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Detection Methods for Food Allergens

Current State and recent Advances

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Outline

- Background
- Properties of Food Allergens
- Analytical Methods - Why are they needed ?
- Methods for detection of allergens
  - Non-specific methods
  - PCR
  - Immunochemical methods, Lateral flow and ELISA
  - Mass Spectrometry
- Harmonization and Guidance
- Conclusions

Hidden Allergens

Most cases of severe food allergic reactions and deaths are due to hidden allergens.

Unexpected presence of an allergen in a food product:

- ❑ unusual ingredient
- ❑ presence of allergen not indicated on ingredients list
- ❑ carry-over or cross-contamination during processing

Major Food Allergens

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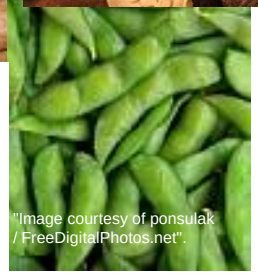
90% of all food induced anaphylaxis are due to the consumption of:

- Cereals containing gluten
- Crustaceans
- Fish
- Eggs
- Peanuts
- Milk
- Tree nuts
- Soy bean
- Molluscs
- Lupine
- Sesame
- Celery
- Mustard
- …and Sulfites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Analytical Methods - Why are they needed ?

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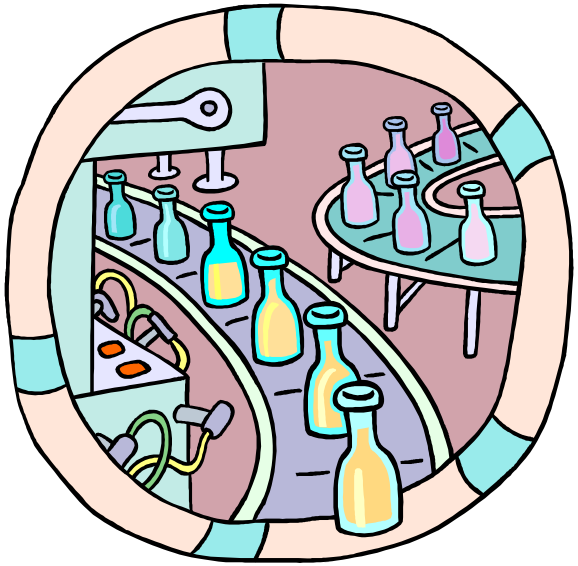
- Regulatory compliance
  - Check end product for label compliance
  - Food recall actions and investigations
  - Investigation and confirmation of consumer complaints

- Control of raw materials/ingredients for cross-contacts (especially for “allergen free” product claims)

- Validate effectiveness of allergen control program, specific cleaning steps and HACCP efforts
  - Food-contact surfaces
  - Rinse-water & push through materials (flour, salt, sugar etc.)
- Research projects and clinical studies e.g. for hypoallergenic foods

Evolution of Allergen Detection Methods

- Before 1990 methods to detect allergens where not established
- Development and use of allergen methods have evolved continuously with increasing awareness and regulation



- Followed by the implemented of allergen control programs in food industry over past 20 years, allergen testing is used for validation thereof

Selection Criteria for Analytical Methods

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How the perfect method would be …

- ❑ Applicable to all food commodities processed or not
- ❑ Highly specific
- ❑ Quantitative (health risk assessment)
- ❑ Highly sensitive (thresholds have not been established)
- ❑ Validated internationally recognized

  (e.g. AOAC, CEN/ISO, CODEX Alimentarius, etc.)

