Detection of Food Allergens – an overview

Alergeny v potravinách a jejich diagnostika
27. duben 2012

Eva Wanzenböck

Romer Labs, Rakousko

Making the World's Food Safer®

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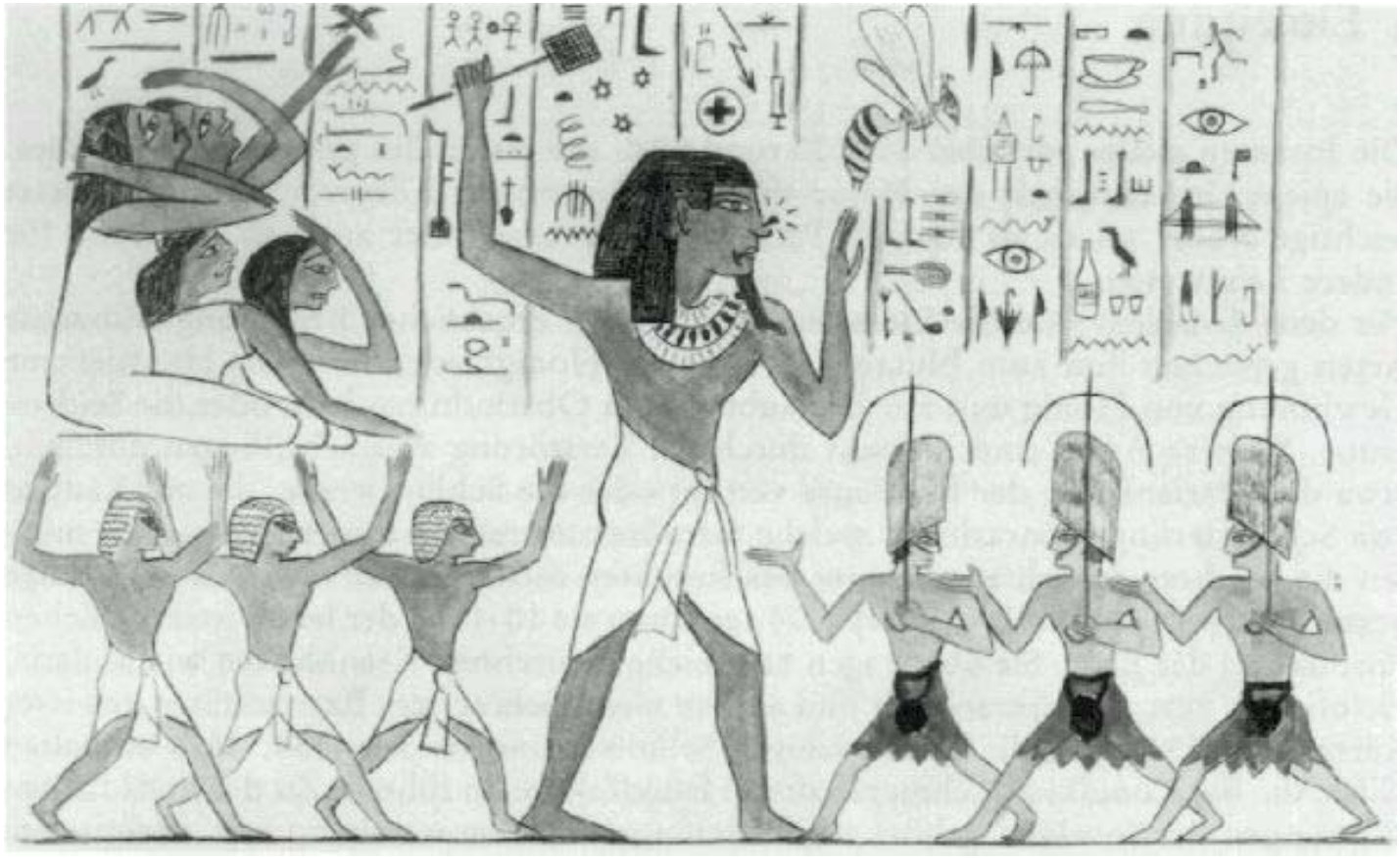
Detection of Food Allergens- an overview

