Allergens
Food Allergy - A Life-threatening Risk

Food allergy affected individuals cannot tolerate the consumption of certain foods. Their body reacts with a specific immune response to the uptake of particular proteins. The symptoms range from mild hives and reactions in the mouth, tongue and throat (swelling) as well as gastrointestinal symptoms (nausea, vomiting, diarrhea) to respiratory symptoms (allergic asthma). The worst case can be life-threatening anaphylactic shock requiring immediate medical treatment.

Therapeutic approach
In general, food allergy cannot be healed in most patients. Although first success has been reported for hyposensitization therapies, this strategy is still controversial. The only suitable approach for affected patients is to avoid the allergen triggering foods. Thus, labeling of food products and reliable analytical methods are required. This has motivated BIOTECON Diagnostics GmbH to develop highly sensitive and specific molecular detection methods for more trusted food allergen labeling.

The Kiss of Death!
In 2002, American physicians reported the case of a 20 year old woman with a known allergy to crustaceans. She nearly died due to allergic symptoms after kissing her boyfriend: He had consumed shrimp some hours before! The kiss transferred traces of allergenic proteins and lead to an intense immune reaction. The symptoms were an angioedema of the lips, a swelling of the throat, FLUSH-symptoms, urticaria, abdominal convulsion, respiratory filibuster and dyspnea.

Source: Steensma, DP: The kiss of death: a severe reaction to a shellfish induced by a good-night-kiss, Mayo Clinic Proceedings, 78(2002), P.221-222
Testing For Food Allergens – Legal Regulations

Modern food industry makes it difficult to identify if a particular product contains a relevant allergen. "Hidden" allergens – allergenic food components which are not identifiable at first glance – are a risk for patients. For example, celery is widely used in spices, convenience products, and ready-to-eat food. Thus, different countries released regulations for the labeling of different allergenic compounds in food products.

- **USA**
  - FALCPA (Food Allergen Labeling and Consumer Protection Act)
  - 8 allergenic foods are subject to labeling
  - Labeling if even cross contact to allergenic substances is possible
  - No thresholds for labeling

- **EUROPEAN UNION**
  - Regulation EU/1169/2011,
    - Directives 2003/89/EU
    - and 2007/68/EU
  - 14 allergenic foods are subject to labeling
  - Only referring to ingredients, not dealing with accidental cross-contamination and traces
  - No thresholds for labeling

- **AUSTRALIA/NEW ZEALAND**
  - VITAL (Voluntary Incidental Trace Allergen Labeling) System
  - 10 allergenic foods are subject to labeling
  - Comprehensive support for risk assessment and labeling of intentional and possible accidental allergens
  - Threshold system for labeling
  - Commercial system

**Special Case GLUTEN:**
In contrast to “gluten-free” labeling of Codex Alimentarius, EU regulation clearly requires labeling of specified gluten-containing cereals. This difference is based on the diseases which are the foundation for the regulations. While the EU directive refers to allergy, Codex Alimentarius aims for coeliac disease affected persons.

- **Risk of cross-contamination is treated very differently**
- **Threshold levels are often lacking**
Most foods have an allergenic potential

The majority of allergenic reactions are caused by a limited number of food allergens

The “Big Eight”

Only a few food materials like rice, leaf lettuce or artichokes do practically never cause allergic reactions. More than 70 different foods have been reported to trigger allergic symptoms. However, the “Big Eight” are responsible for about 90% of all allergenic reactions to food:

Wheat, Peanut, Soya, Milk, Egg, Nuts, Crustacean, Fish

Extended allergen labeling in the EU

The EU defines 14 different food components, which are subject to labeling (see Table 1). Instead of only wheat, the EU requires labeling of gluten containing cereals, namely: wheat, rye, barley, spelt, oats and kamut.