Method Evaluation and Validation Process

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Identification of candidate methods

- Preference for validated methods
- Comparison of method characterisitics with method requirements (LOD, compatible food matrices, cross-reactivities)

Identification of potential “reference” materials

- Commercially available
- Incurred/processed and non - processed material
- Validated/Certified

Evaluation study

- Characterization of the selected material
- Spiking protocols
- Single-laboratory validation

Guidance: Abbott et al. J AOAC Int. 2010, 93: 442-540

Inter-laboratory study (if possible)

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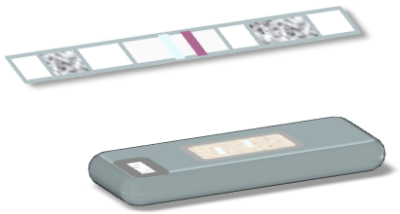
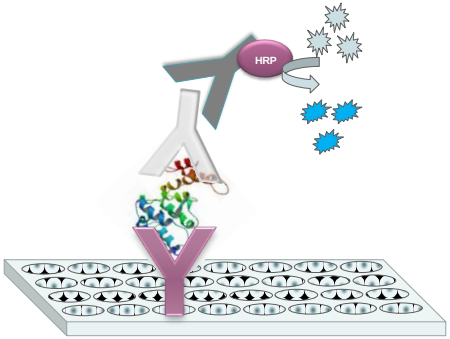
Overall motivation: Provide safe food with informative labels for food allergic consumers, and minimize precautionary labeling

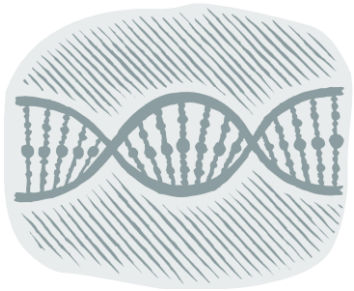
Those are so far screening & semi-quantitative and nowadays confirmatory methods based on mass spectrometry:



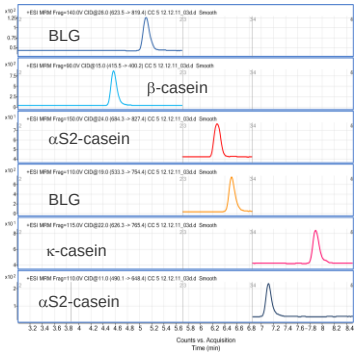
Lateral flow and ATP/Bioluminescence



ELISA or protein assays



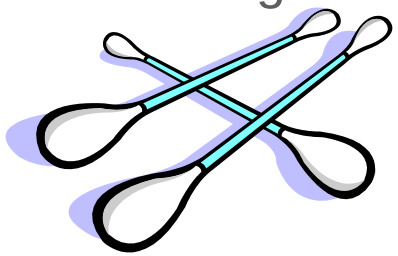
PCR



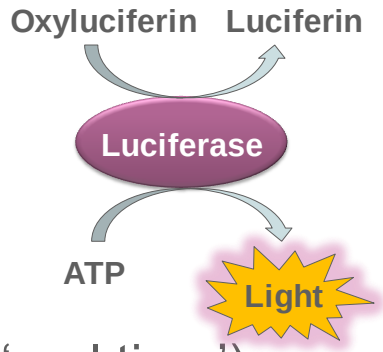
LC-MS

General Protein Tests for Sanitation Effectiveness

- Surface Protein (Allergen) Swab method for detection of protein
  - Detects protein of any source but not specific for a food allergen
  - Based on biuret or BCA reaction
  - Results may not correlate to allergen ELISAs
  - Detection limits are high (~ 3-20 µg protein)



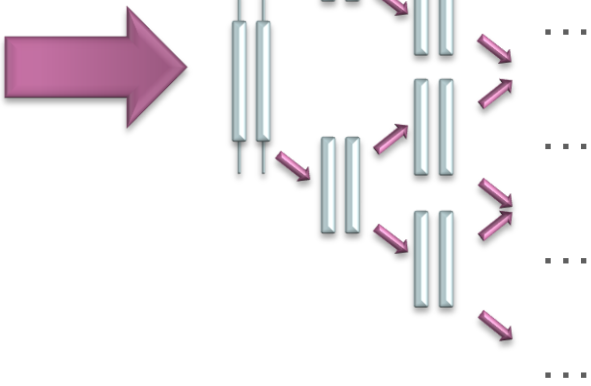
- ATP/Bioluminescence tests
  - Detects ATP from biological sources
  - Not specific for food allergen or even protein
  - ATP levels can vary between foods
  - Results may not correlate to allergen ELISAs
  - Rapid (< 30 sec) and can be performed on-site ('real time')