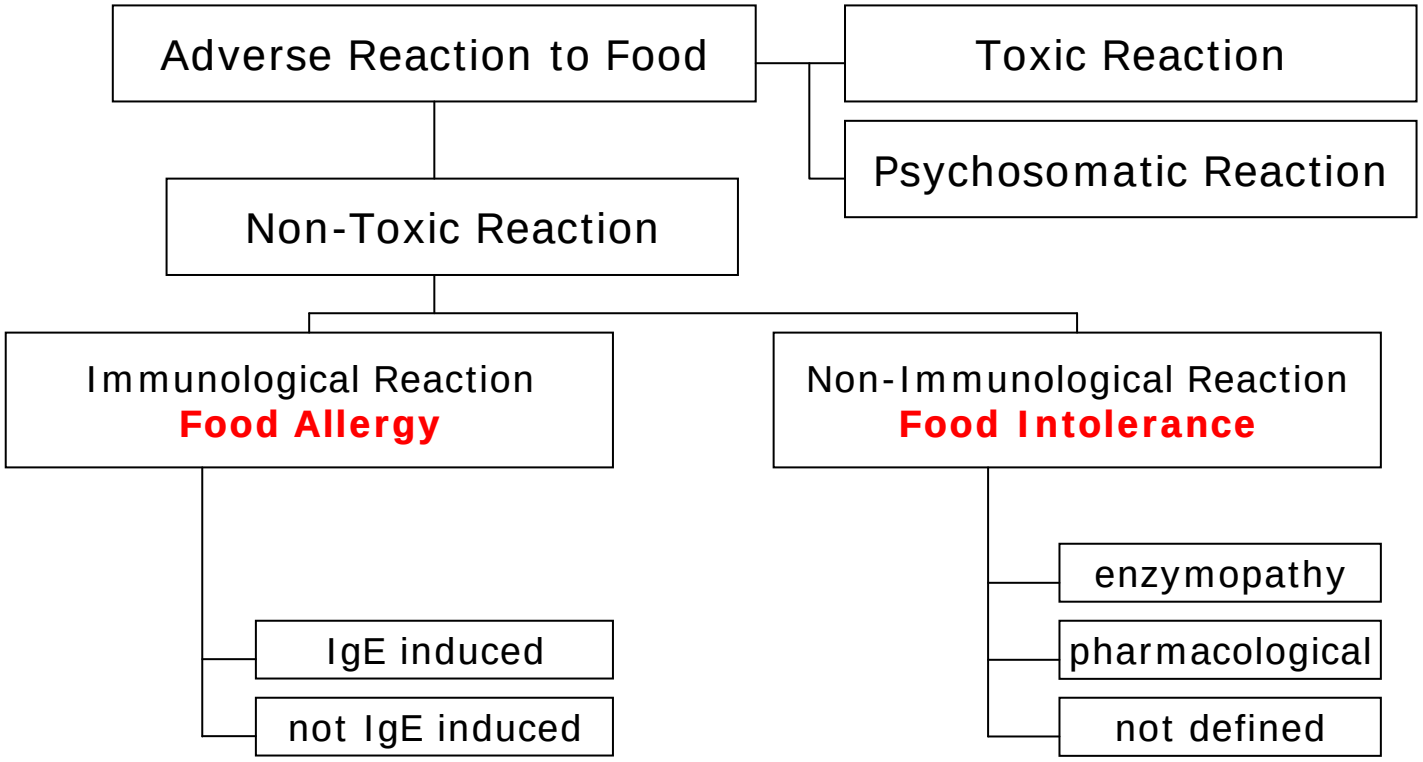
Eva Wanzenböck

1982 – 2012
30 years
Making the World's Food Safer®

Pharao Menes



Adverse Reaction to Food



Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bjorksten B, Moneret-Vautrin D, Wuthrich B. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. *Allergy 1995, 50:623-35.*

