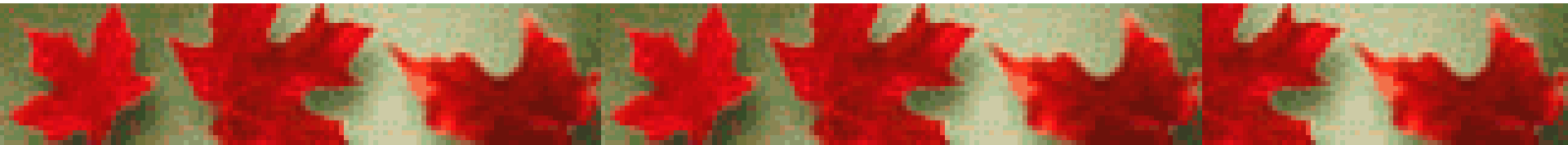
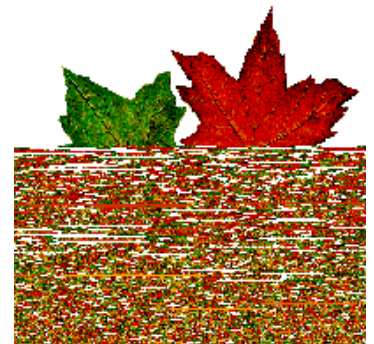
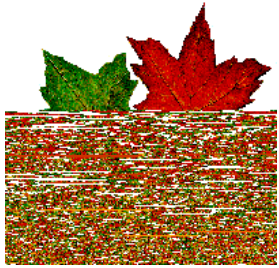


# Validation of Food Allergen Detection Methods



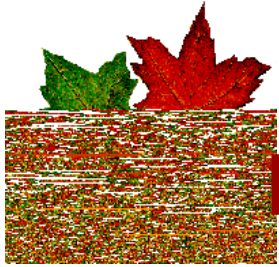
**Michael Abbott**  
Food Allergen Program Manager  
Bureau of Chemical Safety,  
Food Directorate, Health Canada





# Validation of Allergen Methods

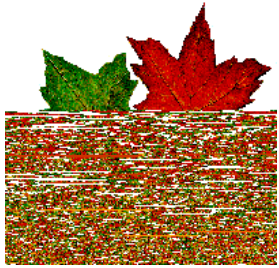
- Allergen testing /methods: critical tool used:
  - To support industry in controlling effectiveness of sanitation protocols (environmental swabbing, control of final foods)
  - To support regulators in investigation of incidents related to undeclared food allergens
- Validation: demonstration of the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



# Reasons for Method Validation

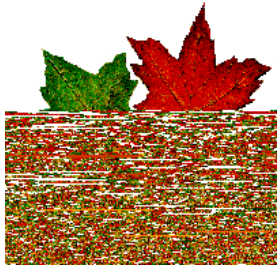
---

- ❑ 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



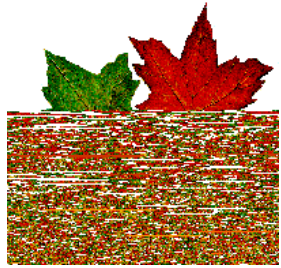
# Reasons for Method Validation

- 
- Allergen Method Users rely on validation:
    - To ensure/support reliability of results
    - To ensure methods perform according to claims/specifications
    - To compare methods ?