Table 1. Food allergens - regional regulation and analytical approach

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Subject to labeling in</th>
<th>Detection by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (and gluten containing cereals)</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Peanut</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Soya</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Milk</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno</td>
</tr>
<tr>
<td>Egg</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno</td>
</tr>
<tr>
<td>Nuts (hazelnut, pecan nut, walnut, brazil nut, macadamia, pistachio, cashew and almond)</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Crustacean</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Fish</td>
<td>EU, CH, USA, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Celery</td>
<td>EU</td>
<td>PCR</td>
</tr>
<tr>
<td>Mustard</td>
<td>EU, CAN</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Sesame</td>
<td>EU, CH, CAN, AUS</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Lupin</td>
<td>EU</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Molluscs</td>
<td>EU</td>
<td>Immuno, PCR</td>
</tr>
<tr>
<td>Sulfide</td>
<td>EU, AUS</td>
<td>Chemical methods</td>
</tr>
</tbody>
</table>

Additional information

It is important to mention that crustacean, fish and molluscs are collective parameters: The many different species in these groups are not named separately and are not differentiated by the available testing methods. In contrast, nuts are clearly defined: almond, hazelnut, walnut, cashew, pecan nut, brazil nut, pistachio and macadamia. There is no testing method for detecting these as a collective parameter and single tests for each nut species must be used.

Abbreviations: European Union (EU), Switzerland (CH), United States of America (USA), Canada (CAN), Australia (AUS), Immunological based methods (Immuno)
Allergen Testing – Why Real-time PCR?

Food allergen analysis aims for the detection of the allergenic component in general since the pathologic potential of a sample is an individual value depending on many factors and cannot be generally determined.

Benefits of testing by real-time PCR
DNA-based real-time PCR methods have been established and accepted in food analysis for many years now. They are used worldwide for the detection and quantification of bacteria, viruses, animal species, GMOs and allergens. Real-time PCR detects and amplifies a target DNA sequence using specific oligonucleotide primers and fluorescence probes. This technology enables a very specific, highly sensitive and – in combination with a suitable reference material – even quantitative detection of the target organism, including food allergens. Real-time PCR is suitable to detect all allergens except milk and egg (low DNA content, PCR is not tissue specific) as well as sulfide/sulfate (chemical parameter). Since DNA is a very stable molecule, PCR can be applied also in highly processed food matrices. Sample preparation is identical for all parameters. This enables time and cost saving workflows. The detection of allergenic food components can easily be integrated in existing PCR routines.

In contrast to protein-based allergen detection methods, real-time PCR is more specific, has a higher dynamic range for quantification and delivers more consistent results.

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th>ELISA</th>
<th>Lateral Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of</td>
<td>DNA</td>
<td>Protein</td>
<td>Protein</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>High - very High</td>
<td>High</td>
<td>Medium - High</td>
</tr>
<tr>
<td>Specificity</td>
<td>Very High</td>
<td>Medium - High</td>
<td>Medium - High</td>
</tr>
<tr>
<td>Quantitative</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Uniform sample prep for all parameters</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Automation for the entire workflow from sample to result</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Direct/Indirect detection of allergenic protein</td>
<td>indirect</td>
<td>Most cases indirect</td>
<td>Most cases indirect</td>
</tr>
</tbody>
</table>

Real-time PCR is ...
... fast, specific, sensitive and safe!
Available parameters:

- Celery
- Soya
- Hazelnut
- Peanut
- Walnut
- Gluten

To protect customers effectively and ensure proper labeling, it is important to detect food allergens reliably, sensitively and specifically. In context with modern food production, it is also important that testing times are short and assays are stable and easy to handle.

**foodproof® real-time PCR kits**

BIOTECN Diagnostics has developed a series of real-time PCR kits to detect allergenic food components in all food matrices and environmental samples. Quantification is possible when used in combination with reference material. All assays are easy to use and can be applied with all common real-time PCR devices able to detect 5'-Nuclease (TaqMan®) probes (e.g. AriaMx®, LightCycler® 480, LightCycler® 96, PikoReal® 24, ABI 7500 etc.). Using one DNA extraction, different allergens can be determined within one PCR run due to harmonized PCR protocols. This saves time and costs for analysis. The methods have been developed and validated based on our long experience in food testing.

