



Legends of Allergy/Immunology: Thomas A. E. Platts-Mills – Allergy 2019

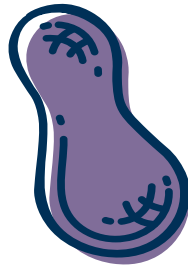
The Case for Monitoring Allergen Content of Food Extracts

DIAGNOSTICS, THERAPEUTICS, VACCINES AND FOOD PRODUCTS

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(mdc@inbio.com)





Conflict of Interest Statement

Martin D. Chapman PhD

This presentation will be evidence based.

Disclosures:

In relation to this presentation, I declare the following real or perceived conflicts of interest:

Financial interest, Co-owner of the InBio companies
Research support, NIH NIAID R01 AI077653-14
Research support, European Union iFAAM project

Case #1 Safety of Oral Food Challenge Materials

Allergists Respond to Death of 3 year-old Boy During Oral Food Challenge

 Ask the Allergist

 Symptom Test

 Find an Allergist

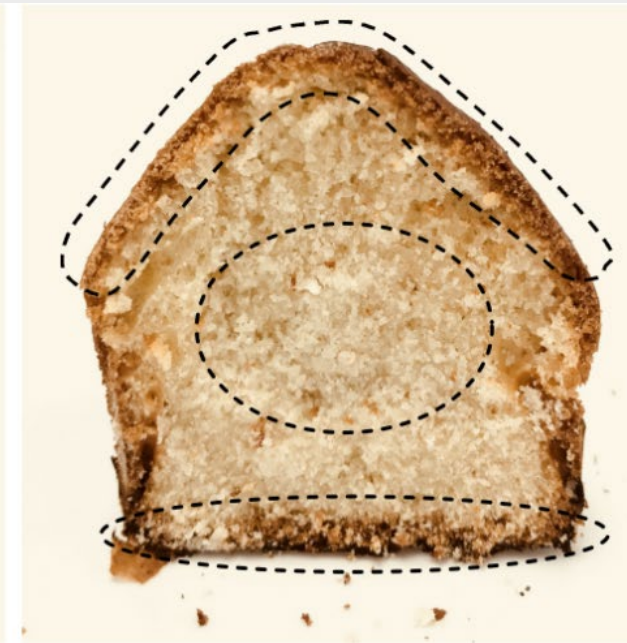


August 4, 2017

Members of the allergy community's hearts go out to 3 year-old Alastair Watson's family and friends. Alastair died during a routine oral food challenge this week. His death is a tragedy, and we cannot even imagine the horror of this loss.

Allergens in Baked Milk Muffins

HEAT STABILITY OF Bos d 11 (CASEIN)



	Mean Allergen Level	
	Bos d 5	Bos d 11
Allergen concentration (µg/g)*	0.17	2,961
Total allergen in baked muffin (µg)**	10.37	180,621

Baked muffins:

- Retain 70% of IgE binding to uncooked muffin mix
- Inhibit IgE binding to uncooked muffin by 80%

Hindley et al, Clin Exp Allergy 2021; 51:132-140

Learning Early About Peanut Allergy: (LEAP - trial of prevention of peanut allergy)



Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy

George Du Toit, M.B., B.Ch., Graham Roberts, D.M., Peter H. Sayre, M.D., Ph.D., Henry T. Bahnson, M.P.H., Suzana Radulovic, M.D., Alexandra F. Santos, M.D., Helen A. Brough, M.B., B.S., Deborah Phippard, Ph.D., Monica Basting, M.A., Mary Feeney, M.Sc., R.D., Victor Turcanu, M.D., Ph.D., Michelle L. Sever, M.S.P.H., Ph.D., Margarita Gomez Lorenzo, M.D., Marshall Plaut, M.D., and Gideon Lack, M.B., B.Ch., for the LEAP Study Team*

POSITION ARTICLE AND GUIDELINES

Open Access

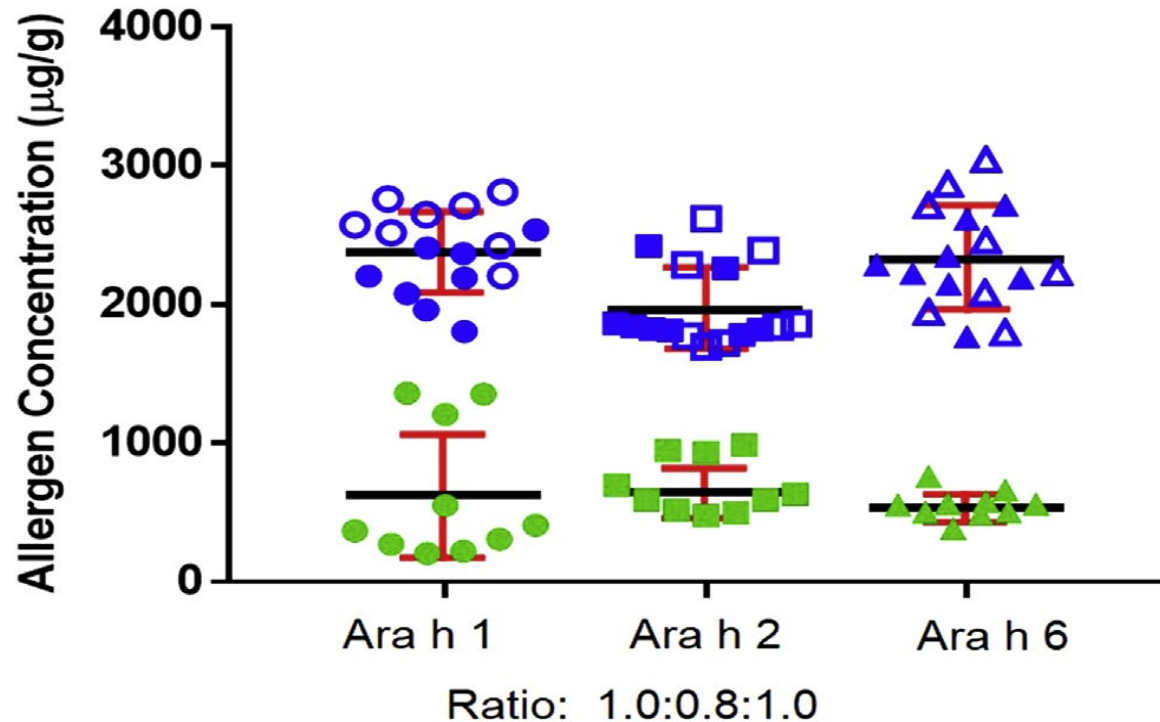
Consensus communication on early peanut introduction and the prevention of peanut allergy in high-risk infants



David M. Fleischer^{1†}, Scott Sicherer^{2†}, Matthew Greenhawt^{3†}, Dianne Campbell^{4†}, Edmond S. Chan^{5†}, Antonella Muraro^{6†}, Susanne Halken^{6†}, Yitzhak Katz^{7†}, Motohiro Ebisawa^{8†}, Lawrence Eichenfield^{9†}, Hugh Sampson^{10†}, FOR THE LEAP TRIAL TEAM and SECONDARY CONTRIBUTORS



Case #2. Doses of peanut allergen in Bamba administered during the LEAP study



Bamba
US/UK
samples

ErdnußLocken
Germany

✓ Bamba is a well formulated peanut product, with each of the three major allergens present in uniform amounts.

