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A T C   G C T T C A T   C A T C C C T A T C   G C T T C C A T C T G C T T C A   T A T C C A
C G T A T C   G C C A T C T T C A T C C G T A T C T G C T T C C A T C T G C T   C C A T C T
A T C C G T A T C T C C A T   T T C A T C C G T A T C   C T T C C A
C A T C G C T T T G C T T C C A T C C C T A T C G C   T G C T T C
C T A T C   G C T A T C A T C C A T C C C T A T C T G C T T C C A T C T G C T   A T C A T C

BIOTECON Diagnostics

BIOTECON Diagnostics

Allergens

Food Allergy – A Life-threatening Risk

Allergens can be a deadly food safety risk

Effective dose may be very low

Proper allergen labeling can save lives

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.

upward tendency. There are a lot of allergen triggering food components.

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.

220 - 250 million people are affected by food allergies

4-8 %

2-4 %

Children

Adults

Food allergies worldwide in 2014, source: WAO.

Food allergy: a worldwide disease

Food allergy is not a rarely occurring phenomenon: different sources estimate the prevalence of food allergy to be 2-4 % among grown-ups and 4-8 % among children with an

!

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.

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.

!

Different countries have implemented rules for labeling of food allergens

Risk of cross-contamination is treated very differently

Threshold levels are often lacking

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

Directive 2013/89/EU, 2007/68/EU, 2000/13/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

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A T C C C T A T C   G C T T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T T
T C A T C C G T A T C T C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C T G

Which Allergens Must Be Tested?

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 allergenic 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.

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.

!

Table 1: Food allergens - regional regulation and analytical approach

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

Abbrevations: European Union (EU), Switzerland (CH), United States of America (USA), Canada (CAN), Australia (AUS), Immunological based methods (Immuno)

C A T C G T A T C C A T C C C A T C A T C G T A T C C A T C C C T A C A T C A T C G T A T C C A T C C A T C A T C G T A T C C A T C C C T A C A T C A T C G T A T C C A T C C
C A T C C C T A T C   G C T T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T
C T T C A T C C G T A T C T C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C T

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.
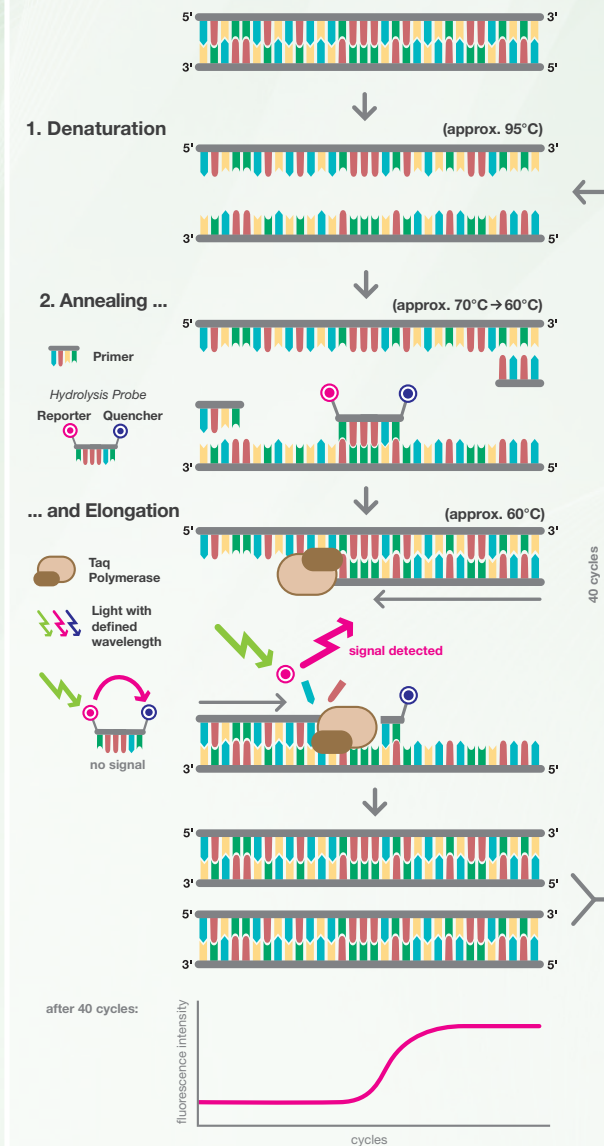
Benefits of testing by real-time PCR

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.

!

Real-time PCR is ...

... fast,
specific,
sensitive
and safe!

