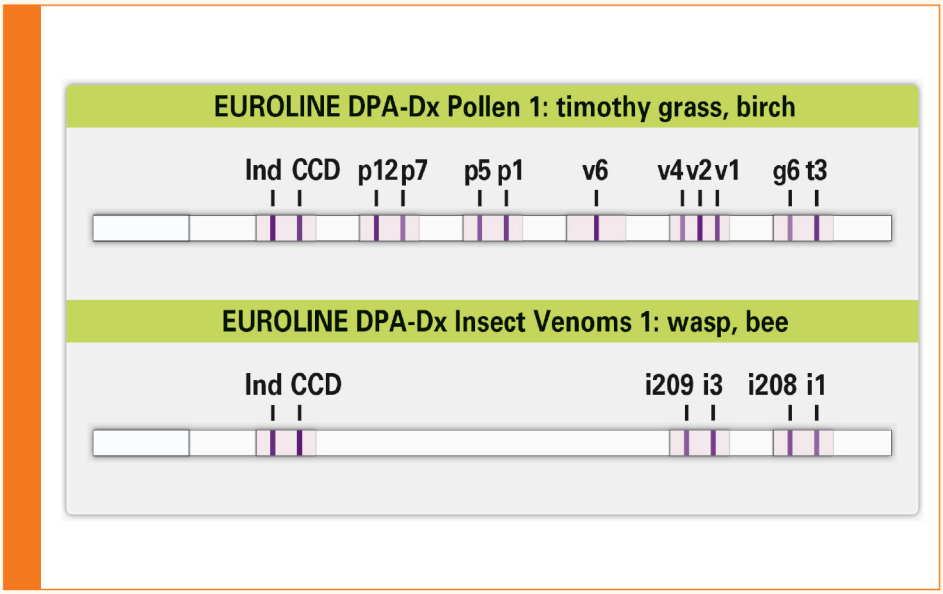

Figure 1. Euroline DPA-Dx profiles

Bee and wasp venom allergies

Bee and wasp venom stings can pose a problem in the summer months. Whereas a normal reaction to a sting involves local swelling, itching and reddening, persons with an allergy can develop severe systemic reactions, including anaphylactic shock. Bee and wasp venom reactions can be identified using the allergen components i208 (bee venom) and i209 (wasp venom). i208 represents the main bee venom marker rApi m1 from the honey bee (Apis mellifera) and i209 is the main allergen rVes v5 from the common wasp (Vespula vulgaris). Both preparations are free of cross-reactive carbohydrate determinant (CCD), providing higher reliability in result interpretation. The DPA-Dx analysis allows true double sensitization to be distinguished from cross reactions between insect venoms. The Euroline DPA-Dx Insect Venoms 1 profile (Figure 1) provides the recombinant antigens i208 and i209 together with the corresponding extracts i1 (bee venom) and i3 (wasp venom), allowing an efficient and comprehensive investigation of bee and wasp venom sensitizations with one test.

Fast and easy test procedure

The molecular allergology immunoblot tests are fast and simple to perform and are suitable for use in any diagnostic laboratory. The test procedure is based on established Euroline technology and consists of three basic steps: serum incubation (60 min), conjugate incubation (60 min) and chromogen substrate incubation (10 min). The in-between washing steps are short, and the entire procedure can be completed in 2.5 to 3 hours. All reagents are ready to use, saving time and reducing the risk of errors.

Only small amounts of sample material, typically 400 µl, are required per test. In a special volume-optimized version of the protocol the test can be performed with as little as 100 µl of patient sample, making it ideal for use in pediatrics.

Since the allergens are configured as a line blot with related allergens grouped together, the evaluation of profiles is effortless. Results are classified according to the RAST/EAST system. All profiles additionally include an indicator band of CCD to aid interpretation

Figure 2. EUROBlotOne

of the relevance of specific IgE results, for example in cases where positive IgE reactions are inconsistent with the clinical picture.

Fully automated processing

The standardized design of Euroline test strips allows automated processing using immunoblot incubators such as the EUROBlotOne (Figure 2). This advanced system automates the entire Euroline procedure from sample entry to report release. The compact, tabletop device has a high walkaway capacity: up to 44 strips can be incubated per run, and different tests can be combined in one run. All dilution, incubation and washing steps are performed automatically, and the integrated barcode scanner ensures that the correct samples are pipetted. User-friendly menus provide easy navigation, and error-detection features ensure high reliability. Test strips are subsequently digitalized using a special camera module.