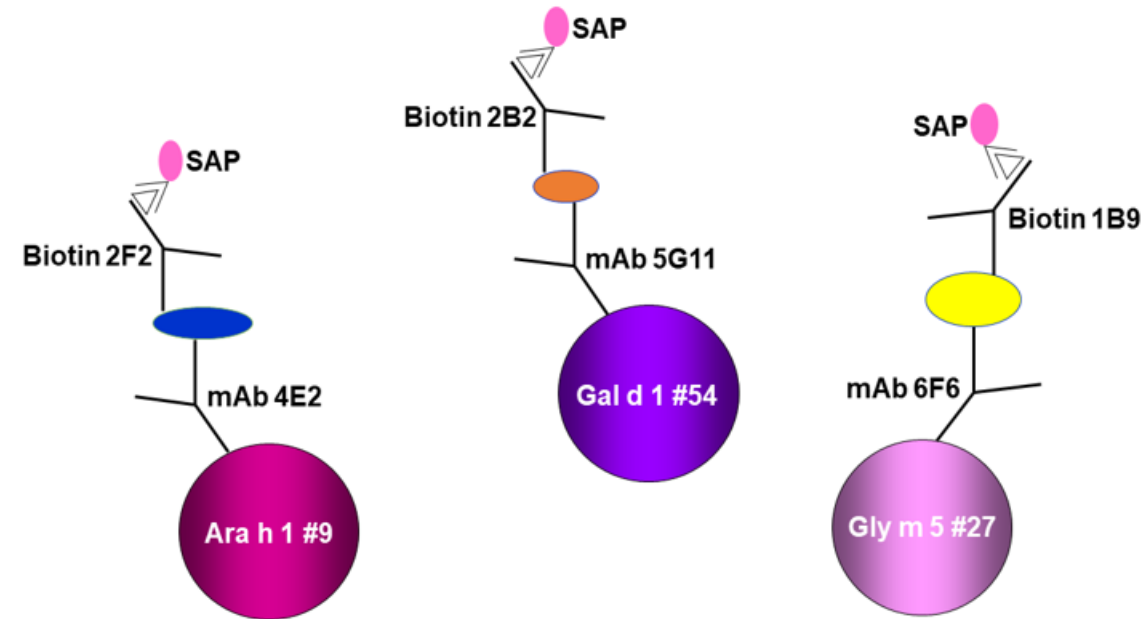
Cumulative weekly doses of **~330 mg peanut allergens** (Ara h 1, Ara h 2 and Ara h 6) are associated with prevention of peanut allergy.

Hindley et al, JACI 2018; 141:780-82

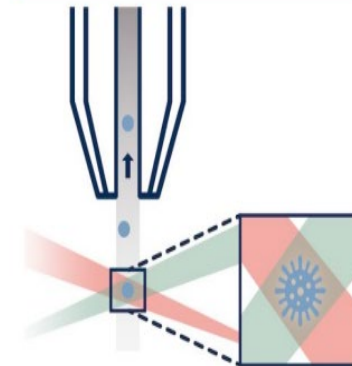
MARIA for Foods

Food allergen specific mAb beads in a suspension multiplex array

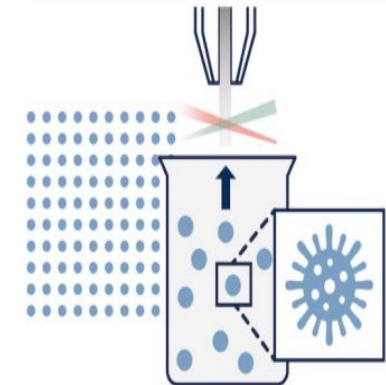
- Multiplex array built on Luminex xMAP technology
- 6.5µm polystyrene beads with internal fluorescent dyes
- Bead surface functionalized with carboxyl groups for covalent coupling
- Beads coupled to capture mAb, incubate with allergen samples
- Detect allergen using biotinylated mAb and streptavidin-conjugated fluorophore
- *Filep et al, Food Chemistry 2022*



Precise Calibration



One Test - Multiple Assays



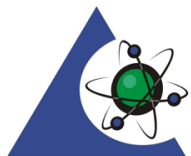
MARIA for Foods Standardized Reagents

ANTIBODIES, BEAD SETS,
ALLERGEN STANDARDS

Target allergens were chosen for
assay development based on:

- Clinical relevance
- Relative abundance
- Purity
- The US Big-9 and EU 14 allergens

Filep et al, Food Chem 2022



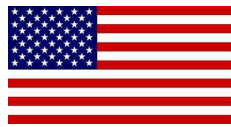
PJLA
Testing

ISO/IEC 17025:2005
Accreditation # 76453

Food	Target Protein	Capture Ab	Allergen Standard	Magnetic Bead Set	Detector Ab
Cashew	Ana o 3	mAb 1H4	rAna o 3	34	mAb 4A11
Peanut	Ara h 1	mAb 4E2	nAra h 1	9	mAb 2F2
Peanut	Ara h 3	mAb 1E8	nAra h 3	30	mAb 4G9
Peanut	Ara h 6	mAb 3B8	nAra h 6	66	mAb 3E12
Celery	Api g 1	mAb 5D4	rApi g 1	43	mAb 7C4
Milk	Bos d 5	mAb NBD5-1	nBos d 5	52	mAb NBD5-2
Milk	Bos d 11	mAb VBIC	nBos d 11	65	mAb CC11
Hazelnut	Cor a 9	mAb 3B6	nCor a 9	36	mAb 6F5
Fish	Cyp c 1	mAb 9A6	rCyp c 1	53	mAb 7C5
Egg	Gal d 1	mAb 5G11	nGal d 1	54	mAb 2B2
Egg	Gal d 2	mAb 1B4	nGal d 2	29	mAb 7D8
Soy	Gly m 5	mAb 6F6	nGly m 5	27	mAb 1B9
Walnut	Jug r 1	mAb 7D7	rJug r 1	44	pAb
Sesame	Ses i 1	pAb	rSes i 1	62	pAb
Mustard	Sin a 1	mAb 3B4	rSin a 1	46	mAb 2B11
Wheat	Tri a 19	mAb 8B10	rTri a 19	7	mAb 3C10
Shrimp	Tropomyosin	mAb 1A6	nShrimp Tropomyosin	78	pAb

MARIA for Foods Includes All Regulated Food Allergens

In the U.S., Europe and Japan



1. Milk: Bos d 5, Bos d 11
2. Eggs: Gal d 1, Gal d 2
3. Fish: Cyp c 1
4. Crustacea: Tropomyosin
5. Tree nuts: Ana o 3, Cor a 9, Jug r 1
6. Peanuts: Ara h 1, Ara h 3, Ara h 6
7. Wheat: Tri a 19
8. Soybean: Gly m 5
9. Sesame: Ses i 1



10. Celery: Api g 1
11. Mustard: Sin a 1

EU 1169/2011;

Off J Eur Union L304, 18-63.

*Food Allergen Labeling and Consumer Protection Act, 2004;
Food Safety and Modernization Act, 2010.

Case #3. Early Introduction Foods

PROMOTED TO CONSUMERS FOR 'PREVENTION' OF FOOD ALLERGY



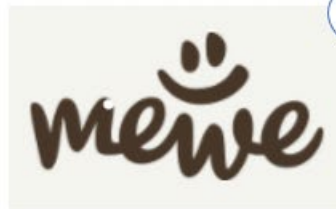
HP



RSF



LMX



MWP



MME



SFO



INS



MYP

InBio has no financial interest in these companies.