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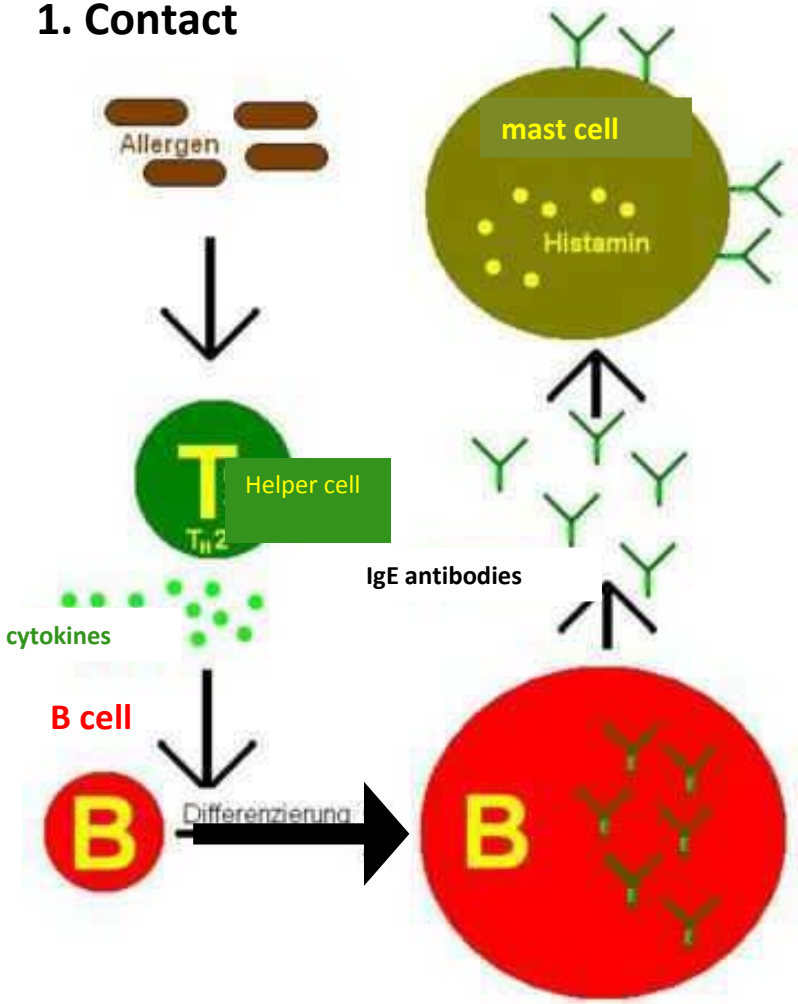
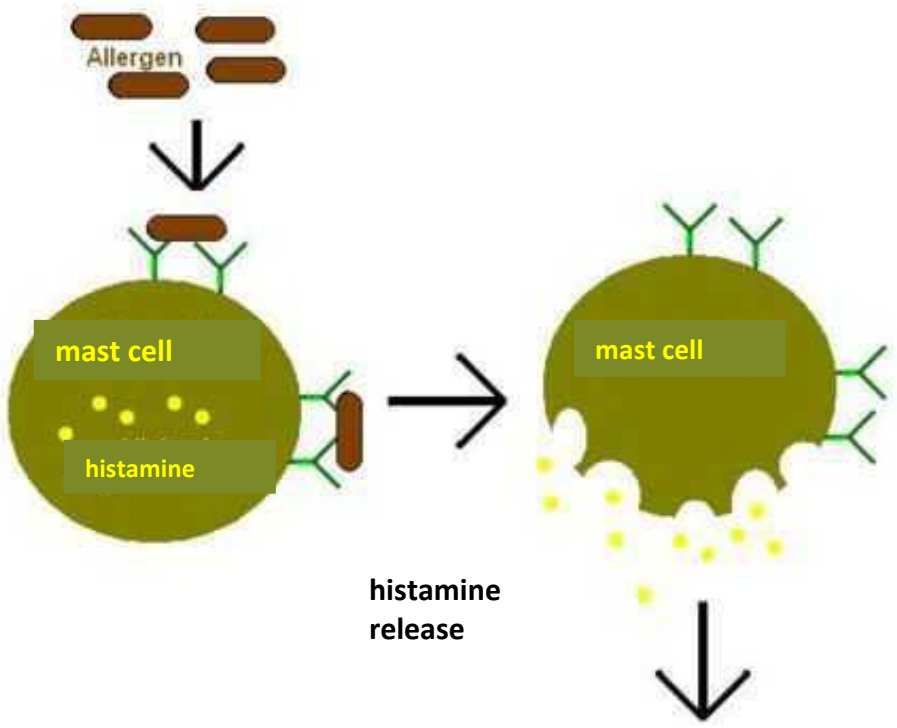
Mechanism of food allergy

1. Contact



2. Contact



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Food Allergy

Clinical Symtoms: eyes, skin, respirotory tract, etc

Global Market

Global Food Allergy & Intolerance products market worth $26 billion (2017)

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How do allergens find their way into products?