PCR Principle

1) DNA-Extraction from sample

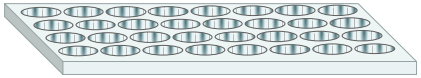
2) DNA amplification



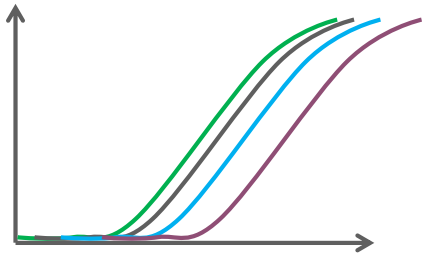
40 cycles
→ 10^{12} copies

3) Detection

Electrophoresis (Agarose gels)



PCR ELISA

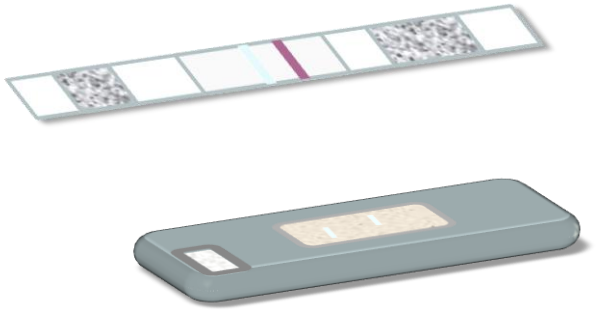


real-time PCR

- Where it works well
  - Detection of the allergenic food source material: food species of plant or animal origin (complies with labelling requirements)
  - DNA is a very stable molecule (food processing)
  - Available for many allergenic food sources + GMO
  - Sensitive (2.5 and 10 mg/kg allergenic food/total food)
  - Semi-quantitative
  - Rapid detection
  - real-time PCR allows multi-allergen detection up to 4-6 analytes (e.g. detection of several tree nuts)
  - Useful in cases where ELISAs are not available or results questionable (e.g. hydrolyzed proteins)
- Limitations
  - Cannot distinguish between milk and beef or egg and chicken
  - Equipment expensive and not available in all labs
  - Absence of DNA does not indicate absence of protein

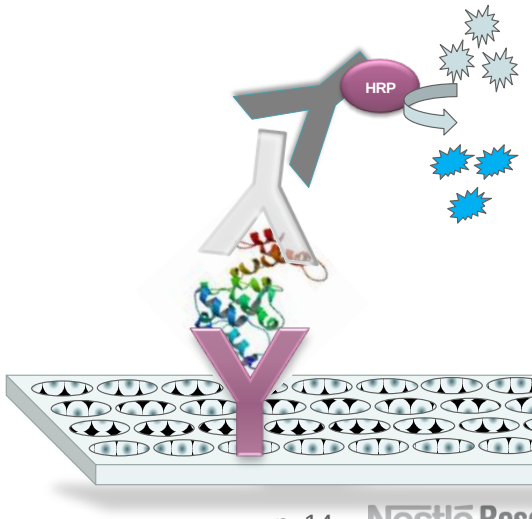
Lateral Flow Devices (LFD) and Dipstick Tests

- Antibody-based like ELISA methods
- Typically used for environmental sampling, cleaning verification & screening of foods
- Qualitative (semi-quantitative with a reader)
- Available for many food allergens
- Rapid on-site analysis (< 5 min)
- Less expensive than ELISA
- Sensitive (LOD ~ 5 ppm)
- Some are designed for rinse water only but not for food matrices
- Require confirmatory methods