EIF Study Overview

SAMPLES AND PROTOCOL

Protocol:

- EIF from 9 manufacturers and 4 controls were compared
- Between 1-8 samples of 32 EIF were analyzed for 17 allergens by MARIA for Foods.
- Ara h 2 was measured by ELISA 2.0
- In total, 86 samples were tested for a dataset of n=1548
- *Filep S, Chapman MD JACIP 2022*





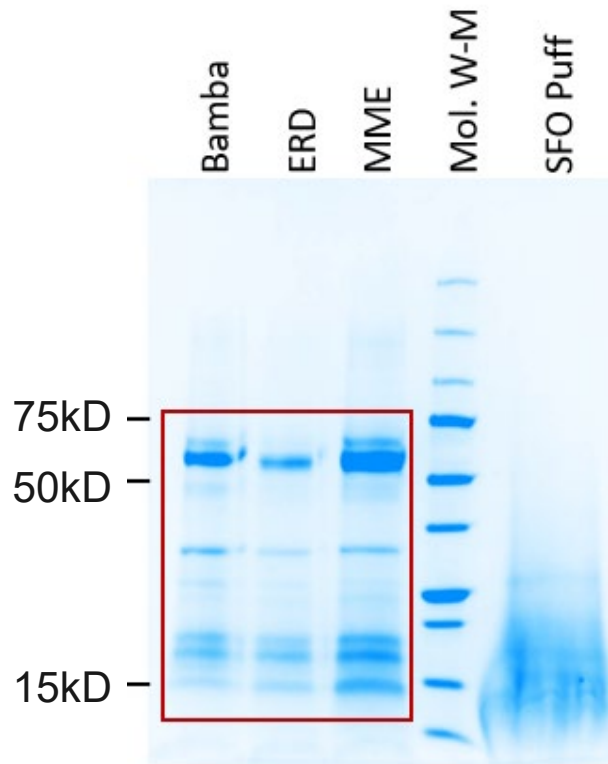
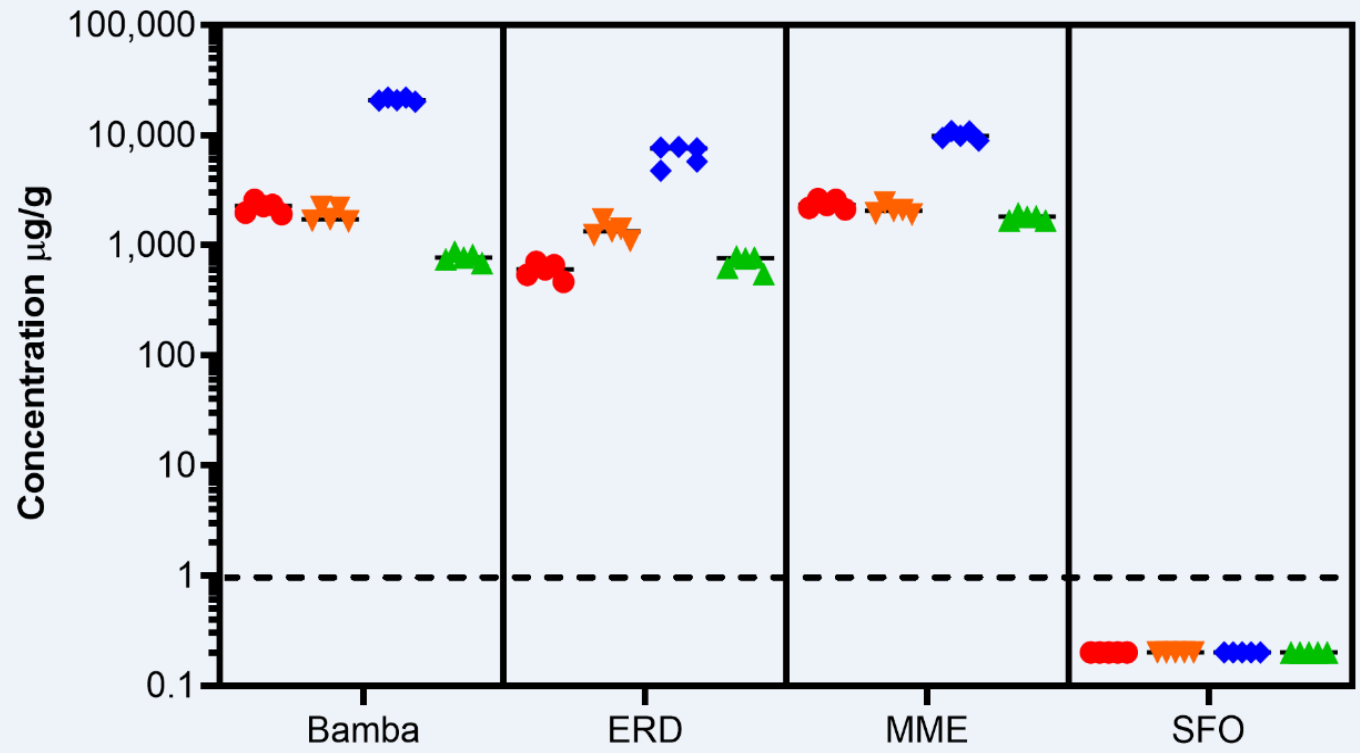
Doses of Food Allergens in Early Intervention Products

KEY FINDINGS

- Peanut puffs – 3 of 4 products had ‘consistent’ allergen profiles
- Peanut powders – 4 of 7 products had ‘consistent’ allergen profiles
- Up to 100-fold differences between allergen levels in different products
- Up to 100-fold variability in levels between multi-allergen products
- Cross-contamination – e.g. trace amounts of milk and egg allergens in peanut products
- Inconsistent allergen profiles in products from 3 companies