# 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: US FDA, HC, EU JRC
    - ▲ International organisations: AOAC international, AOAC RI

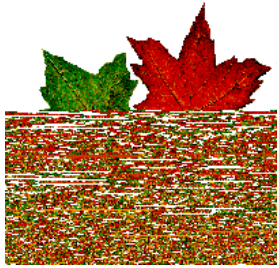


# Validation of Allergen Methods: existing processes

---

- 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

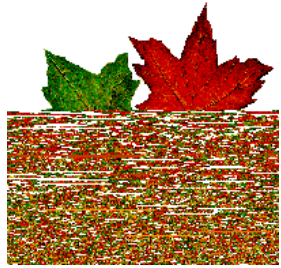




# Need to « identify validation procedures/protocols »

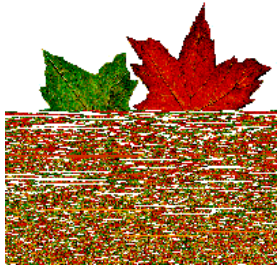
---

- To ensure availability and propagation of allergen testing methods:
  - Processes need to:
    - ▲ Be more timely
    - ▲ Less expensive
    - ▲ More predictable
    - ▲ (more) international



# Recognition of Validated Methods

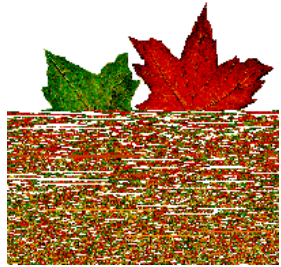
- 
- Possible to gain accreditation from a recognized body such as AOAC (PTM, or RI)
  - Publication of validation study results in scientific journals



# Challenges for validation of allergen detection methods

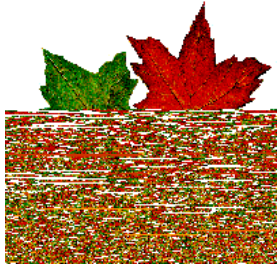
---

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



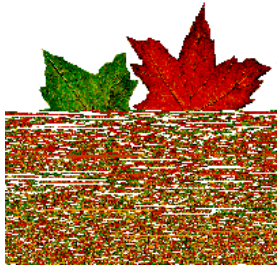
# Reference Materials

- ❑ Choice of reference material can be very challenging
- ❑ Different species of the same food commodity can have different protein profiles
- ❑ Processing can have dramatic impact on protein content, conformation, solubility and reactivity
- ❑ 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.



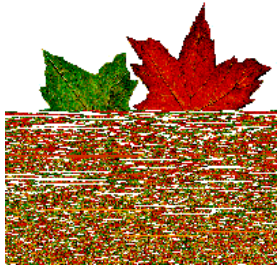
# Reference Materials

- 
- Unlikely one material can represent many possibilities (variety, processing etc.) at once
  - Widely available reference material can provide a common reference point for data comparison purposes



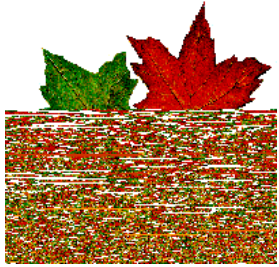
# Spiking or Fortification Methods

- Food samples are “spiked” with a known amount of a food allergen then analyzed to see the result
  - ▲ Can use the reference material itself (better) or
  - ▲ An extract of the reference material
  
- For some kinds of samples (such as liquids or fine powders) it may be possible to prepare a large batch of a food sample which has been homogeneously fortified at a specific level with a food allergen



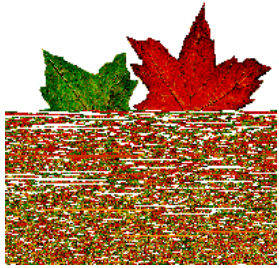
# Spiking or Fortification Methods

- For other kinds of samples, due to difficulty in achieving homogeneity at very low spiking levels, spiking of individual test portions is recommended
- May be very difficult to spike at low (ppm) levels with the reference material itself
- It should be noted that spiked samples may result in artificially high recoveries



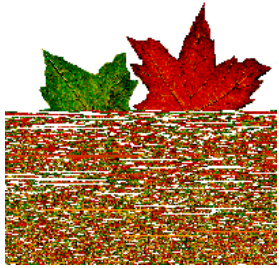
# Incurred products

- ❑ Manufactured under “real-life” conditions to contain a known amount of a particular food allergen (ex. peanut in cookie, milk in chocolate)
- ❑ Not readily available (yet)
- ❑ Provides better data on real life performance than spiking