ELISA Methods

- ELISA is still the favourite to food industry
- Highly specific to the allergenic food
- Quantitative or qualitative designs available
- Sufficiently sensitive (fits with existing threshold information and reference doses)
- Compatible with ingredients, finished products, rinse water, swabs/environmental samples
- 30 min-6 hours total analysis time
- Dedicated kits for processed and non-processed foods partially available



Proteomic MS Approaches

- The early beginning
  - Camafeita et al., 1997: The first non-immunological alternative attempt to quantify gluten gliadins in food samples (MALDI-TOF MS)

- Until today
  - 1998 - 2012: ~ 30 publications (milk, soybean, cereals, tree nuts, crab, fish, lupin, egg, and N-in-1)

- Proteomic methods like LC-MS/MS can be used in different ways
  - Multiallergen screening using ESI-Q-IT MS/MS
  - « Absolute quantification » using ESI-QqQ MS/MS
  - Semi-quantitative screening using High-resolution MS/MS
  - Guidance: Johnson, P., et al. *J AOAC Int*, 2011, 94(4), 1026-1033

Allergen quantification using ESI-QqQ MSMS and Stable Isotope Dilution

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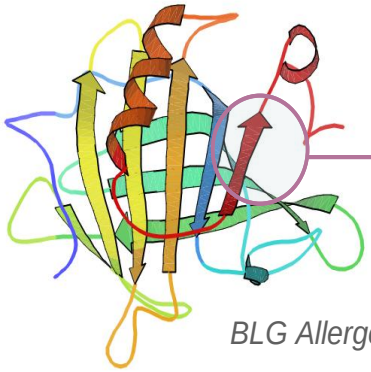
- Determination of selected milk proteins/peptides using LC-MS/MS and ***Stable Isotope Dilution*** after trypsin digestion

Select allergenic protein



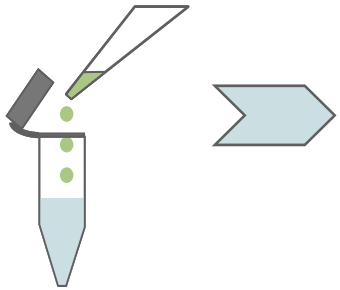
K.VLVLDTDYK.K

BLG Allergen=Bos d 5

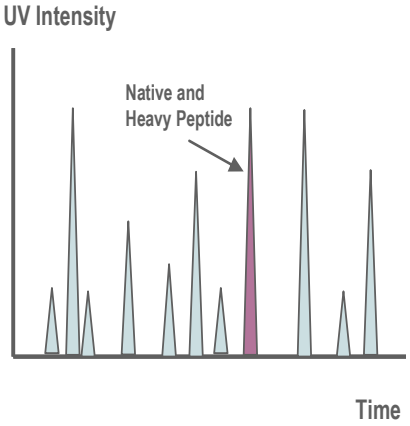
Synthesise heavy peptide analogon (IS)

