

Food Allergen Detection

Methods

Eric A.E. Garber, Ph.D.

Division of Natural Products, OPDF

Center for Food Safety and Applied Nutrition

Food and Drug Administration

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GOAL

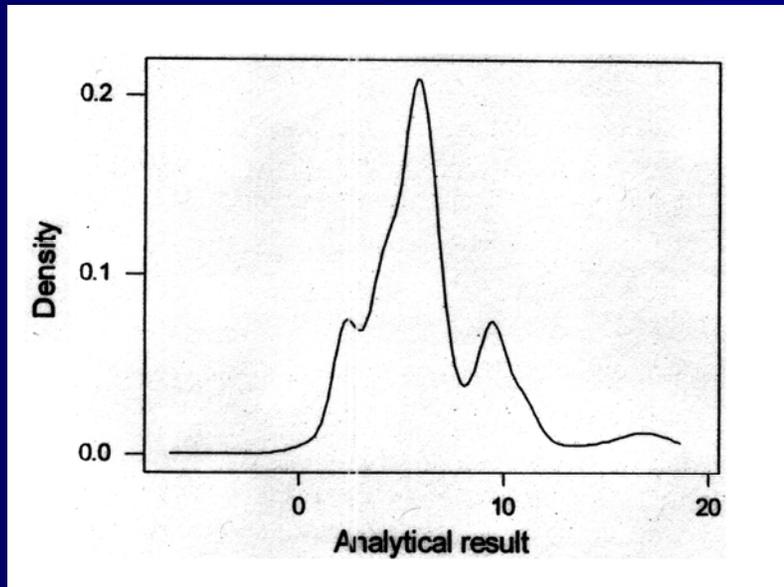
Validated Methods



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

	casein	Milk whey	casein-whey	Egg white	Wheat gliadin	Soy
ELISA Systems	S	S	-	S	-	S
Morinaga	S	S	-	S	S	
Neogen	S	S	S	S	S	S
ProLab Diagnostics	D	D	-	S	-	
R-Biopharm	C	C	-	S	S	A, P
SafePath	-	S	-	S	-	S
Tecra	-	-	-	S	-	
Tepnel	C	C	D	S	S	C
Diffchamb	-	-	-	-	S	

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

Lowest Level of NIST Whole Egg Powder Detected ^a in Spiked/Prepared Food

Test Kit	Bread	Muffins	Dressing	Pasta		Fr. Vanilla	PBS
				cooked	uncooked	Ice Cream	
	($\mu\text{g/g}$)						
ELISA Systems	>100	>100	2	5	5	5	5
Morinaga Institute	2	2	2	2	2	2	2
Veratox [®] (Neogen)	>100	>100	5	100	5	5	10
Prolisa [™] EggPAK [™]	>100	100	2	10	2	5	2
RIDASCREEN [®]	>100	>100	5	>100	5	5	10
TECRA [®] Egg VIA	>100	100	5	5	2	2	2
BioKits (Tepnel)	>100	100	2	5	2	2	5

^a μg NIST SRM #8415 per gram needed to generate a response 1.9-times the response observed with the 0 $\mu\text{g/g}$ sample



Challenge Library of Food Extracts

Amaranth

Barley

Buckwheat

Corn

Millet

Oat

Rice

Rye

Sorghum

Soy

Spelt

Teff

Wheat

Wheat Gluten

Chick pea

Garfava Flour

Green Pea

Lentils

Lima (butter) Bean

Pinto Beans

Soybean (soya flour)

Poppy

Pumpkin

Sesame

Sunflower

Nuts

Almond

Brazil

Cashew

Chestnut

Coconut

Hazelnut

Macadamia

Peanut

Pecan

Pistachio

Pine Nut (kernel)

Walnut

Skim milk powder

Cocoa

Lecithin

Bovine gelatin

Porcine gelatin

Ice cream

Infant formula - soy



Milk Evaluation

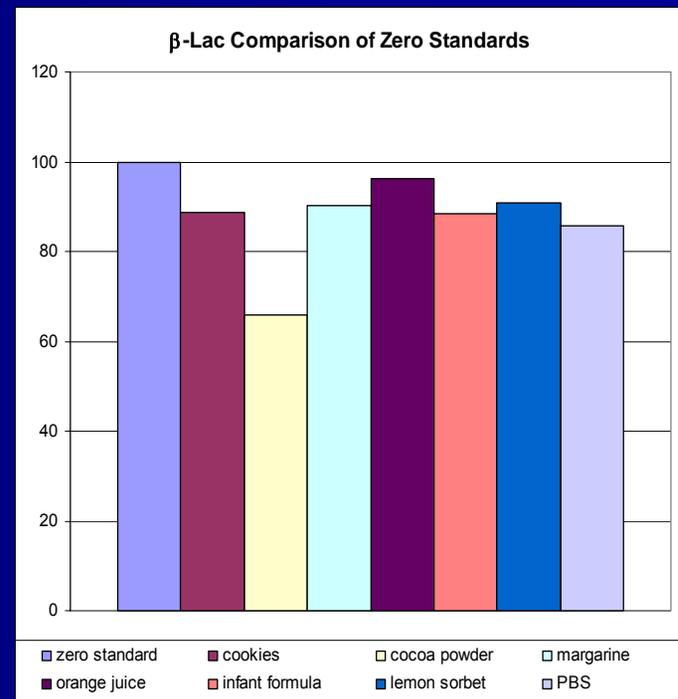
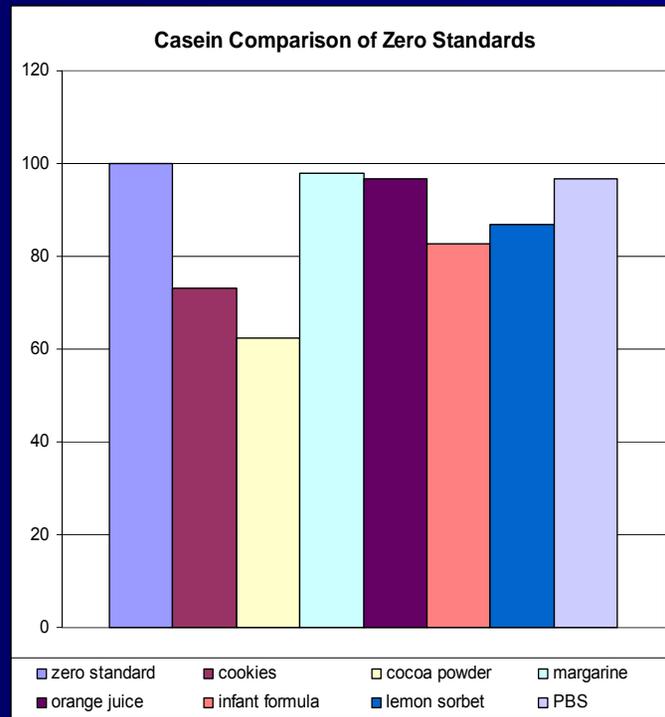
In - House

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 begin upon completion of egg test kit
validation



Non-specific Binding Problems with Competitive ELISAs



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 \neq Antigenicity \neq Bioavailability \neq Detectability



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