Major Peanut Allergen Levels

FOOD PUFFS

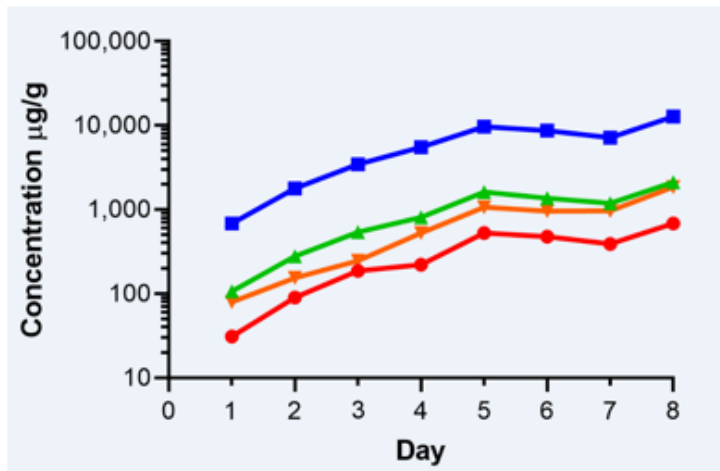


- Ara h 1
- ▼ Ara h 2
- ◆ Ara h 3
- ▲ Ara h 6



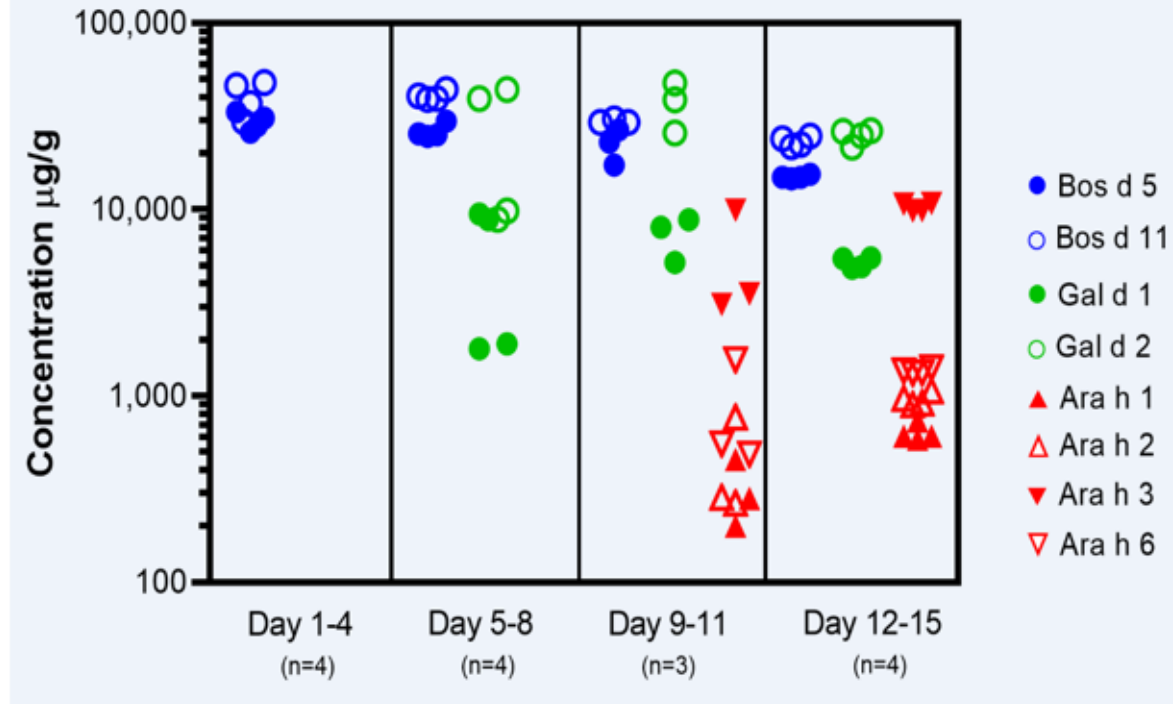
Allergen concentration by day in selected EIF

POWDER PRODUCTS



■ Ara h 3, ▲ Ara h 6, ▼ Ara h 2, ● Ara h 1

HP Peanut Powder

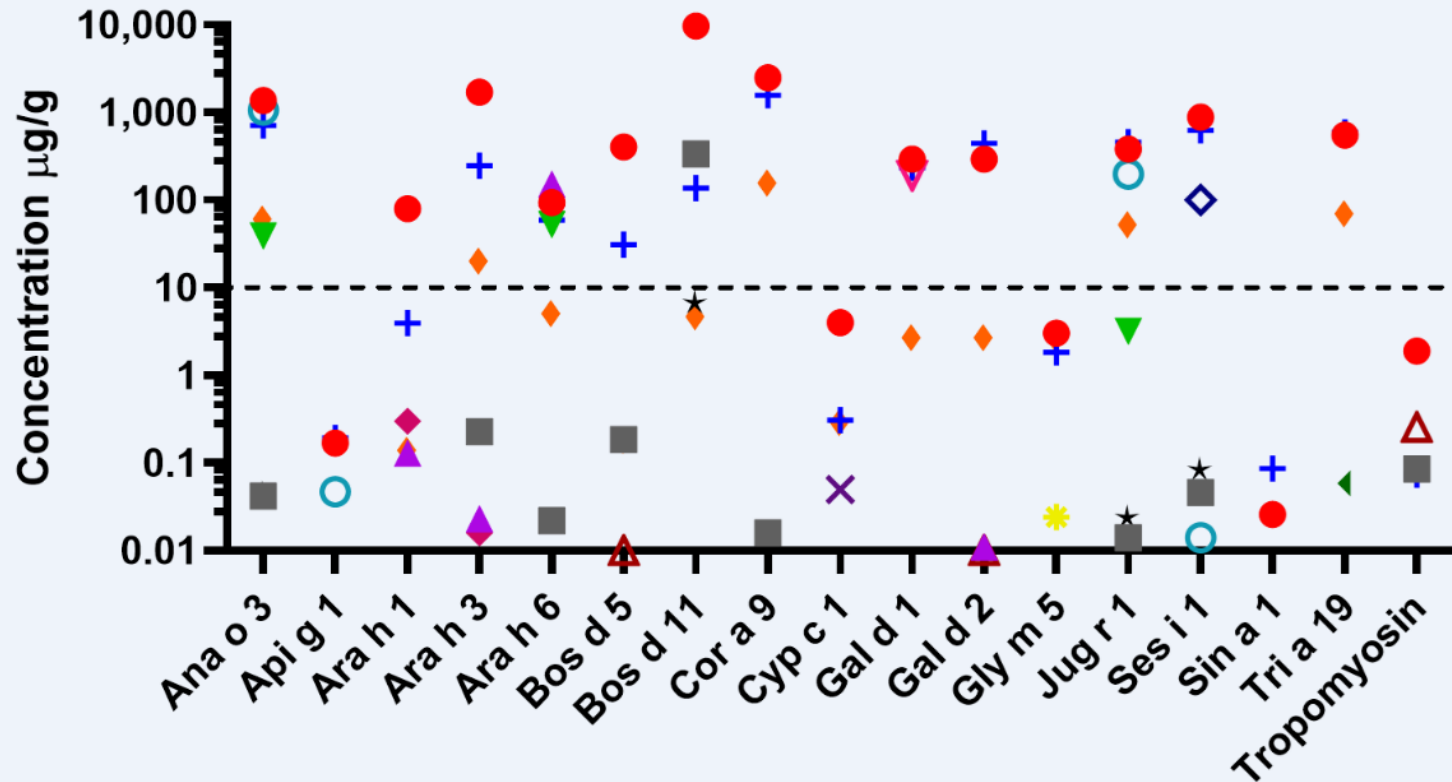


RSF Milk, Egg and Peanut Powder

Filep and Chapman JACIP 2022

Variability in allergen levels

Multi-blend EIF and Fruit Sauces



- SFO ● + ■ ★ ◆
 - MYP ▲ ▼
 - INS ◆ ○ * △ ▽ ◆ ◀ ✕
- Dashed line at $10\mu\text{g/g}$ indicates 'trace' level of allergen.

Early Intervention Foods – Combined Doses

CUMULATIVE DOSES OF SPECIFIC ALLERGENS

EIF Product		Dosing Schedule	Cumulative Allergen Dose*		
			Peanut	Milk	Egg
LMX	Peanut Powder	One serving, 3x per week	1,977	NA	NA
MWP	Nut Butter	One serving, 3x per week	2,946	NA	NA
HP	Peanut Flour	3x per week	208	NA	NA
RSF	Milk, Egg, Peanut Powder	Total allergen, day 1-15	159	1,245	715
SFO	16 Blend Food Powder	One serving, 7x per week	28	140	8.4
MYP	Peanut	One serving	29	NA	NA
INS	Peanut + Apple	One serving	14	NA	NA

*Based on the dosing recommendations provided by the manufacturer on the product insert or website. NA = Not applicable.