- **Recipe** (Ingredients, pre-mix ingredients)
- **Cross-contaminations**
  - Storage ingrediens
  - Production (weighing, mixing, production line – inapropriate cleaning, packaging)
  - Carry over – cleaning
  - Staff

→ Uneven distribution of allergens

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LEGISLATION

EU Directive 2003/89/EC – Annex IIIa

- **Cereals containing gluten** (i.e. wheat, rye, barley, oats, spelt, kamut or their hybridised strains) and products thereof
- **Crustaceans** and products thereof
- **Eggs** and products thereof
- **Fish** and products thereof
- **Peanuts** and products thereof
- **Soybeans** and products thereof
- **Milk** and products thereof (including lactose)
- **Nuts** i. e. Almond (*Amygdalus communis L.*), Hazelnut (*Corylus avellana*), Walnut (*Juglans regia*), Cashew (*Anacardium occidentale*), Pecan nut (*Carya illinoiesis (Wangenh.) K. Koch*), Brazil nut (*Bertholletia excelsa*), Pistachio nut (*Pistacia vera*), Macadamia nut and Queensland nut (*Macadamia ternifolia*) and products thereof
- **Celery** and products thereof
- **Mustard** and products thereof
- **Sesame** seeds and products thereof
- **Sulphur dioxide** and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO_2

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EU Directive 2006/142/EC

COMMISSION DIRECTIVE 2006/142/EC

of 22 December 2006

amending Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council listing the ingredients which must under all circumstances appear on the labelling of foodstuffs

The following ingredients shall be added to Annex IIIa of Directive 2000/13/EC:

- **Lupin** and products thereof
- **Molluscs** and products thereof

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Overview Regulations

Allergens	EU	US	Canada*	Japan	Australia / NZ
Egg	✓	✓	✓	✓	✓
Milk	✓	✓	✓	✓	✓
Fish	✓	✓	✓		✓
Crustaceans	✓	✓	✓		✓
Tree Nuts	✓	✓	✓		✓
Peanuts	✓	✓	✓	✓	✓
Wheat	✓	✓	✓	✓	✓
Soy	✓	✓	✓		✓
Celery	✓				
Mustard	✓				
Sulfites	>10 mg/kg		✓		>10 mg/kg
Sesame	✓		✓		✓
Buckwheat				✓	
Molluscs	✓				
Lupines	✓				

*Canadian legislation still a draft

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„Does she really read the ingredients listed on the label.“

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DETECTION METHODS

Methods Overview

- PCR, Real Time PCR
  - Polymerase Chain ReactionRT-PCR
- Mass Spectrometry
- ELISA
  - Enzyme linked immunosorbent assay
- LFD
  - Lateral flow device

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Polymerase Chain Reaction

- The Polymerase Chain Reaction (PCR) is an in-vitro copy machine for specific DNA sequences
- two primers (starter molecules), specific for the beginning and the end of the target DNA sequence, are needed
- building blocks of the DNA (desoxyribonucleosid-triphosphate) for the DNA-synthesis are needed
- a thermostable polymerase uses the primer and the building blocks to copy the DNA

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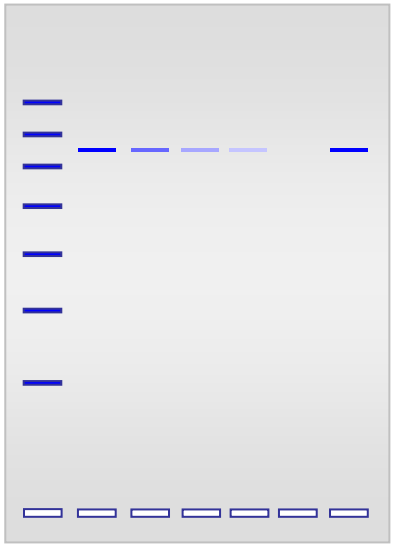
Gel Electrophoresis of PCR product



Detection (gel electrophoresis)

Principle of Hybridization and real-time PCR

- **hybridization** probes are used to prove the specific amplification of the target DNA sequence during the PCR.
- this detection method avoids false positive results.
- in the **real-time PCR** the products are detected during the amplification (real-time) by using sequence-specific or hybridization probes or non-specific dyes.
- at the present time the real-time PCR is the most exact and the most reliable method for quantitation of target copy numbers.