K.VLVLDTDY[$K^{13}C^{15}N$].K

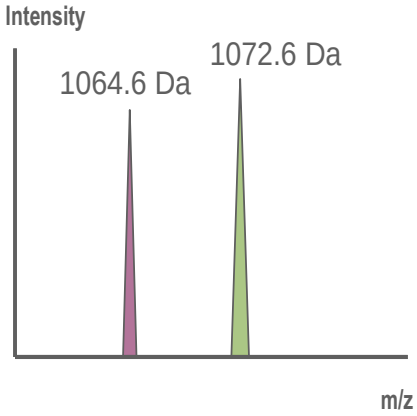
Spike IS into analyte



Separate by HPLC



Quantify by using MS as detector



Selection of Proteins/Peptides for Quantification

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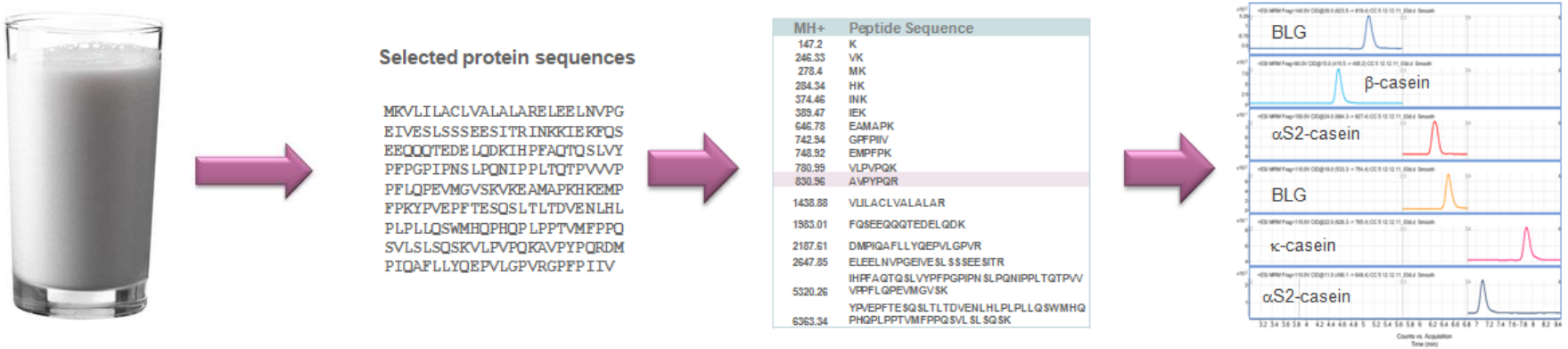
- Selection of “marker proteins”
  - Specific for milk proteins from different species (cow, buffalo) but not specific for other food ingredients (e.g. egg)
  - Ideally 2-3 marker proteins per allergenic compound
  - Good extraction properties and solubility (no membrane proteins)
  - None to few posttranslational modifications, modifications during food processing



Lutter P et al. *Journal of AOAC International* 2011; **94**, 1043-1059.

Confirmatory & rapid screening methods for food allergens by LC-MS/MS

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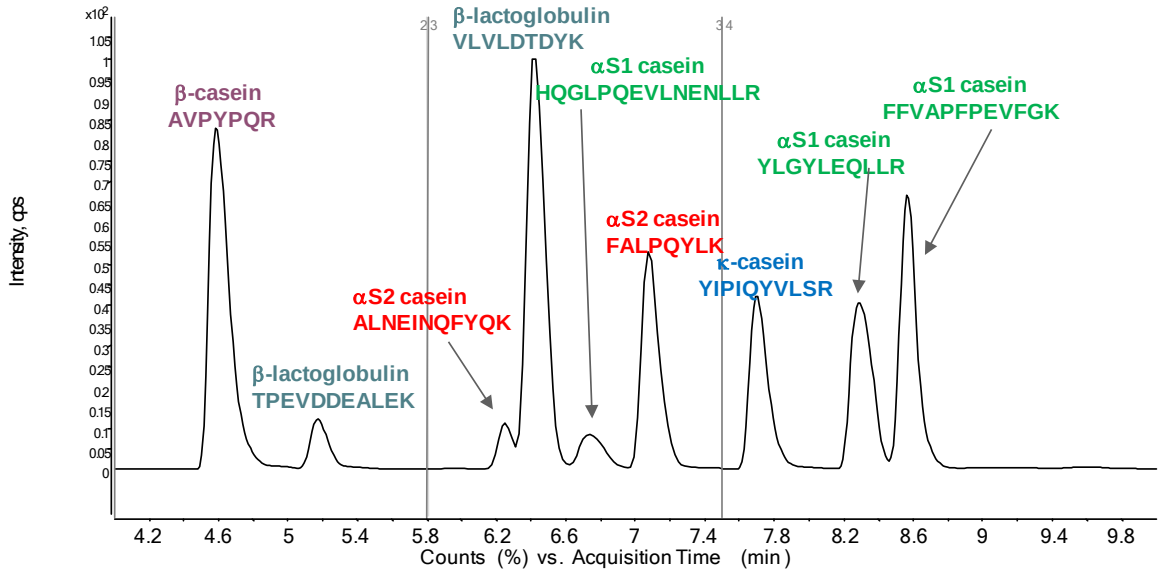
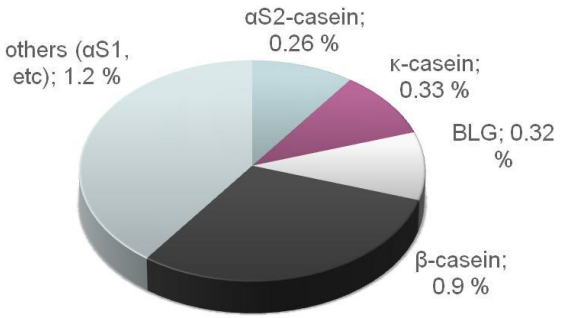
- LC/MS/MS offers the unique advantages of multi-allergen screening & quantification
  - High sensitivity and specificity enables multiple allergens in a single analysis
  - Not dependent on proper folding of protein → less affected by food processing (cooking)
  - Internal standard addition provides improved precision & reliability
  - No need for antibody production → rapid set-up
  - Multiplexing allows time and cost reduction





