Molecular allergology: component-resolved diagnostics

Overview
Sensitisations to birch and grass pollens – two of the most frequently occurring inhalation allergies – can be characterised to a new level of detail using a novel multiparameter, component-resolved immunoblot test system from EUROIMMUN. The molecular allergology assay supplements the EUROLINE Allergy range, which comprises a comprehensive spectrum of application-oriented profiles designed for use in any diagnostic laboratory.

What is molecular allergology?
Molecular allergology or component-resolved diagnostics is a cutting-edge approach to allergy diagnostics, whereby single purified allergen components (SPAC) are used for specific IgE detection in place of the usual whole extracts. This powerful technology introduces a new dimension to differential allergy diagnostics.

How does this technology enrich allergy diagnostics?
The raw allergen preparations of substances such as pollen that are traditionally used for in vitro allergy diagnostics are generally not well characterised and are thus difficult to standardise. 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 sensitisation. The in-depth profiling enables allergologists to:
- Identify disease-causing allergens
- Assess the risk of cross reactions
- Determine patients’ suitability for specific immunotherapy

Which components are used to diagnose birch and grass pollen allergies?
The combination of purified recombinant birch pollen allergens Bet v1, Bet v2, Bet v4, Bet v6 and grass pollen allergens Phl p1, Phl p5, Phl p7, Phl p12 is sufficient to identify all cases of birch and grass pollen sensitisation. The EUROLINE SPAC Pollen 1 Profile (Figure 1) contains all of these allergens, allowing these allergies to be diagnosed and characterised with a single incubation.

The efficacy of the assay has been confirmed by clinical studies. The assay successfully verified sensitisations to birch or grass pollen in 77 patients with clinically and anamnestically diagnosed allergies (1). Furthermore, the test system correlated well with comparable commercial assays, demonstrating an EAST class correlation of 95-97% for each of the allergen components.

How is the immunoblot test performed and evaluated?
The immunoblot test is fast and simple to perform and is suitable for use in any diagnostic laboratory. The 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-optimised version of the protocol the test can be performed with as little as 100 μl of patient sample, making it ideal for use in paediatrics.

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 cross-reactive carbohydrate determinant (CCD) to aid interpretation of the relevance of specific IgE results, for example in cases where positive IgE reactions are inconsistent with the clinical picture.

Can the procedure be automated?

The entire EUROLINE procedure from incubation to evaluation and archiving of results can be automated using innovative instruments and software designed and developed by EUROIMMUN.

Incubation of immunoblot strips can be fully automated using the EUROBlotMaster (Figure 2). Up to 44 strips can be incubated per run, and different tests can be combined in one run. User-friendly menus provide easy navigation, and integrated error-detection features ensure high reliability. Test strips are subsequently digitised using a special camera (EUROBlotCamera) or scanner (EUROBlotScanner) module (Figure 3). An advanced version of the EUROBlotMaster, which includes an integrated camera and provides fully streamlined processing, will be introduced soon.

Evaluation and archiving of results can be entrusted to the EUROLineScan program. The software automatically identifies, quantifies and assigns bands, and a full results report is available within minutes of completing the incubation (see EUROLineScan results sheet at the end of the article). The extensive individual data is administered and documented by the system, and all images and data are tronically archived, eliminating the need to store potentially infectious blot strips. EUROLineScan can be easily integrated into LIS software, for example the EUROLabOffice system, for a smooth daily laboratory routine.

What other EUROLINE allergy profiles are available?

The new molecular allergy test is 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 elecportfolio over 200 different allergens, comprising both SPAC and native extracts which have been extensively purified and carefully quality controlled to ensure consistency. All profiles are application-oriented, with each designed to address a particular diagnostic inquiry.

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
- **Paediatrics**: allergens that commonly trigger childhood allergies
- **Country or region-specific**: allergen combinations tailored to particular geographical areas

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 SPAC range will soon be expanded to include further test systems based on this cutting-edge technology.

Reference

EUROLineScan result sheet

Patients IDs: LB15
Name: LB15
Date of birth: 01.05.1945
Lab number: 234587
Number: 7
Test: Food-224-29
Strip number: A080630AA
Testkit: A080630AA
Date of receipt: 30.07.2008
Results from: 14.08.2008

Automatic evaluation of test strips using the EUROLineScan software

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

Signature: ____________________________