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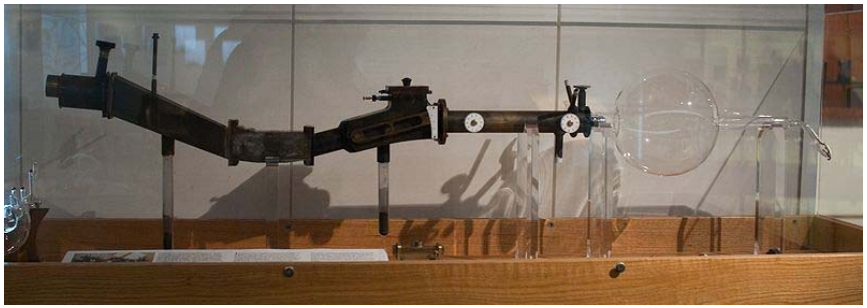
Realtime PCR

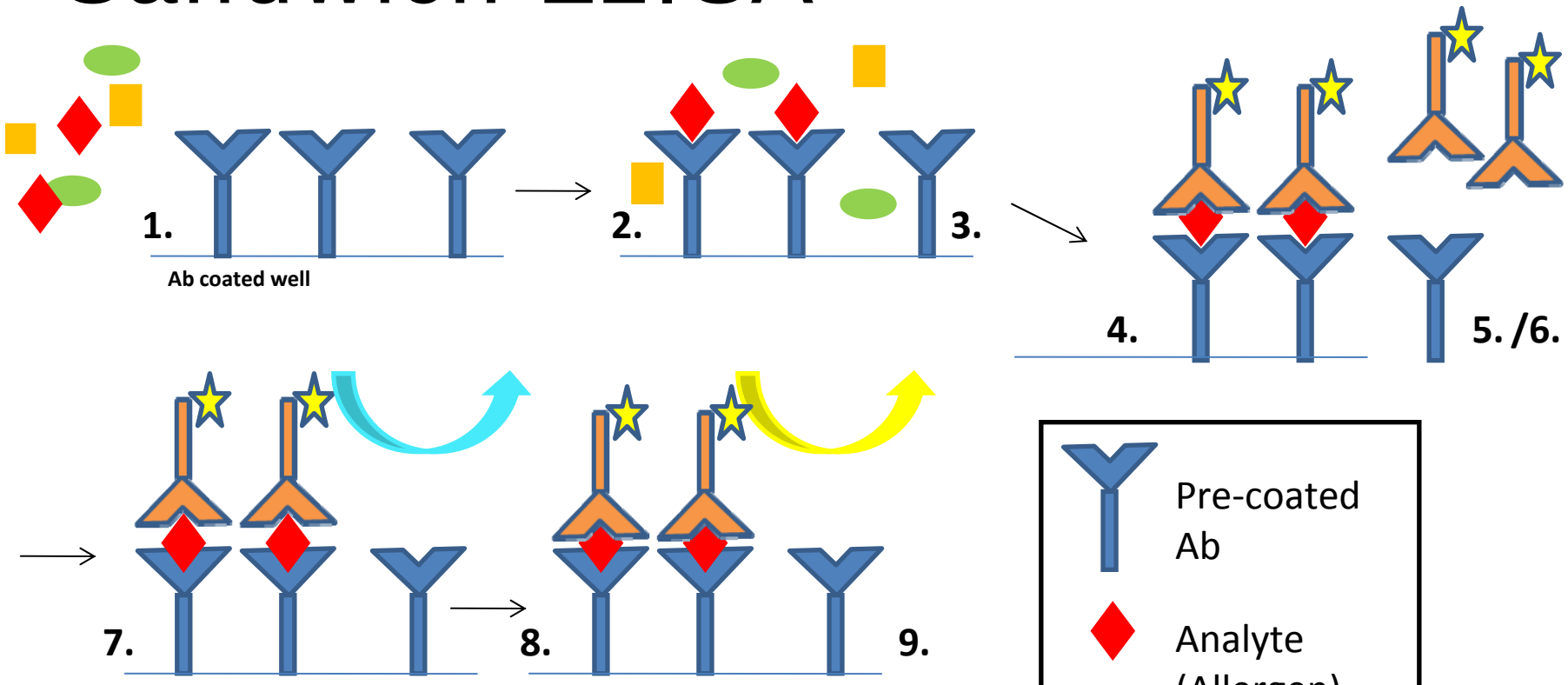
- Realtime PCR is a **quantitative** method
- Additional to common PCR there is a DNA probe that is specific to the target DNA.
- The **probe anneals to the target DNA and is fluorescing** when polymerase cuts the Reporter from the Quencher on the probe.
- During the reaction in the thermocycler the fluorescence is measured.
- The **amount of original DNA can be calculated with internal standards**.

