FOOD ALLERGEN TESTING

Are all allergens detection methods created equal?

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Food Allergens in Context
What are food allergens?

FOOD
- DNA
- Vitamins
- Minerals
- Lipids
- Fats
- Carbohydrates
- Proteins

DETECTION
- Allergens
Targeting food allergens

DNA

DETECTION

Allergens

Translation

Proteins

FOOD
STANDARDISATION OF METHODS
Standardisation in Europe

European Level:

CEN: European Committee for Standardisation

National Level:

DIN: Deutsches Institut für Normung

BSI: British Standardisation Inst.

AENOR: Asociación Española de Normalización y Certificación
Standardisation in Europe

Standardisation bodies main function:

Agree on common standards or methods.

Here: agree on common allergen methods
But: where is method validation done?

Example Germany:

German book for food law:
LFGB (Lebensmittel und Futtermittel Gesetzbuch)

§64 LFGB: Methods for food analysis

BVL (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit) host all these groups.

In Germany for allergens: §64 LFGB food allergens
§64 LFGB Methods for Food Allergen Detection

**PCR Methods:**
- **L 08.00-53**  Soy in sausage
- **L 08.00-56**  Celery in sausage
- **L 08.00-58**  Lupine in sausage
- **L 08.00-59**  Mustard and soy in sausage
- **L 44.00-8**  Hazelnut in chocolate
- **L 44.00-11**  Peanut in chocolate

**ELISA Methods:**
- **L 00.00-69**  Peanut contamination in food
- **L 44.00-7**  Hazelnut in chocolate
And at CEN Level?

1) EN 15842:2010 Foodstuffs - Detection of food allergens - General considerations and validation of methods

2) EN 15633:2009 Foodstuffs - Detection of food allergens by immunological methods – Part 1: General Considerations

3) EN 15634:2009 Foodstuffs - Detection of food allergens by molecular biological methods – Part 1: General Considerations

STATUS: STANDARD
And at CEN Level?

1) EN 15842:2010 Foodstuffs - Detection of food allergens - General considerations and validation of methods

2) EN 15633:2009 Foodstuffs - Detection of food allergens by immunological methods – Part 1: General Considerations
   a) CEN/TS 15634-2 Foodstuffs - Detection of food allergens by molecular biological methods - Part 2: Celery (Apium graveolens) - Qualitative determination of a specific DNA sequence in cooked sausages by real-time PCR

3) EN 15634:2009 Foodstuffs - Detection of food allergens by molecular biological methods – Part 1: General Considerations
   a) CEN/TS 15633-2 Foodstuffs - Detection of food allergens by immunological methods - Part 2: Quantitative determination of hazelnut with an enzyme immunoassay using monoclonal antibodies and bicinchoninic acid-protein detection
German §64 LFGB Methods adopted at CEN Level

**PCR**

CEN/TS 15634-2 Celery = L 08.00-56 Celery in sausage

**ELISA**

CEN/TS 15633-2 Hazelnut = L 44.00-7 Hazelnut in chocolate
Analytical Techniques – What to watch out for
ELISA
Targeting proteins: ELISA

POSITIVE SAMPLE

NEGATIVE SAMPLE

Test 1

Test 2
LC-MS/MS (Mass Spectrometry)
1. walnut
2. hazelnut
3. soy
4. soy
5. milk
6. soy
7. peanut
8. almond
9. peanut
10. hazelnut
11. soy
12. hazelnut
13. peanut
14. milk
15. egg
16. almond
17. almond
18. egg
19. egg
20. peanut
21. egg
22. almond
23. hazelnut
24. milk
25. milk
Targeting DNA: PCR

DNA

PCR

$2^1 = 2$

$2^2 = 4$

$2^3 = 8$

$2^4 = 16$

$2^n$

…
Recap Techniques

Techniques applied for allergen detection in industry and gov’t labs:

DNA-Based: PCR
Protein-based: ELISA/LFD
Mass Spectrometry
Where Some Technologies Fail
PCR – Possible False Negatives
**Analysis: Protein (ELISA/MS) vs. DNA (PCR)**

- **Protein:** Target the source of the problem (allergen)
- **Challenge:** Correlation DNA : Protein (allergen) concentration

**Case 1: Egg and milk - DNA & Protein**

**Egg White:**
- no DNA
- High protein/allergen concentration

**Egg Yolk:**
- Only 1 DNA copy
Analysis: Protein (ELISA/MS) vs. DNA (PCR)

Case 2: Protein concentrates and fractionated products

Soya Milk (caseinates) → Soy, milk protein Concentrates

Protein
DNA

↑↑ Protein
↓↓ DNA

Chile August 2013
PCR – Possible False Positives
Problems associated with the analytical method:

