Food Allergen Detection

Methods

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April 27, 2006
GOAL

Validated Methods
WHY?
Kit Performance Variability

"...using the target standard deviation predicted by the Howitz equation, the distribution had modes that corresponded to each of the major ELISA kit brands." - FAPAS® Allergens Report 2708, Peanut (2003), p 7.

Milk: 5 different kits, no consensus level
Egg: 5 different kits, no consensus level
Focus: Consumer's Perspective

Detection of the Food
  one of the food allergens or
  a non-allergenic marker unique to the allergenic food reflects allergenicity

Effects of Preparation
Qualitative versus Quantitative
  Definitive Detection
  Signal / Noise (Bkgd) versus Recovery
  Reproducible

Allergenicity, Antigenicity, Bioavailability, Detectability
Methods

**P C R**
- commercial assays for legumes (peanuts & soy) and nuts
- simultaneous detection of multiple grains published

**Mass Spectrometry**
- publications

**Immunosorbent**
- ELISAs and Test strips commercialized

*application of commercially available methods as first approximation*
## Test Kit Availability

<table>
<thead>
<tr>
<th></th>
<th>casein</th>
<th>Milk whey</th>
<th>casein-whey</th>
<th>Egg white</th>
<th>Wheat gliadin</th>
<th>Soy</th>
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<tbody>
<tr>
<td>ELISA Systems</td>
<td>S</td>
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<tr>
<td>Morinaga</td>
<td>S</td>
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<tr>
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<tr>
<td>ProLab Diagnostics</td>
<td>D</td>
<td>D</td>
<td>-</td>
<td>S</td>
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<tr>
<td>R-Biopharm</td>
<td>C</td>
<td>C</td>
<td>-</td>
<td>S</td>
<td>S</td>
<td>A, P</td>
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<td>SafePath</td>
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<td>S</td>
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<tr>
<td>Tecra</td>
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<tr>
<td>Tepnel</td>
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<td>C</td>
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<td>S</td>
<td>C</td>
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<td>Diffchamb</td>
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<td>-</td>
<td>-</td>
<td>S</td>
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</table>

S- Sandwich ELISA; C- Competitive ELISA; D- Under Development; A- Antisera; P- PCR
Potential Sources of Error

- Sample Collection
  
  maintain representative nature of original sample

- Sample Preparation - Extraction

- Analysis

- Data Interpretation

Validated Method established level of confidence
& defined acceptable levels of variance
Extraction

- Dissolve/partition into an aqueous solvent
  - buffer plus blocking agent/stabilizer
  - 40 - 60% alcohol included to extract gluten
  - temperature (room temperature, 60 °C …)
  - shaking (continuous, periodic)
  - remove particulates (filter, centrifuge, 'let-sit')

- Reducing - denaturing conditions
  - SDS / β-mercaptoethanol (Morinaga Kits)
  - Mendez Cocktail (gluten)

_The Morinaga Assay detects the reduced / denatured antigen_
Evaluation Goals

- **LODs and LOQs**
  - **Appropriate standards**
    - NIST SRMs not necessarily designed for protein analysis
    - If unavailable, deposit alternative with NIST
    - Establish ‘conversion factors’ for other standards
  - Effects of preparation (baking, boiling, freezing)

- Survey Commodities

- Specificity / Cross - Reactivity
Approaches Employed

In-House Evaluative Studies

Working Group
- Industry, Academia, Health Canada, EU, & AOAC
- Inclusive approach
- Maximize expertise, resources, and impact

Work with Test Kit Manufacturers
- Goal to maximize number of available methods
- Share results of evaluations
- Suggest possible solutions to scientific problems
- Share needs of the agency (target levels, matrices, etc.)
What Has Been Done

3 Peanut Test Kits AOAC Validated
AOAC Recognition of Validated Gluten Test Kits & Extension of 991.19

Egg Evaluation / Validation underway
- in-house evaluations completed
- Working Group evaluations completed
- alternative reference material characterized for depositing with NIST
- specificity / cross-reactivity
  - use of encoded challenge library as part of validation
  - negative, positive, and concentration dependent controls
  - all samples analyzed at 3 levels that reflect 1, 0.1, 0.01% focus
- need to
  - finalize target level
  - draft & submit validation protocol to AOAC
  - conduct multi-lab validation at 95% confidence level

Milk scheduled to begin upon completion of egg using protocols and procedures developed for egg
## Detection Of NIST Egg SRM Spiked Into Food & Prepared