Mass Spectrometry

- Mass Spectrometry is a technique to determine extremely accurate mass of molecules. Accurate mass is useful in a variety of different fields in science
- The key concept is ionization. The ionization process imparts a charge on the molecule which can be measured by the instrument.
- Mass Spectrometry started with the work of Sir Joseph John "JJ" Thomson. His work on conduction of electricity through ionized gasses lead to his being awarded the Nobel Prize for Physics in 1906, though his best known work on mass spectrometry came later in 1911. Later his son, John Paget Thomson, won the Nobel Prize for Physics in 1937



Sandwich ELISA



1. Add sample/standards
2. Incubation
3. Washing
4. Add conjugate
5. Incubation
6. Washing
7. Add substrate
8. Add stopp solution
9. Read

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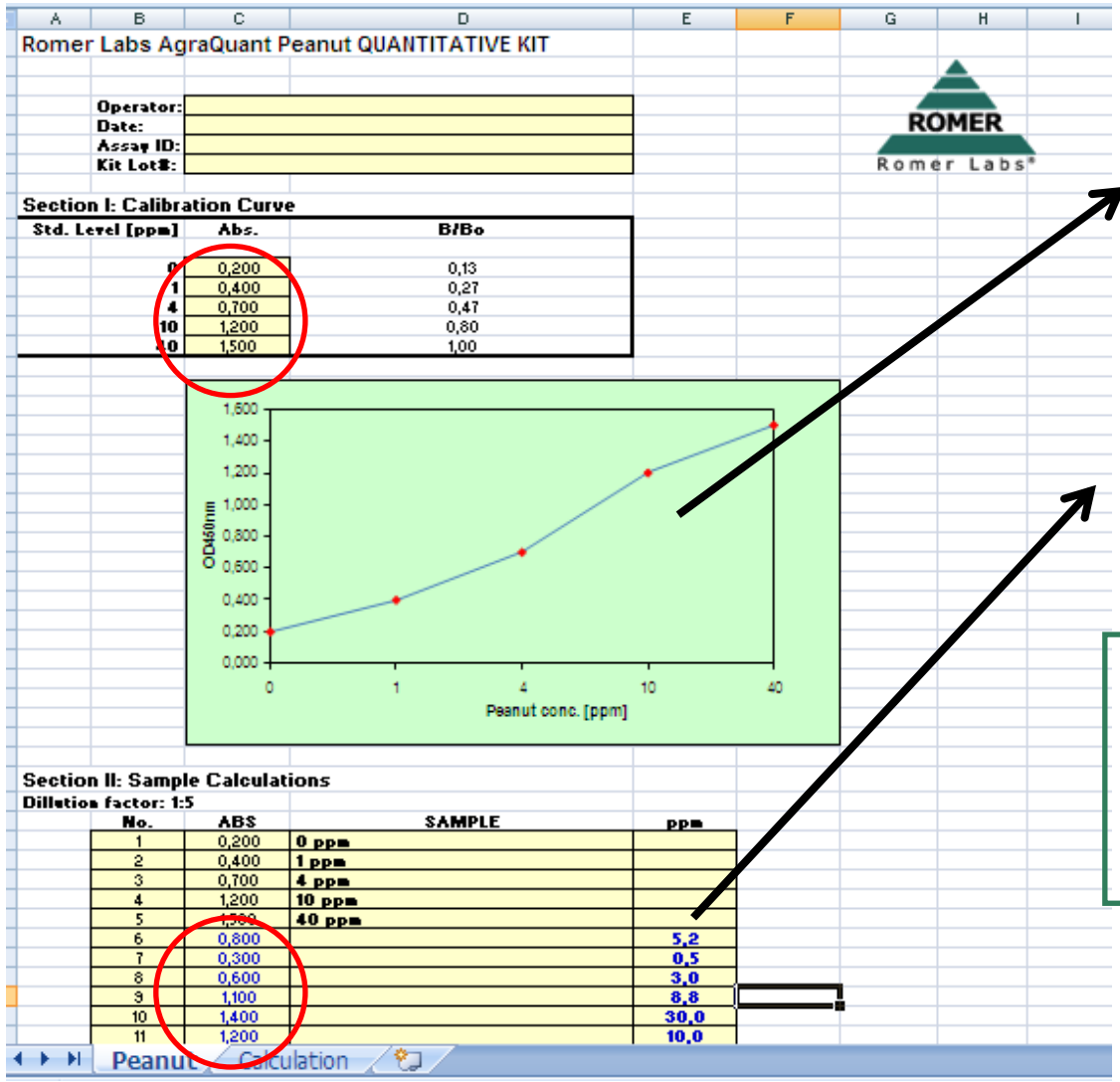
Spreadsheet