- Cross-reactivity
- Cross-specificity
Specificity – X-reactivity / X-specificity

- Responsible for false positives
- Occurs among
  - Taxonomically related proteins - Impact on ELISA and MS
  - Taxonomically related genes – Impact on PCR
- Examples of known cross-specificity:
  - Tropomyosin from shrimp and cockroach (ELISA)
  - Pecans and walnuts (ELISA)
  - Celery and Parsley (PCR)
- Cross-specificity is in some cases desirable:
  - Gluten from wheat, rye and barley
## The Parsley Paradoxon

<table>
<thead>
<tr>
<th></th>
<th>Parsley</th>
<th>Celery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Results</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>German §64 official method</strong></td>
<td>OK</td>
<td>✓</td>
</tr>
<tr>
<td><strong>In-house method</strong></td>
<td>OK</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Commercial kit</strong></td>
<td>NOT OK</td>
<td>X</td>
</tr>
</tbody>
</table>
ELISA – Possible False Negatives
Problems associated with the sample:
Food composition + Food processing = Matrix effect
Structure modifications

ELISA is sensitive to protein modifications

Examples:
- Heated products (baked, roasted, pasteurized…)
- Extruded products (cornflakes)
- Low pH
Food Processing – Hydrolysis, fragmentation

Protein

DNA

ELISA

PCR
Hydrolized products

- Fermented products:
  - Beer, miso
- Flavor enhancers:
  - Vegetable and cereals boiled in acid solutions
Interaction with other food components

- Interact with target:
  - Proteins
  - DNA

- Affects the method
  - ELISA (antibodies)
  - PCR (polymerase)
  - MS

Examples
- Chocolate (polyphenols)
- Fat
- Sugars (maillard reactions)
Polymeration – Egg white proteins (OVA)

Examples:
Heated products: Bakery
Very acidic food products: salad dressing
Egg and milk in processed products are barely or not at all detected by ELISA/LFD and PCR.

No good method for egg and milk in processed materials available.
<table>
<thead>
<tr>
<th>Study</th>
<th>Test Method</th>
<th>LoD Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garber 2005</td>
<td>ELISA</td>
<td>LoD egg in bread: &gt;100ppm</td>
</tr>
<tr>
<td>Taylor 2010</td>
<td>ELISA</td>
<td>LoD milk in baked: &gt;250ppm</td>
</tr>
<tr>
<td>Diaz Amigo 2010</td>
<td>ELISA</td>
<td>Egg in Cookies 15’ baked: of 15,000 ppm only 500 detected</td>
</tr>
<tr>
<td>Diaz Amigo 2010</td>
<td>ELISA</td>
<td>Milk in Cookies 15’ baked: of 220 ppm only 32 detected</td>
</tr>
<tr>
<td>Khuda 2011</td>
<td>ELISA</td>
<td>Milk Cookins 30’ baked: of 350 ppm only 5 detected</td>
</tr>
</tbody>
</table>
Where the difference matters
May 2012...

A Kindergarten...
May 2012…

…and some pancakes
...but: ELISA can still work, if you choose the right one!
# Processed Foods - ELISA / MS comparison

<table>
<thead>
<tr>
<th>Target Allergen</th>
<th>ELISA (ppm)</th>
<th>MS (peak area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>Kit C: &lt;LoD</td>
<td>❌</td>
</tr>
<tr>
<td></td>
<td>Kit D: &lt;LoD</td>
<td>❌ ❌</td>
</tr>
<tr>
<td></td>
<td>Kit E: &lt;LoD</td>
<td>❌ ❌ ❌</td>
</tr>
<tr>
<td></td>
<td>Kit F: 700 ppm</td>
<td>✓</td>
</tr>
</tbody>
</table>

1000 ppm milk
1000 ppm egg
1000 ppm soy
Some General Guidance on Method Choice
Two major points

Always try to analyse raw materials (unprocessed or lightly processed materials)

If you have to analyse processed products, obtain as much information about matrix as possible and choose your analytical method accordingly
PCR Summary

PLANT MATERIAL

NUTS

SEA-FOOD

EGG MILK

ACIDIC PRODUCTS

HYDROLYSED PRODUCTS
ELISA/LFD Summary

RAW MATERIALS

EGG MILK

BAKED PRODUCTS
LC-MS/MS Summary

PLANT MATERIAL

NUTS

SEA-FOOD

EGG

PRODUCTS
Method Selection

Decision on best method requires:

- Expertise on food allergen field
- Deep knowledge on:
  - Food processing, food chemistry, biological analytical techniques and MS
  - Understanding of industrial manufacturing practices
- Experience on the analysis of numerous matrices

Food allergen analysis is NOT straightforward
**Summary**

All methods have their place

All methods have pros and cons

Labs and Food Manufacturers need to know