**Allergen RM 800:**

This innovative reference material contains all allergenic food components in a non-allergenic matrix. The content is adjusted precisely to 800 ppm. For quantification of food allergens, this material can be extracted in parallel with the samples and measured in a dilution series. Therewith, the allergen content of a food or raw material sample can be reliably determined.

**Technical Data:**

- 64 reactions/kit
- Ready-to-use master mix including all reagents and Taq polymerase
- 5’Nuclease probes
- Detection of target (FAM-channel) and internal control (HEX-channel)
- Limit of detection: 0.1 ppm (referring to **foodproof® Allergen RM 800**)
- Limit of quantification: 0.8 ppm (referring to **foodproof® Allergen RM 800**)
Kits for manual and automated DNA extraction are available

High-throughput: instruments for DNA extraction and PCR setup clearly increase productivity

foodproof® Sample Preparation Kits

The basis for reliable detection of food allergens by real-time PCR is efficient DNA extraction. Since allergenic food components are effective in low concentrations, it is necessary to extract and purify even small amounts of DNA from processed food and raw materials.

foodproof® Sample Preparation Kits

As perfect addition to our real-time PCR kits, the foodproof® technology comprises easy-to-use solutions for quick and effective extraction of relevant DNA. The foodproof® Sample Preparation Kit III is a column based extraction system for manual application. After thermal and chemical lysis, DNA is bound to the silica matrix of spin filter tubes and purified in several washing steps before the clean DNA is dissolved in an elution buffer. This process is easy to handle, needs little laboratory equipment and takes less than one hour.

Scale up your throughput

For a high number of samples, the foodproof® Magnetic Preparation Kit III in combination with a KingFisher Flex instrument offers a high-throughput method for DNA extraction used for food allergen detection. Up to 96 samples can be processed in approximately 30 minutes. Following cell lysis, the DNA is bound to the surface of magnetic beads, which are automatically transferred to washing and elution buffers. This reduces manual effort and speeds up analysis.

A further improvement of productivity is achieved by automated PCR setup using the foodproof® RoboSet®.
**Workflow**

**Sample Preparation**
- **Start**
- **Manual**: approx. 10 min
- **Automated**: approx. 90 min

**Detection and Quantification Kits**
- **A 500 13**: Allergen RM 800
- **R 302 60**: foodproof® Celery Detection Kit
- **R 302 61**: foodproof® Soya Detection Kit
- **R 302 62**: foodproof® Hazelnut Detection Kit
- **R 302 63**: foodproof® Peanut Detection Kit
- **R 302 64**: foodproof® Gluten Detection Kit
- **R 302 65**: foodproof® Walnut Detection Kit

**Sample Preparation Kits**
- **S 400 06.1**: foodproof® Sample Preparation Kit III
- **S 400 13**: foodproof® Magnetic Preparation Kit III, 96 isolations
- **S 400 13 L**: foodproof® Magnetic Preparation Kit III, 480 isolations

**Instruments**
- **D 220 01**: KingFisher® Flex
- **Z 600 06**: KingFisher® Flex Consumable Pack
- **D 230 01**: foodproof® RoboSet®
- **D 230 01 HE**: foodproof® RoboSet® High-End Version

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**BIOTECON Diagnostics GmbH**
Hermannswerder 17
14473 Potsdam
Germany
Phone: +49 (0) 331-2300-200
Fax: +49 (0) 331-2300-299
bcd@bc-diagnostics.com
www.bc-diagnostics.com

**Analysis Results in < 2.5 h**

**PRODUCTS & SERVICES**
- foodproof & microproof Detection Kits
- DNA Extraction Kits
- Real-time PCR Cyclers
- Automation
- Service Lab