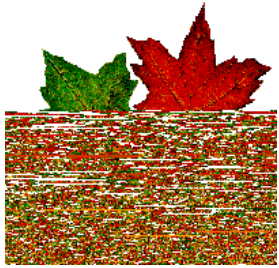
# Choice of Calibrators and Reporting Units

- 
- Different methods may be calibrated against the different standard materials, making comparison of results from different tests difficult
  - Must be very careful about reporting units – ppm (of what?, whole food?, protein?, soluble protein?)



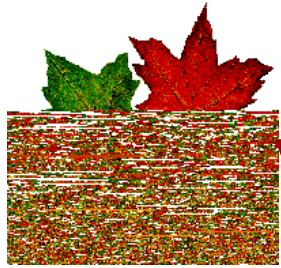
# Choice of matrices to include in a validation study

- Results from ELISA methods can be influenced by the matrix being tested
  - Good performance in one matrix does not guarantee good performance in other matrices
- Want to select a range of different matrices which are most likely to be contaminated with the allergen
  - ▲ Ex. Dark chocolate as matrix for milk test



# Cross Reactivity

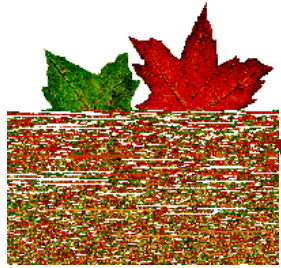
- ELISA methods can be susceptible to cross reactivity (a positive response to something other than the analyte of interest)
  - ▲ Rare for today's commercial methods
- Validation must include testing the method for cross reactivity against many different matrices to determine if the antibodies react to any of them



# Internal vs. Interlaboratory Validation Studies

## Internal studies

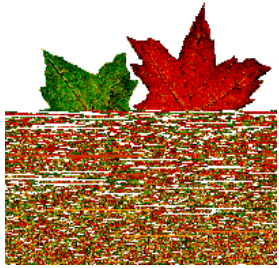
- In-house studies performed by a method developer
- Generally include testing for cross reactivity and recovery studies using spiked or fortified samples in a variety of matrices
  - ▲ May include other aspects such as ruggedness, lot to lot variability, stability over time,



# Internal vs. Interlaboratory Validation Studies

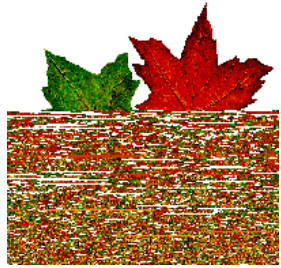
## Interlaboratory studies

- Validation studies performed in a variety of different laboratories
- Generally include recovery studies using spiked or fortified samples or incurred samples in a few different matrices
  - ▲ Provides information on the variability of results between different labs



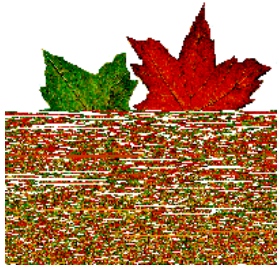
# Past Validation studies for allergen methods

- 
- ❑ Previous validation studies have been conducted on commercial peanut kits, gluten tests and a few other commercial ELISA kits
  - ❑ Study design varied from one study to the next as no set guidance for allergen method validation exists.
    - ▲ Difficult to compare results from different studies



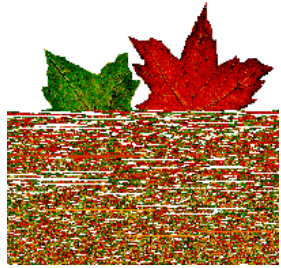
# Existing Validation Protocol Documents

- 
- 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.”