Voluntary Lot Withdrawal of Allergenic Extract – Pecan nut (*Carya illinoensis*) – For Diagnostic Use Only, Manufactured by ALK- Abelló, Inc. for Increased Reports of False Negative Test Results

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September 22, 2023

The following lot of Allergenic Extract – Pecan nut (*Carya illinoensis*) – For Diagnostic Use Only, manufactured by ALK-Abelló, Inc. has been voluntarily withdrawn by the manufacturer due to increased postmarketing adverse event reporting of false negative skin test results, with one case reported of life-threatening anaphylaxis from subsequent pecan nut exposure.

The AAAAI and ACAAI Response (March 2023)

- Concern that labeling all food extracts for skin testing in this manner is unnecessary
- New label statement could also be applied to food-specific serum IgE testing
- New labeling could be over-interpreted, resulting in:
 - over-testing
 - over-diagnosis
 - reduced quality of care
 - increased health care costs
- AAAAI and ACAAI requested further discussion with FDA concerning these statements.

Case #4 Peanut Diagnostic Products

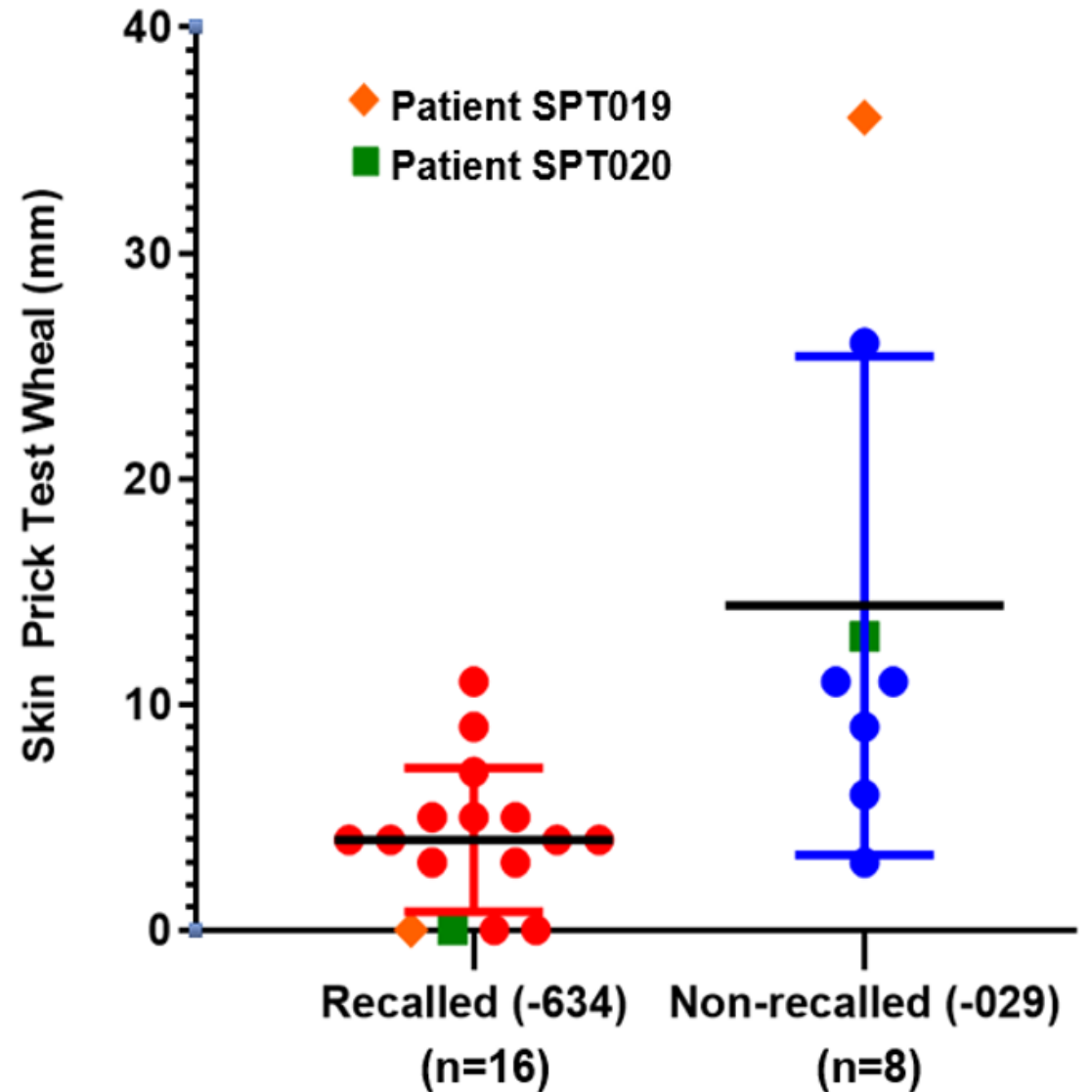
POTENCY OF RECALL AND
NON-RECALL LOTS

Mean Wheal Diameter

Recall: 4mm, SD 3mm

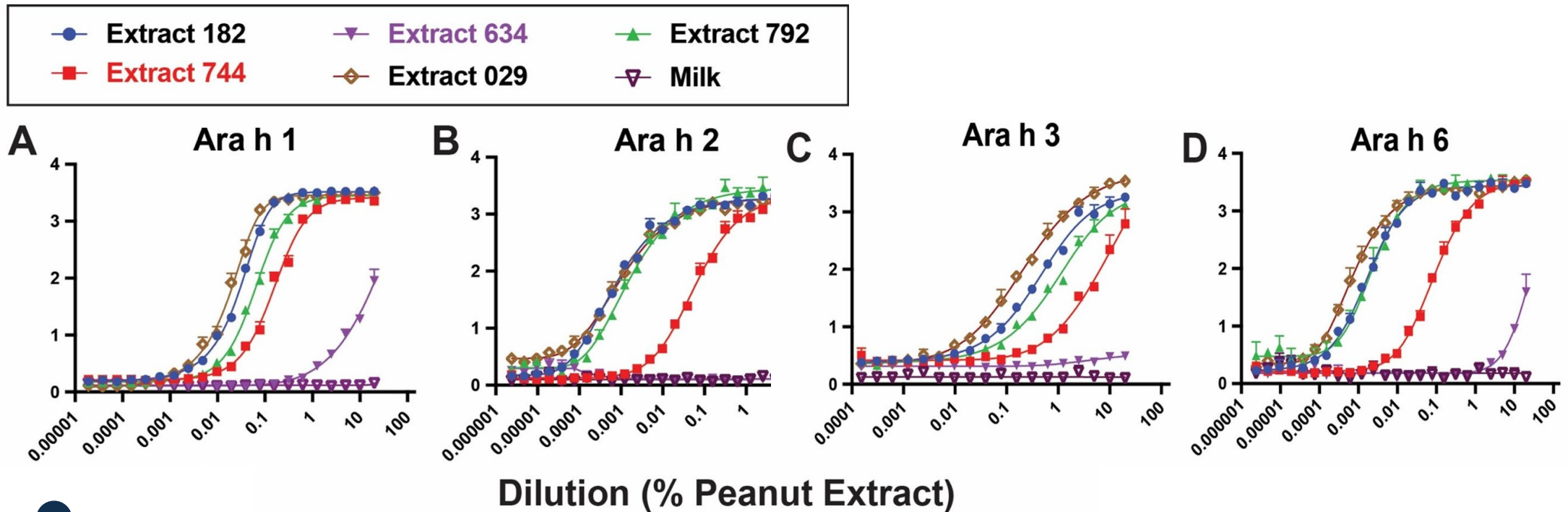
Non-Recall: 14mm, SD 11mm

Stone, Hemler et al, JACIP 2023



Allergen analysis using hIgE mAb to peanut allergens

DOSE RESPONSE CURVES USING RECALL AND NON-RECALL LOTS



Allergen concentration within recall and nonrecalled peanut lots

Two site mouse IgG mAb ELISA 2.0

		Ara h 1	Ara h 2	Ara h 3	Ara h 6
	Sample ID	µg/ml	µg/ml	µg/ml	µg/ml
R	ALK Lot# 0004014634	<0.31	<0.02	<0.01	0.001
R	ALK Lot# 0004218744	<0.31	1.09	0.29	3.73
	ALK Lot# 0003290182	7.5	76	2.2	229
	ALK Lot# 0003853792	1.9	19	1	146
	ALK Lot# 0004328029	24	261	3	485
	LOD (µg/ml)	<0.31	<0.02	<0.01	<0.001

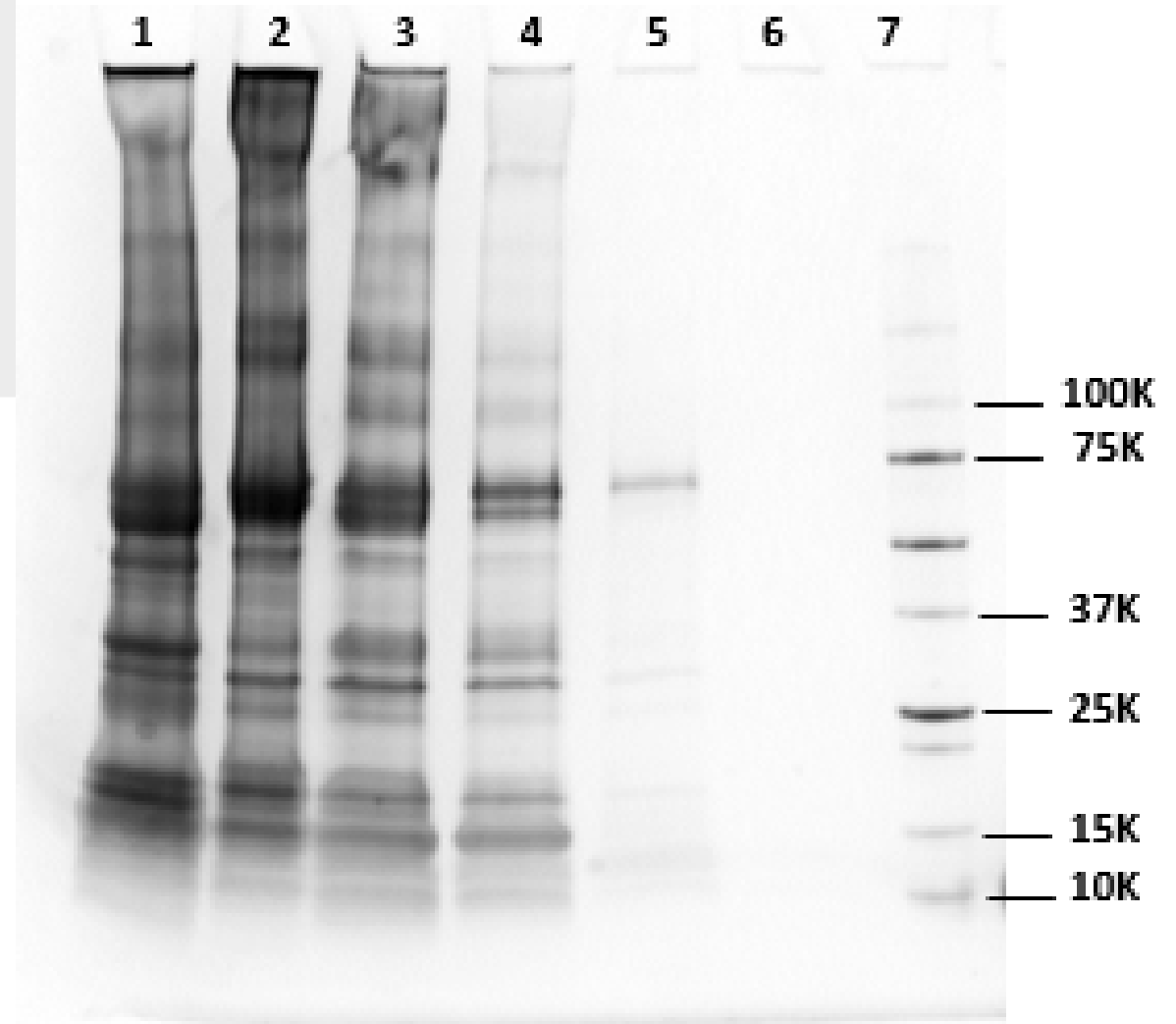
- Filep et al, JACI 2018; Stone et al JACIP 2023

SDS-PAGE analysis

RECALL AND NON-RECALL LOTS

SDS-PAGE analysis of **10uL** of each of the ALK peanut extract lots:

1. ALK Lot# L0441 (Exp. 4/2015)
2. ALK Lot# 0004328029
3. **ALK Lot# 0003290182**
4. ALK Lot# 0003853792
5. **ALK Lot# 0004218744**
6. **ALK Lot# 0004014634**
7. MW



