# Validation of ELISA-based Methods for Food Allergens – Towards increased harmonization

*Technical and Information workshop for application of analysis to allergen management*

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# Validation of Allergen Methods

- Allergen testing /methods: critical tool used:
  - To support industry in controlling effectiveness of sanitation protocols (environmental swabbing, control of final foods)
  - To support regulators in investigation of incidents related to undeclared food allergens
- Validation: demonstration of the reliability of the method performance for the purpose it was developed for.




# Validation of Allergen Methods

- Validation studies:
  - Conducted as part of the method development process by test kit manufacturers
  - Conducted by method users to implement the methods in their own laboratories (part of QA/QC requirements)
  - Conducted as part of various processes:
    - Led by regulatory agencies: HC, EU JRC, Australia NMI, US FDA
    - International organisations: AOAC international, AOAC RI




# Reasons for Method Validation

- To ensure a method is fit for a particular purpose
- To show the method performs well in the hands of different users
- To provide impartial data with regards to method performance under specified conditions
- To compare performance of methods (vis-à-vis « fit for purpose »)




# Current Validation Processes

- Driven by regulatory agencies:
  - Japanese Government
  - Health Canada/CFIA
  - US FDA (AOAC-RI)
  - EC JRC : ring trials
  - CEN-driven processes

- AOAC – AOAC/RI driven processes:
  - PTM status
  - Official Methods




# Need for Validated Allergen Methods

- Validation of methods is critical to propagate use of allergen methods amongst **industry**:
  - As part of allergen prevention plans applied by industry
  - Amongst regulators (as part of food allergy prevention strategy)




# Challenges for validation processes

- Methods are mainly « bio-analytical methods »
  - ELISA
  - PCR
- Confirmatory / reference methods are not established
  - MS-based techniques are still under development
- Absence of clearly « targeted analytes » - Various markers for the same food commodity




# Efforts of Harmonization/ Guidance

- Clear guidance on validation protocols is needed to enable generation of validation data
  - CEN : Harmonization at the European level
  - AOAC Presidential taskforce
    - Initiated discussions on development of validation protocols for ELISA-based techniques (2007- 2010)




# Context of work….

- AOAC /MoniQA





# Objectives of harmonization

- To reduce duplication of effort and help ensure that validation studies for food allergen detection methods receive maximum recognition

- To provide additional guidance relevant to food allergen detection methods which recognizes that
  - ELISA based methods have specific characteristics
  - Detection of food allergens poses specific challenges




# Using existing Guidance

- AOAC Official Methods of Analysis Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis (2002 AOAC International).

- IUPAC “Protocol for the Design, Conduct, and Interpretation of Collaborative Studies,” and the “Harmonized Protocols for the Adoption of Standardized Analytical Methods and for the Presentation of their Performance Characteristics.”




# Key Considerations

- Need to have input/agreement from various stakeholders on the protocol , particularly method developers and regulatory agencies /other end users

- Design protocol such that it was possible for data generated using the validation protocol to be submitted for accreditation to certification bodies (e.g. AOAC, government organisations…)




# Key Considerations

**Specific Characteristics of ELISA-based detection methods**

- Some inherent variability in results (higher than for traditional GC or LC methods)
- Matrix effects may be more difficult to overcome (performance of test may vary depending on matrix being analyzed)
- May cross react with other analytes




## Need for more specific guidance

- Lack of reference materials
- Lack of “incurred” products
- Spiking or fortification methods
- Choice of calibrators, reporting units
- Choice of matrices to include in validation




# PROCESS / ENGAGEMENT

- Development of discussion points
- Engagement at different meetings:
  - MoniQA meeting – Marloie, Belgium – July 2007
  - AOAC allergen taskforce meeting –Anaheim, CA, USA, Sept 2007
  - HC/MoniQA meeting, March 2008
  - HC/FARRP Allergen workshop, May 2008
  - AOAC Allergen Taskforce meeting – Sept 2008
- Development of a Manuscript for publication in J. AOAC. Int. – 2009 (including continued consulations)
- Publication of a Guidance document in April 2010




# Key Considerations

- Focus on key method information to be provided by method developers (proprietary methods)
  - Including food allergen-specific information

- Focus on conditions of conduct and analysis of outcomes of Inter-laboratory Validation

- Allergen Specific Criteria:
  - ✓ Initial examples are: Egg, milk




# Example of Information to be provided

**Information is mostly allergen-specific**

- Antibody Information
- Information on Calibrators
- Information on Matrices
- Limit of Detection / Limit of Quantitation
- Cross Reactivity
- Ruggedness / Lot to lot variability




# Antibody Information / Calibrator information

## Information on Antibodies

- Monoclonal or polyclonal ?
- Does the Ab target a single protein or multiple proteins ?
- Was the target protein fractionated, modified or synthesized ?
- Balance between providing enough information to characterize method and not to reveal proprietary information

## Information on Calibrators

- Preparation and characterization of calibrator solution
  - ✓ Raw of processed material
  - ✓ Extraction and purification
  - ✓ Buffers used for solution
- Clear indication on the unit used to express the
  - ✓ whole commodity, level of protein (total, soluble) and how level of protein was determined




# ELISA Parameters to provide

## LOD / LOQ / Lower Limit of Application

- Limit of detection (LOD) is defined as the concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level.
- Limit of Quantitation (LOQ) is the lowest level of analyte in a sample that can be reasonably quantified, at a specified level of precision.
  - Estimation of LOD made through statistical analysis of the calibration data according to the ISO standard ISO11843-2 (6) for linear data, or ISO 11843-5 (7) for linear and non-linear data
- Method developers are free to define a Lower Limit of Application (LLA) at whatever level of confidence they choose, which provides a level below which they do not support/recommend the use of the method.