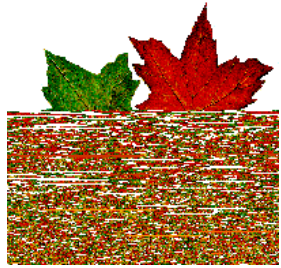
## Need for Guidance specific to Validation of Food Allergen Detection Methods

- ❑ 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



# Towards Harmonized Validation Procedures

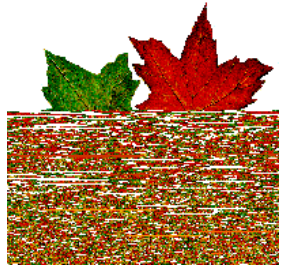
- 
- Development of discussion points
  - Meeting for discussion – Belgium (July 2007)
  - AOAC presidential taskforce meeting – Anaheim (September 2007)
  - Meeting for discussion – Vancouver (March 2008)
  - Meeting for discussion – Halifax (May 2008)
  - AOAC meeting – Dallas (September 2008)
    - And followup teleconferences on statistical interpretation of study results



# Acknowledgments

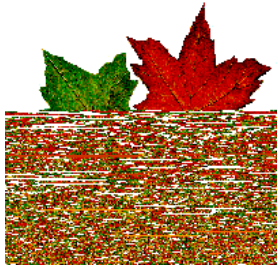
---

Michael Abbott	Health Canada	Samuel Godefroy	Health Canada
Phil Goodwin	Hallmark	Steve Taylor	FARRP
Bert Popping	Eurofins	Masahiro Shoji	Morinaga
Tatsuya Fujimura	Morinaga	Jupiter Yeung	GMA
Steve Musser	USFDA	Mohamed Abouzied	Neogen
Mike Ryan	Elisa Systems	Tony Lupo	Neogen
Petra Urban	R-Biopharm	Kurt Johnson	R-Biopharm
James Roberts	NMI – Australia	Richard Fielder	Tepnel Biosystems
Phillipe Delahaut	CER	Valéry Dumont	CER
Anne Lepage	CER	Helen Nicolidakis	Health Canada
Terry Koerner	Health Canada	Arjon Van Hengel	EC-JRC
Franz Ulberth	EC-JRC	Paul Wehling	General Mills
Carmen Westphal	USFDA	Stephen Hayward	Health Canada
Jeff Ammann	TTB	Christiane Faeste	NVI, Norway
Sigrid Haas-Lauterbach	R-Biopharm	Hiroshi Akiyama	NIHS, Japan
Lynn Niemann	FARRP	Debrah Lambrecht	FARRP
Marc Burke	Tepnel	Elizabeth Berryman	Tepnel
Armen Mirzoian	TTB		



# Key Considerations

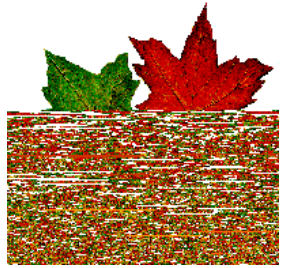
- 
- Need to have input/agreement from various stakeholders on the protocol , particularly method developers and regulatory agencies /other end users
  - Design protocol such that it was possible for data generated using the validation protocol to be submitted for accreditation to AOAC or other certification body



- 
- Manuscript entitled :

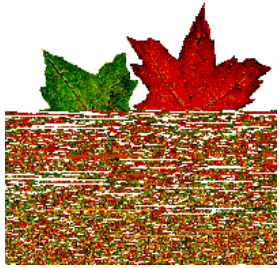
**Validation Procedures for  
Quantitative Food Allergen  
ELISA Methods: Community  
Guidance and Best Practices**

was submitted to the Journal of  
AOAC International in April 2009



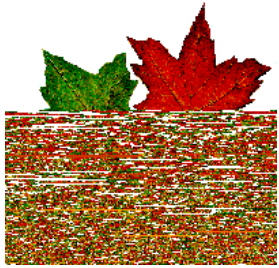
# Highlights of the Validation Procedures document