Current Diagnostic Peanut Extracts

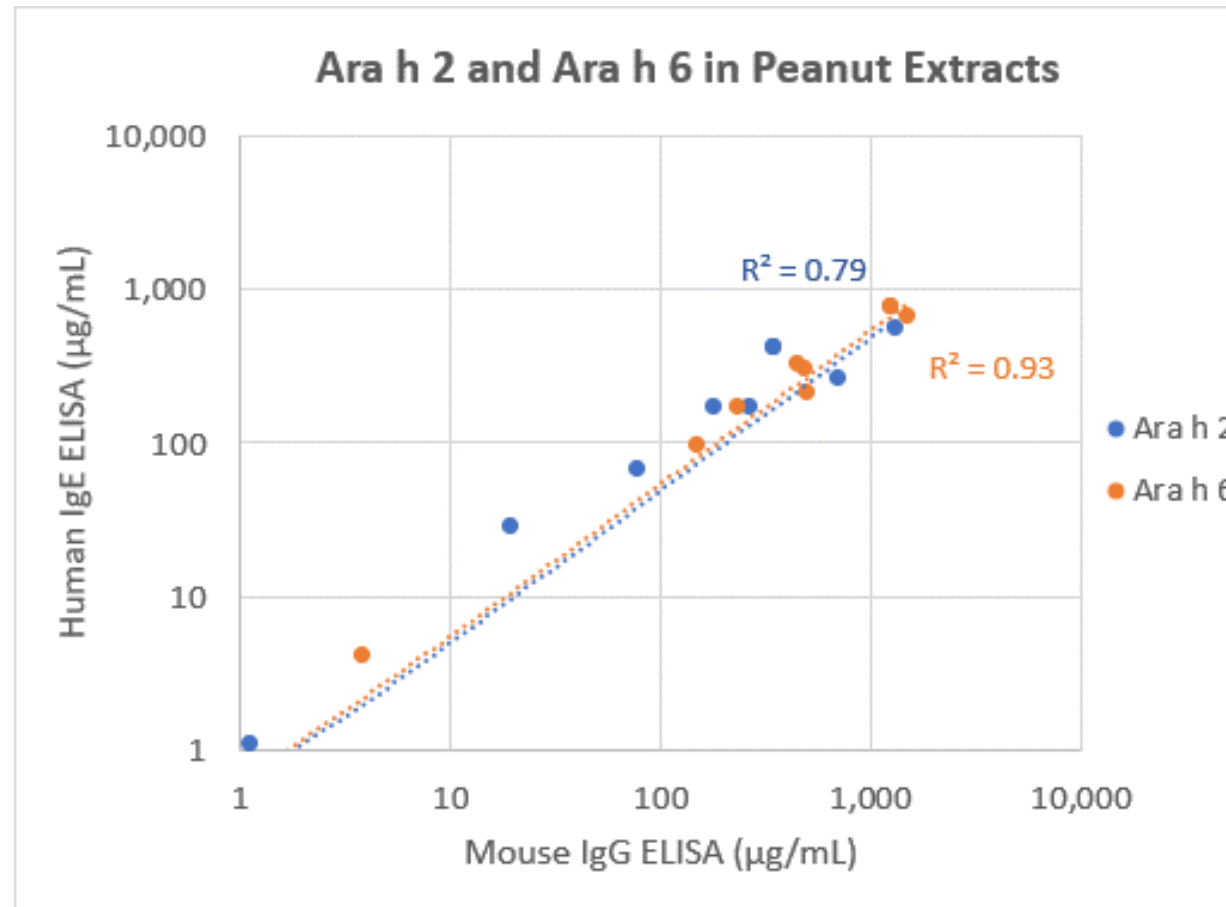
COMPARISON OF
ALLERGENIC POTENCY

Manufacturers	Lot #	Ara h 1 µg/ml	Ara h 2 µg/ml	Ara h 3 µg/ml	Ara h 6 µg/ml
Diagnostic Peanut Extracts					
ALK	0004328029	24	261	3	485
ALK	0004451535	65	177	10	439
Lofarma	I1622.01	126	692	156	492
Stallergenes Greer	417151	52	1,285	584	1,473
Other Peanut Products					
InBio Peanut Protein	46147	1,310	680	2,100	380
Bamba (µg/g)*		2,211	1,884	21,072	778
PB2 (µg/g)*		5,377	5,748	52,547	5,890
LOD (µg/ml)=		<0.31	<0.02	<0.01	<0.001

Filep at al Food Chem 2022; Filep & Chapman JACIP 2022

Correlation between murine IgG and hIgE mAb immunoassays

ARA H 2 AND ARA H 6 ELISA





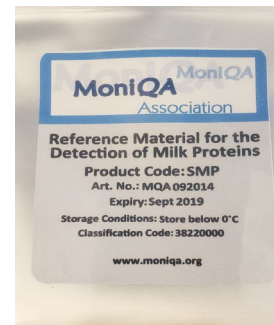
Allergen Standardization

A MULTI-FACETED APPROACH

- Biochemical analyses.
- Immunoassays for precise measurement of specific allergens:
 - ELISA, MARIA for Foods – Multiplex array technology for simultaneous allergen measurements.
- Mass spectrometry for detecting multiple allergens.
- Human IgE monoclonal antibodies from allergic patients.
- Customized methods for novel therapeutic products
- Standardization based on measuring allergens - the active pharmaceutical ingredients (API).

Case #5 NIST Food Reference Materials

9-PLEX ANALYSIS



	Allergen Concentration (µg/g):								Shrimp Tropomyosin
	Ana o 3	Ara h 3	Ara h 6	Bos d 5	Cor a 9	Gal d 1	Gal d 2	Gly m 5	
MoniQA - Milk	BD	BD	BD	21,370	BD	BD	0.01	BD	BD
NIST SRM 1549a - Milk	BD	BD	BD	7,000	BD	BD	BD	BD	BD
NIST SRM 8445 - Egg	BD	BD	BD	BD	BD	11,970	109,700	BD	BD
NIST SRM 3234 - Soy	BD	BD	BD	BD	BD	BD	BD	45,314	BD
NIST SRM 2387 - Peanut Butter	BD	59,250	1,820	BD	BD	BD	BD	BD	BD
NIST RM 8405 - Hazelnut	BD	BD	BD	BD	175,340	0.20	0.01	BD	BD

Filep et al, Food Chemistry 2022

The Utility of Mass Spectrometry

READY FOR PRIMETIME?

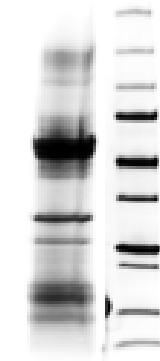
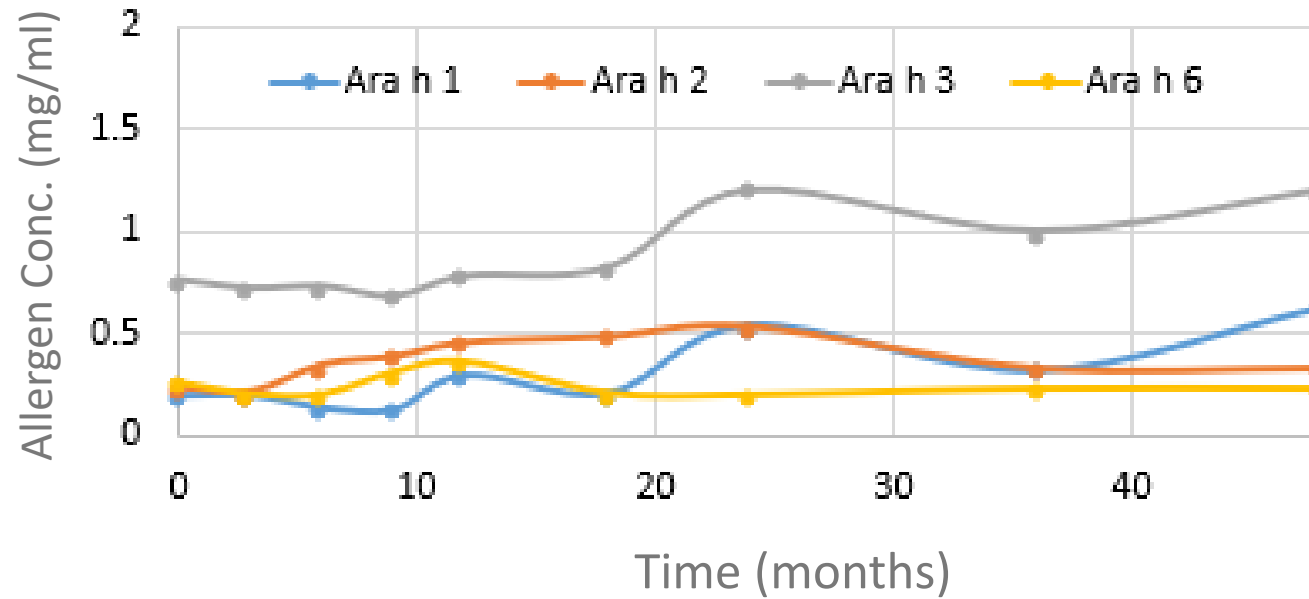
- Purity assessment of allergen molecules
- Absolute quantification of individual allergens
- Relative abundance of allergens e.g. in therapeutics and foods
- Already being considered by regulatory authorities (FDA, PEI)