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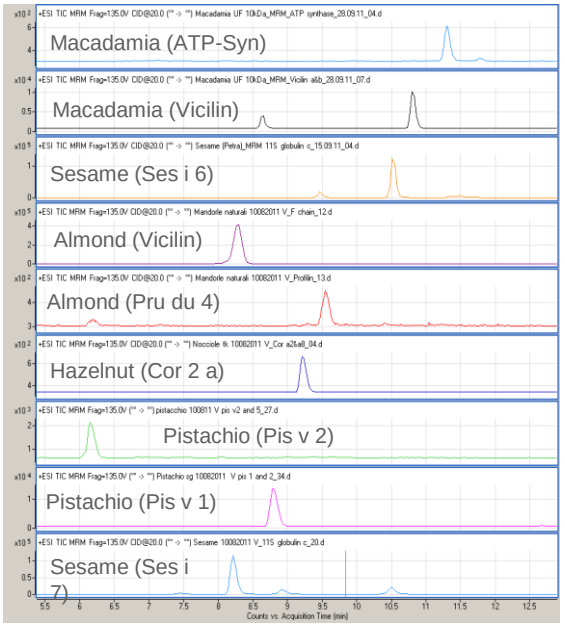
In Theory any Combination is possible with MS

LC-MS/MS multiplex analysis allows flexible set-up and combination of allergen targets based on needs.

Examples:

- Confectionary set: tree nuts + sesame + peanut (10 in 1)
- Culinary set: lupine + mustard + celery + soy + gluten + egg
- Infant nutrition set: whey + casein + soy + gluten
- Other …

Confectionary Design Multi-Reaction-Monitoring (MRM)



Efforts of Harmonization and Guidance

- Guidance on validation protocols is needed to enable generation of validation data
- Reduction of dublication of efforts and ensurance of maximum recognition
- Examples:
  - AOAC/MoniQA: e.g. Abbott et al. J AOAC Int. 2010
  - CEN: Harmonisation at European level
  - iFAAM project (EU): Method validation for hazelnut, peanut, walnut, egg & milk multi-allergen method by LC-MS/MS

Methods for Food Allergen Testing - Conclusion

- Many tools are available for detection of allergens or allergenic foods
- Immunochemical methods the most common and still most favored by food industry
- Choice of method depend on specific use like type of food matrix, analytical capabilities
- Require “in-house” validation
- More than one method may be needed per food allergen
- Need for reference standards for harmonisation and comparison