# Cross Reactivity

- Method developers must test their method for cross reactivity
- Should include a wide selection of foods and ingredients, particularly those that are genetically similar to or food matrices likely to be matrices of focus
- The greater the number of samples tested for cross reactivity the better.
- Cross reactivity testing should be based on full strength extracts – if a positive result is obtained the extract should be diluted and rerun to characterize extent of cross reaction




# Recommended Cross-reactivity testing for egg ELISAs

| | | | | |
|---|---|---|---|---|
| Adzuki beans | Almond | Barley | Beef | Brazil nut |
| Buckwheat | Cashew | Chestnut | Chick peas | Chicken |
| Cocoa | Coconut | Corn | Crustacean/ prawn/shrimp | Fish/salmon/ tuna |
| Gelatin (bovine) | Hazelnut | Kidney beans | Kiwi | Lecithin |
| Lentils | Lima beans | Linseed | Macadamia nut | Milk |
| Oats | Octopus | Peanut | Peas | Pecans |
| Pine nut | Pistachio | Poppy seeds | Pork | Pumpkin seeds |
| Rice – white and brown | Rye | Sesame | Soybean | Split peas |
| | Sunflower seeds | Walnut | Wheat | |




# Guidance on Ruggedness testing

- **Ruggedness :** ability to resist changes in final results when minor deviations are a made in experimental conditions
- In particular deviations in incubation times, reagent volumes and extraction conditions (times and temperature) should be investigated
- Deviations of time and volume of ±5% or more and incubation temperatures of ±3°C or more are recommended for ruggedness testing
- If an experimental condition is particularly important for achieving consistent results this should be mentioned in the kit instructions




# Key elements of Interlaboratory Validation

- Guidance stemming from : (AOAC Official Methods of Analysis Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis (2002 AOAC International).)

- Detailed guidance provided on design of inter-laboratory collaborative studies, specifically on :
  - Number of laboratories required
  - Number of matrices, spiking levels and replicates required
  - Data analysis
  - Acceptance Criteria




# Allergen Specific Criteria: Egg and milk

## Reference materials:

- In general, a **reference material is a material that is representative of the allergenic food commodity, that is well characterized, that can be produced or supplied with robust reproducible characteristics, and which can be used as a calibration standard, control or spiking material.**

### Suggested reference material for egg is

NIST RM-8445 Spray dried whole egg for allergen detection.

- The first NIST reference material specifically intended for use in food allergen testing

### Suggested reference material for milk is

NIST SRM-1549 Non-fat milk powder

- Not specifically intended for use in food allergen testing, but has been found to perform well as a reference material for milk ELISAs in previous studies




# Spiking or fortification methods

- Fortification methods :
  - By far, best source of information on method performance is incurred samples and these should be used whenever possible
  - Validation studies using spiked samples are still considered an acceptable way to generate some information on method performance
  - It should be recognized that spiked samples may result in artificially high recoveries




# Food Matrices

- Matrix being analyzed can have large impact on the results
  - Good performance in one matrix does not guarantee good performance in another matrix
- Choice of matrices is left to the method developer or study lead
- There are matrices of particular interest for each food allergen
  - Based on which food products are most likely to be contaminated with a particular food allergen
  - Method developers are encouraged to include as many of these as possible in the validation study




# Matrices of Interest

## Egg

- Chicken
- Cookies
- baked goods
- Fish
- Ice Cream
- Pasta
- Salad Dressing
- Wine

## Milk

- Cookies, baked goods
- Beef
- Chicken
- Dark Chocolate
- Drink Mixes(ex alcoholic beverage premix)
- Orange Juice
- Infant Formula
- Pork
- Soy
- Milk
- Wine




# Next Steps

- **Implementation of the guidance document to gather data supporting validation of test kits for egg and milk**
  - Health Canada's Food Directorate has endorsed the guidance document and the corresponding criteria as a reference for its acceptance of allergen methods to be included in the compendium of allergen methodologies

- **A similar guidance document needs to be developed for Gluten methods to**
  - Address multiplication of gluten test kits
  - To support innovation in the food processing sector enabling more gluten free kits with the appropriate level of control.




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# Thank-you!

**For more information, please visit us @**

www.healthcanada.gc.ca/foodallergies

www.Moniqa.org





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고맙습니다
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děkuji
Thank You
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köszönöm
gracias
Ευχαριστώ
merci
どうもありがとう
danke