Chapman & Briza CAAR 2012
Mindaye et al, Clin Exp Allergy 2017

Real time stability testing of peanut flour protein

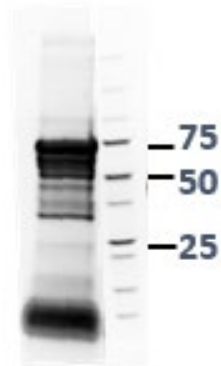
GOLDEN PEANUT CO, 12% LIGHT ROAST (DEFATTED)



60 months

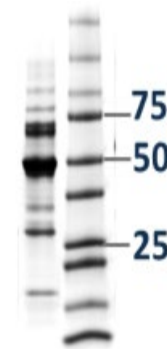
Allergen composition of food reference materials

RELATIVE ABUNDANCE BY MASS SPECTROMETRY



Allergen	Relative Abund. (%)
Cor a 1.0401	0.2%
Cor a 8	4%
Cor a 9	51%
Cor a 11	13%
Cor a 14	15%
Cor a 16	17%

Hazelnut Flour Protein



Allergen	Relative abundance (%)
Gly m 4	0.03%
Gly m 5	34%
Gly m 6	46%
Gly m 7	0.1%
Gly m 8	2.5%
Other	17%

Soy Flour Protein

Wuenschmann et al, AAAAI 2024

Manufacturing processes of peanut (*Arachis hypogaea*) allergen powder-dnfp

Stephanie A. Leonard¹, Yasushi Ogawa², Paul T. Jedrzejewski², Soheila J. Maleki³, Martin D. Chapman⁴, Stephen A. Tilles^{2*}, George Du Toit⁵, S. Shahzad Mustafa⁶ and Brian P. Vickery⁷

Screening of drug substance:

- Relative potency by ELISA
- Protein integrity/content by HPLC
- Aflatoxin
- Color, fat content, moisture
- Bacteria, yeast and mold counts

Up to 60% of peanut source material lots (N=34) failed to meet acceptance criteria

Case #6 Potency ranges of peanut drug substance

PTAH; Palforzia[®]

Clinical Study	Number of Drug Product Batches	Ara h 1		Ara h 2		Ara h 6	
		Potency Range ^a	Ratio ^b	Potency Range ^a	Ratio ^b	Potency Range ^a	Ratio ^b
PALISADE ^c (ARC003)	8	0.68–1.59	2.34	0.72–1.34	1.86	0.63–1.26	2.00
ARTEMIS ^d (ARC010)	11	0.68–1.25	1.83	0.93–1.34	1.44	0.75–1.26	1.68
Total	19	0.68–1.59	2.34	0.72–1.34	1.86	0.63–1.26	2.00

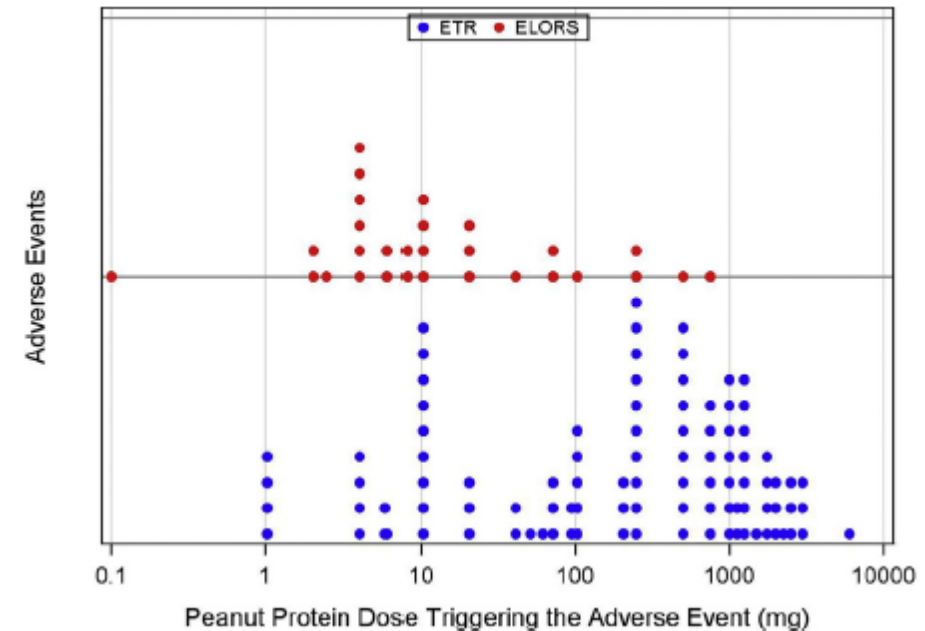
Real-World Experience with Peanut Oral Immunotherapy: Lessons Learned From 270 Patients



Richard L. Wasserman, MD, PhD^{a,b}, Angela R. Hague, PA-C^a, Deanna M. Pence, RRT^a, Robert W. Sugerman, MD^{a,b}, Stacy K. Silvers, MD^{b,c}, Joanna G. Rolan, PA-C^a, and Morley Herbert, PhD^d *Dallas and Austin, Texas*

- Retrospective review of allergy clinic patients receiving peanut OIT (2009-17).
- 79% of patients achieved maintenance dose (2000mg) and 105 patients received 3 or more years of maintenance.
- 23% of patients had adverse reactions that required treatment with epinephrine (ETR)
- 14% had other adverse reactions (ELORS)

JAIP 2019.



Captain Crunch and the Kool-Aid!

HOME BREW, OFF-LABEL
PEANUT OIT THERAPY



Captain Crunch

Escalation Day: ¼-2 pieces, 2-16mg @ 20 min int

Build Up Protocol, Bi-weekly up dosing with:

Captain Crunch – 16-128mg

PB2 - tsp 15-2000mg

Peanut butter – tsp 18-2300mg

Nuts.com peanut flour

~10g + 50ml dH₂O = 250mg/ml

Six Serial dilutions down to 2.5µg/ml in dH₂O

11 doses, 0.1-20mg peanut protein solution dissolved
in Kool-Aid powder

Up dosing:

12 doses peanut fragments or peanuts, up to
challenge with 24 peanuts (6000mg peanut protein)

2000mg maintenance dose for 3 years



Future Priorities for Allergen Standardization

VALIDATION, METHODS DEVELOPMENT, REGULATORY APPROVAL

- Standardization and harmonization of peanut and other food allergen diagnostic products is a high priority.
- Simplified and improved multiplex immunoassay platforms.
- Validated mass spectrometry methods that can be incorporated into multi-center ring trials.
- Validation of *in vitro* methods for assessment of biologic potency
- Fast track processes for allergen standards, measurement methods and potency assessment to speed regulatory approval.

The Case for Monitoring Allergen Content of Food Extracts

CLOSED!

- Assessment of the allergen profile, composition and quality of natural and processed foods.
- Defining the 'active pharmaceutical ingredients' (API) of foods.
- Potency and/or tolerogenicity assessment of oral food challenge materials, early introduction foods, and next-gen allergen therapeutics.
- A must for clinical trials.
- Quality control and management of diagnostic and therapeutic products.
- Development of robust food reference materials for standardization and harmonisation of food extracts.

Acknowledgements

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University of Salzburg

Lorenz Aglas

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