Advances in Detection and Measurement Technologies that Support Validation and Verification of Allergen Controls

IAFP European Symposium on Food Safety
Athens 2016

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12th May 2016
Allergen Control

- Raw product
- Production
- Retail

Risks:
- Raw materials
- Shared lines
- Transport
- Packaging
- Storage
- Staff
- Cleaning

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Allergen Detection Methods

Protein-based

• ELISA, LFD
• mass spectrometry (MS)
• […]

DNA-based

• (Realtime) PCR
• […]

Image adapted from: National Human Genome Research Institute.
Detection of proteins using immunological methods

Targets
- allergenic protein or representative protein from the allergenic food
- stable during processing (heat, pH, high-salt)

Antibodies
- Polyclonale antibodies produced in animals
- Monoclonale antibodies produced *in-vitro*
**Sandwich ELISA**

1st antibody **(green)** against allergen bound at surface

Addition of sample extract (containing allergen - **pink**)
1st antibody **(green)** binds allergen

2nd antibody **(yellow)** against allergen binds at another site (epitope)
2nd antibody connected to enzyme **(grey)**

Addition of substrate **(blue)**

Enzyme **(grey)** convert substrate **(blue)** into a visible dye **(yellow)** – quantification possible!
ELISA - Advantages

- fast
- ready-to-use kits & most allergens available
- semi-quantitative
- low LoQs (0.1 – 1 ppm)
- low investment costs
- automatisation
LFD (Lateral Flow Device)

Qualitative Quick test
Application mainly in industry *on-site* (cleaning control, check of raw materials)

Liquid flows through the stripe
Advantages

- very fast (15 min)
- available for many allergens
- low LoDs (1-25 ppm)

...but:

- false-negatives possible at high allergen concentration (hook/prozone effect)
- prone to matrix effects (sticky, strong colour etc.)
- „super easy“?
Protein extraction
- e.g. high fat content, pH
- unsoluble aggregates (Maillard)
- phenolic substances / tannines e.g. in wine, tea, dark chocolate

Antibody recognition
- degraded epitope
- Cross-reactions with similar epitopes (related species)
- matrix effects: binding might be inhibited or unspecific

⇒ adapted protocols (detergents, blocking additives etc.)

Quantification
- Protein content in calibration material may not represent sample
- ELISA result vary between kits due to different calibration materials
  ⇒ Reference material (CRM & RM) required e.g. MoniQA
ELISA cross reactions

Starting Feb 2015
Several recalls due to presence of almond in paprika
Almond ELISA: positive results

Later it reveals
positive findings due to unknown cross reaction with mahaleb (*Prunus mahaleb*)
- closely related to almond (*Prunus dulcis*)
- used as spice

Main problem is the intransparent supply chain!

→ ELISA Kit „validation upgrade“
→ routine testing on spice encreased
Comparison of commercial ELISA kit performance in (heat) processed food

Incurred bakery products

Cereal Bar: 177° C / 30 min
Muffin: 177° C / 48 min
Spiking level: 5000 ppm
Allergen: nonfat dry milk

Parker et al. 2015 JAFC (modified)
Detection of species-DNA

PCR (Polymerase Chain Reaction)

- DNA Extraction
- Amplification of a specific DNA region
- Detection (agarose gel or fluorescent probe)

Advantages
- sensitive (5-20 ppm)
- many kits available / papers + sequence data base information
- Multiplexing - detection of multiple allergens in one reaction
PCR – Challenges / Limitations

**DNA-Extraction (purity & quality)**

- Polysaccharides and polyphenoles inhibit PCR reaction
- DNA – Degradation by acid, heat possible

→ DNA-extraction from food samples is complex and time consuming

**Low (no) DNA in major allergens like milk and egg**

Presence of DNA = Presence of allergen? Conversion?
Exact mass detection

- Protein extraction + digestion to peptides
- Separation of protein fragments in LC
- MS/MS Ionisation and detection
LC-MS/MS - Advantages

- **qualitative multi screening 7 allergens:**
  - milk, egg, soy, peanut, walnut, hazelnut, almond
- **specific**
  - 4 peptides per allergen / 2 MRM per peptide
- **reliable**
  - Heat treated/highly processed product are detected

... but:
- only a limited number of matrices are validated so far
- Quality of protein database is important
- Quantification difficult:
  - quantitative enzymatic digestion?
  - conversion peptide → allergen?
Sampling allergens
disperse distribution / spot contamination

Sample

Allergen

Product A

Product A

Product A

Subsample

! Homogenize sample
=> Analyze representative sample

! Do replicates (repeat inconsistent)
! Upscale the subsample
To improve quality and efficiency of allergen testing:

- Risk assessment has to cover the whole process (from raw material to product)
- Control plans must include appropriate sampling and choice of methods

- Detailed matrix validation are required:
  - raw material (also minor components e.g. food colour)
  - processed products
Thank you very much for your attention!

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