- 
- Allergen-Specific Information to be provided on the ELISA method
  - Key elements of Interlaboratory Validation
  - Allergen Specific Criteria



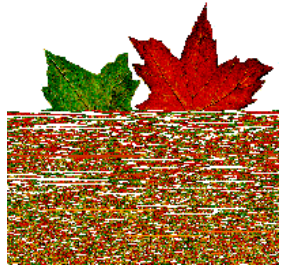
# Allergen-Specific Information to be provided on the ELISA method

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



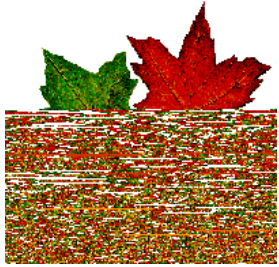
## Key elements of Interlaboratory Validation

- Number of laboratories required
- Number of matrices, spiking levels and replicates required
- Acceptance criteria
- Data Analysis
- Follows general guidelines in AOAC Appendix D and IUPAC



# Allergen Specific Criteria

- 
- Reference materials
    - ▲ Suggested reference materials for eggs and milk
  - Spiking or fortification methods
  - Food matrices



## Next Steps

- 
- Once published the procedures will be tested by running a validation study or studies implementing the guidance
  - Egg or milk methods will be used
    - ▲ Some preliminary discussions have taken place but no specifics yet

# MoniQA

## Network of Excellence

Monitoring and Quality Assurance in the Food Supply Chain



Funded by the European Commission  
within the Sixth Framework Programme (FP6)  
Topic T5.4.5.1: Quality and safety control strategies for food (NOE)

### Coordinator:

Roland Ernest Poms, ICC – International Association for  
Cereal Science and Technology, Vienna, Austria

**33 member institutions** from 20 countries / 4 continents  
***Currently: 92 registered institutions from 35 countries***

A total of **155 researchers** including 40 doctoral students

Duration: **5 years** – starting 1 February 2007

**EU funding: 12.3 M €**



SIXTH FRAMEWORK  
PROGRAMME



[www.moniqa.org](http://www.moniqa.org)

# MoniQA

## MoniQA Network of Excellence

Towards harmonisation of analytical methods to monitor and control quality and safety in the food supply chain

### **...in a nutshell**

**Scientists from 20 countries collaborate in the MoniQA network to harmonize worldwide food quality and safety monitoring and control strategies**

**Harmonised food quality and safety control adds value in the food chain and will improve consumer confidence**

**Socio-economic impact assessment will enhance effectiveness and efficiency of new food quality and safety regulations**



[www.moniqa.org](http://www.moniqa.org)

# Motivation for MoniQA

- **New EU Regulations**

(e.g. food allergens, mycotoxins, other food contaminants)

- **Fragmentation of research and standardisation**

(e.g. little or no communication between research centers and standardisation bodies, duplication of work, more collaboration, decreasing costs...)

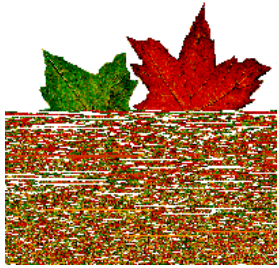
- **Limited validity of standardisation/validation certificates**

- **Need for rapid methods**

- **Lack of appropriate validation protocols for new and rapid methods**

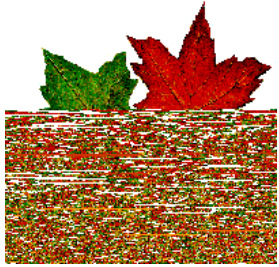
- **Lack of reference methods and reference materials for some analytes**

- **Dependance on voluntary work**



# Conclusions

- 
- Recognition of importance of method validation is growing
    - ▲ Provides assurances to method users
  - Number of international collaborative initiatives taking place in this area
    - ▲ Validation guidance document and future implementation
    - ▲ MoniQA, including efforts to develop incurred samples and reference materials



---

Thank you for  
your attention!