<table>
<thead>
<tr>
<th>Test Kit</th>
<th>Bread (µg/g)</th>
<th>Muffins (µg/g)</th>
<th>Dressing cooked (µg/g)</th>
<th>Pasta uncooked (µg/g)</th>
<th>Fr. Vanilla (µg/g)</th>
<th>Ice Cream (µg/g)</th>
</tr>
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<tbody>
<tr>
<td>ELISA Systems</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>2</td>
<td>5</td>
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<tr>
<td>Morinaga Institute</td>
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<tr>
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<td>10</td>
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<tr>
<td>RIDASCREEN®</td>
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<tr>
<td>TECRA® Egg VIA</td>
<td>&gt;100</td>
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<td>5</td>
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<td>BioKits (Tepnel)</td>
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<td>5</td>
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<td>5</td>
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</tbody>
</table>

*a* µg NIST SRM #8415 per gram needed to generate a response 1.9-times the response observed with the 0 µg/g sample
### Challenge Library of Food Extracts

<table>
<thead>
<tr>
<th>Amaranth</th>
<th>Garfava Flour</th>
<th>Coconut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>Green Pea</td>
<td>Hazelnut</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>Lentils</td>
<td>Macadamia</td>
</tr>
<tr>
<td>Corn</td>
<td>Lima (butter) Bean</td>
<td>Peanut</td>
</tr>
<tr>
<td>Millet</td>
<td>Pinto Beans</td>
<td>Pecan</td>
</tr>
<tr>
<td>Oat</td>
<td>Soybean (soya flour)</td>
<td>Pistachio</td>
</tr>
<tr>
<td>Rice</td>
<td>Poppy</td>
<td>Pine Nut (kernel)</td>
</tr>
<tr>
<td>Rye</td>
<td>Pumpkin</td>
<td>Walnut</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Sesame</td>
<td>Skim milk powder</td>
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<tr>
<td>Soy</td>
<td>Sunflower</td>
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<tr>
<td>Spelt</td>
<td>Nuts</td>
<td>Lecithin</td>
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<tr>
<td>Teff</td>
<td>Almond</td>
<td>Bovine gelatin</td>
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<td>Wheat</td>
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<tr>
<td>Wheat Gluten</td>
<td>Cashew</td>
<td>Ice cream</td>
</tr>
<tr>
<td>Chick pea</td>
<td>Chestnut</td>
<td>Infant formula - soy</td>
</tr>
</tbody>
</table>
Milk Evaluation

**In-House**

- comparison of NIST SRM # 1549 with a commercial product
- casein and whey specific assays evaluated
- combination casein-whey assays under development
- only one commercial combo-assay available

Working Group evaluations & validation schedule to being upon completion of egg test kit validation
Non-specific Binding Problems with Competitive ELISA SAs

Samples with responses < 100 appear as false positives.
Wheat / Gluten

In-House evaluations (V.A. Brewer exp. Lead)
   cross-reactivity (quantitative) with various grains*
   use of reducing / denaturing methods for extraction
      Mendez cocktail
      Morinaga assay

Morinaga assay reported to detect Oats
   - wheat-free based on PCR analysis
   - in-house confirmation underway

*gratitude is expressed to the USDA for providing wheat-free samples of various grains
Areas of Concern

Quantitation of allergenic foods used in whole or part milk, casein, or whey
   egg, egg white, or egg yolk
Quantitation of related, cross reacting foods
   wheat, rye, barley, triticale, spelt, etc.
   relating assay responses to ppm of a grain vs. ppm gluten
   celiac versus (classical food) allergies
Detection of Oats
Reduced/denatured extracts have been effective in analyzing prepared foods for proteins. Is this approach universally feasible? (soya)

Allergenicity ≠ Antigenicity ≠ Bioavailability ≠ Detectability
Acknowledgements

- Samantha P. Amato (JIFSAN intern)
- Gregory M. Orlowski (JIFSAN intern)
- Jenny X. Nuygen (JIFSAN intern)
- Vickery A. Brewer (FDA)
- Jeffrey R. Ammann, Ph.D. (TTB)
- Susan L. Hefle, Ph.D. (U. Nebraska)
- Jupiter Yeung, Ph.D. (FPA)
- Michael Abbott Ph.D. (Health Canada)
- Adrianus van Hengel, Ph.D. (IRMM, EU)
- Douglas L. Park, Ph.D. (FDA, retired)
- L. Rust, Ph.D. (NIH)
- David M. Peterson, Ph.D. (USDA, ARS, retired)
- Mohamed Abouzied & P.J. Terwillegar (Neogen Corp.)
- Kurt Johnson (R-Biopharm Inc.)
- Phil Goodwin (Hallmark / HAVen)
- Thomas Grace (Tepnel BioSystems Ltd.)
- Bruce Ritter (ELISA Technologies)
- Michael Ryan (ELISA Systems)
- Hans Moederzoon & Robert Rae (ProLab Diagnostics)
- Hema Shah (Morinaga, Crystal Chem Inc.)
- Ian Garthwaite (Tecra International Pty. Ltd.)
- Kauko Haapasaari (Diffchamb Sverige AB)