Point-to-Point Calculation

Results reported
<LOD
>upper limit of quantification

Point-to-Point Mode on Statfax Reader

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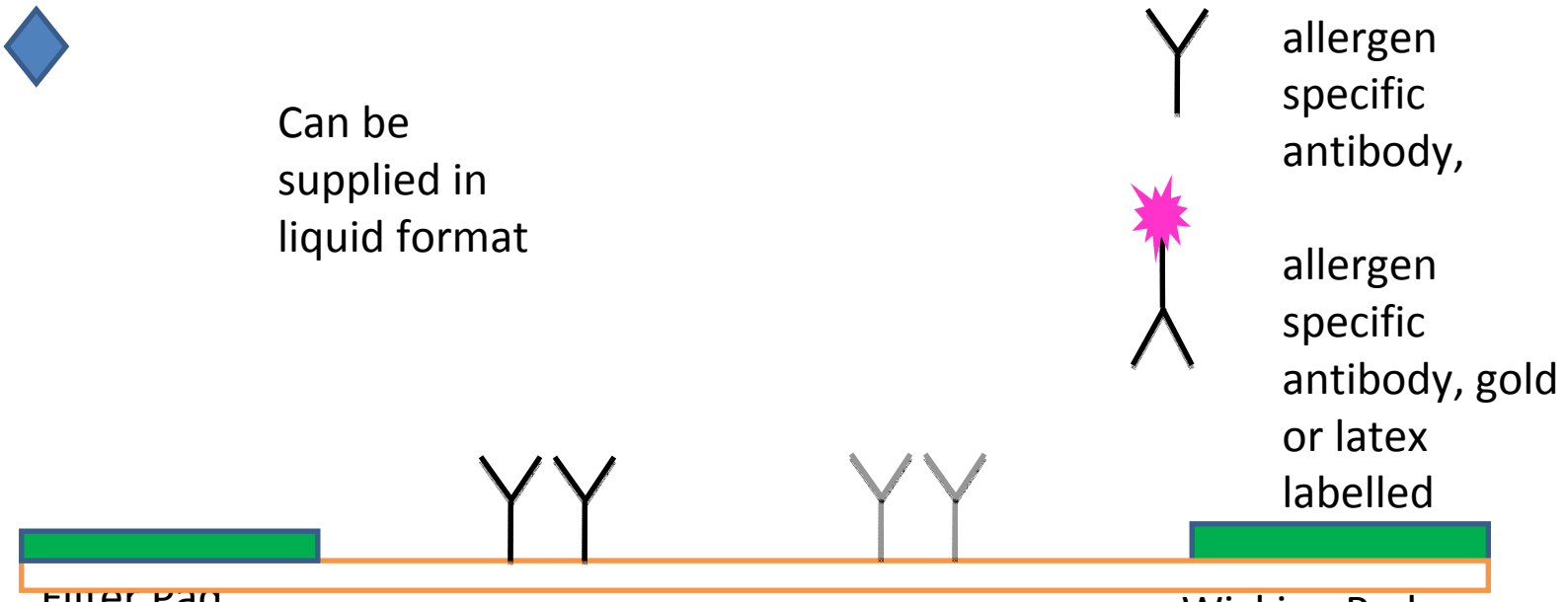
Sandwich LFD

Allergen in sample

Can be supplied in liquid format



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Challenges in Allergen Analytics

- No reference material available
- No certified standards available

- Spiking very difficult
  - Spiking with protein extract (what is detected?)
  - Spiking with allergen (food)
  - Spiking extract
  - Spiking sample