Table 2: Allergen detection methods - properties and limitations

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

Real-time PCR - Hydrolysis Probes

1. Denaturation (approx. 95°C)

2. Annealing ... (approx. 70°C → 60°C)

Primer

Hydrolysis Probe
Reporter Quencher

... and Elongation (approx. 60°C)

Taq Polymerase

Light with defined wavelength

signal detected

no signal

40 cycles

after 40 cycles:

fluorescence intensity

cycles

A T C G T A T C C A T C C C A T C A T C G T A T C C A T C C C T A C A T C A T C G T A T C C A T C C A T C A T C G T A T C C A T C C C T A C A T C A T C G T A T C C A T C C C
A T C C C T A T C   G C T T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T T
T T C A T C C G T A T C T C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C T G

foodproof® **Allergen Detection Kits**

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**
BIOTECON 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.

foodproof® **Allergen Detection Kit**

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 **food**proof® Allergen RM 800)
- Limit of quantification: 0.8 ppm
  (referring to **food**proof® Allergen RM 800)

C A T C G T A T C C A T C C C A T C A T C G T A T C C A T C C C T A C A T C A T C G T A T C C A T C C A T C A T C G T A T C C A T C C C T A C A T C A T C G T A T C C A T C C
C A T C C C T A T C   G C T T A T C C A T C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T A T C C A T C C C C T A T C   G C T T C A T   C A T C C C T A T C   G C T
C T T C A T C C G T A T C T C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C C C A T C T T C A T C C G T A T C   G C C A T C T T C A T C C G T A T C T

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 **food**proof® technology comprises easy-to-use solutions for quick and effective extraction of relevant DNA.
The **food**proof® 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 **food**proof® 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 **food**proof® RoboSet⁺.
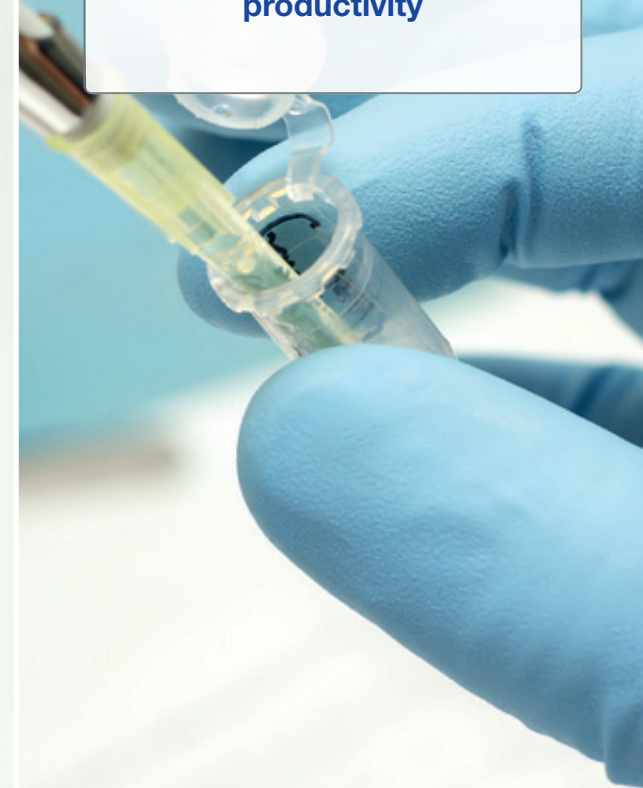
Kits for manual and automated DNA extraction are available

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

Manual DNA Extraction:

Automated DNA Extraction:

Automated PCR Setup:

foodproof®
Sample Preparation Kit III

foodproof®
Magnetic Preparation III

KingFisher® Flex

foodproof® **RoboSet⁺**

Workflow

Manual / **Automated**

Manual / **Automated**

Analysis Results in < 2.5 h

| Sample Preparation Start | DNA Extraction approx. 30 min | PCR Setup approx. 10 min | Real-time PCR Run 90 min |

PRODUCTS & SERVICES

- foodproof & microproof Detection Kits
- Real-time PCR Cycler
- foodproof DNA Extraction Kits
- Automation
- Service Lab

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www.bc-diagnostics.com

Detection and Quantification Kits

A 500 13   Allergen RM 800
R 302 60   **food**proof® Celery Detection Kit
R 302 61   **food**proof® Soya Detection Kit
R 302 62   **food**proof® Hazelnut Detection Kit
R 302 63   **food**proof® Peanut Detection Kit
R 302 64   **food**proof® Gluten Detection Kit
R 302 65   **food**proof® Walnut Detection Kit

Sample Preparation Kits

S 400 06.1   **food**proof® Sample Preparation Kit III
S 400 13   **food**proof® Magnetic Preparation Kit III, 96 isolations
S 400 13 L   **food**proof® Magnetic Preparation Kit III, 480 isolations

Instruments

D 220 01   KingFisher® Flex
Z 600 06   KingFisher® Flex Consumable Pack
D 230 01   **food**proof® RoboSet+
D 230 01 HE **food**proof® RoboSet+ High-End Version