Results are then automatically evaluated and archived using the worldwide-established and user-friendly EUROLineScan software. The software automatically identifies, quantifies and assigns bands, and a full results report is available within minutes of completing the incubation (Figure 3). The extensive individual data is administered and documented by the system, and all images and data are electronically archived, eliminating the need to store potentially infectious blot strips. The software can be easily integrated into LIS software, for example the EUROLabOffice system, for a smooth daily laboratory routine.

EUROIMMUN Medizinische Labordiagnostika AG

Automatic evaluation of test strips using the EUROLineScan software

	Concentration	Class
Egg white (f1)	< 0.35 kU/l	0
Egg yolk (f75)	< 0.35 kU/l	0
Milk (f2)	< 0.35 kU/l	0
Yeast (f45)	1.1 kU/l	2
Wheat flour (f4)	20 kU/l	4
Rye flour (f5)	5.5 kU/l	3
Rice (f9)	22 kU/l	4
Soybean (f14)	14.5 kU/l	3
Peanut (f13)	50 kU/l	5
Hazelnut (f17)	50 kU/l	4
Almond (f20)	19 kU/l	4
Apple (f49)	28 kU/l	4
Kiwi (f84)	2.5 kU/l	2
Apricot (f237)	15.5 kU/l	3
Tomate (f25)	7.5 kU/l	3
Carrot (f31)	5.5 kU/l	3
Potato (f35)	80 kU/l	5
Celery (f85)	22 kU/l	4
Codfish (f3)	0.47 kU/l	1
Shrimp (f23)	< 0.35 kU/l	0
CCD (CCD)	7.5 kU/l	3

Concentration [kU/l]	Class	Explanation
< 0.35	0	No specific antibodies detected.
0.35 - 0.7	1	Very weak antibodies detected, frequently no clinical symptoms where sensitisation is present.
0.7 - 3.5	2	Weak antibodies detected, existing sensitisation, frequently with clinical symptoms in the upper range of class.
3.5 - 17.5	3	Significant level of antibodies detected. Clinical symptoms usually present.
17.5 - 50	4	High level of antibodies detected. Almost always with clinical symptoms.
50 - 100	5	Very high antibody titre.
> 100	6	Very high antibody titre.

Figure 3. EUROLineScan result report

EUROLINE PROFILE AREAS:

- **Inhalation**: grass, tree and weed pollens, mites, animal hair, moulds
- **Food**: egg, milk, grains, seeds, legumes, nuts, fruits, vegetables, fish, shellfish
- **Atopy**: key inhalation and food parameters for screening
- **Cross reactions**: allergens that typically induce cross reactivity
- **Insect venoms**: bee and wasp venoms
- **Pediatrics**: allergens that commonly trigger childhood allergies
- **Country or region-specific**: allergen combinations tailored to particular geographical areas

Comprehensive Euroline allergy range

The new molecular allergy tests are part of the established Euroline allergy range, which provides efficient multiparameter analysis of IgE antibodies against up to 36 different allergens in parallel. The immunoblots are composed from a wide portfolio of allergens, comprising both allergen components and native extracts which have been extensively purified and carefully quality controlled to ensure consistency. All pro-files are application-oriented, each one being designed to address a particular diagnostic inquiry.

The Euroline system offers a very competitive price per allergen, making this system the ideal choice for laboratories wanting to perform state-of-the art allergy diagnostics on a small budget.

Perspectives

The advent of molecular allergology technology represents a quantum leap for allergy diagnostics. Component-resolved allergy test systems are unrivalled in the depth of diagnostic information they deliver and hence the level of support they provide for therapeutic decision-making. The Euroline DPA-Dx range will soon be expanded to include further test systems based on this cutting-edge technology.

References

1. Weimann *et al.* 30th Annual Congress of the EAACI, Istanbul, Turkey, June 2011.
2. Weimann *et al.* 20th IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine (EuroMedLab), Milan, Italy, May 2013.