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Check-Sample-Survey

2011-2012

- Oct. 2012: in process
- May 2012: Gluten in snack sample
- Oct. 2011: Casein in a rice sample

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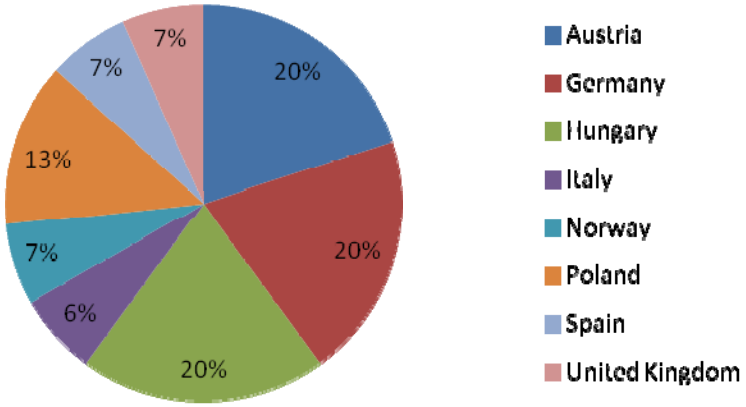
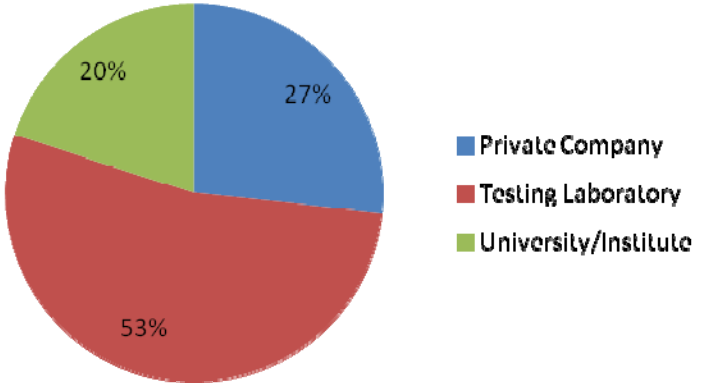
- Launched in Oct 2011 with Casein CSS

Participating Countries



Participant by Industry



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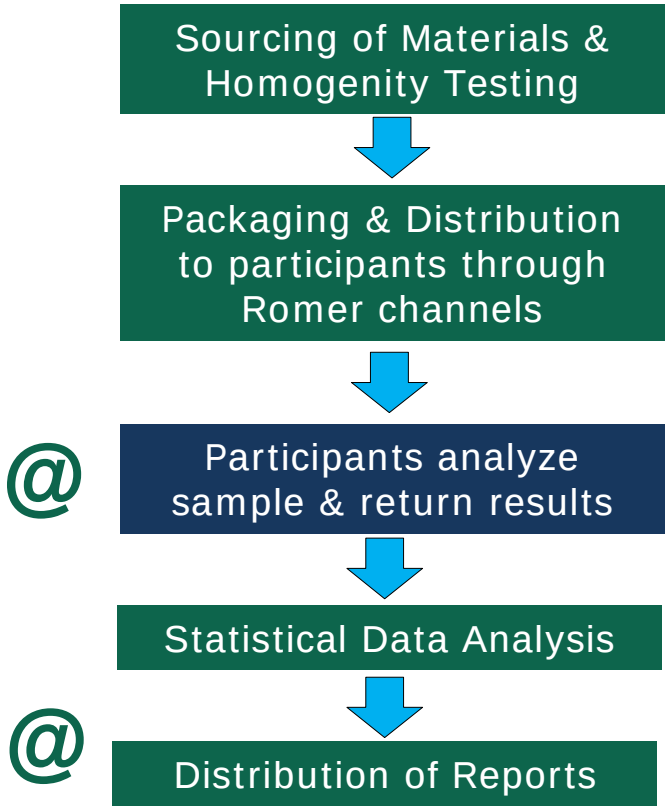
Organisation of Romer CSS



- State-of-the-art statistical methods
- Robust packaging to guarantee sample consistency
- Highly efficient distribution network

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Documentation of Romer CSS

- Concept Letter
- Accompanying Letter with Sample
- Final Report: Detailed description of statistical analysis and guideline to interpret results as well as information on the methods employed

Thank you for your attention