Allergenicity Risk Assessment of Novel Proteins (GMOs & New Foods)

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Celiac database is included in AOL

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RE Goodman's Experiences (for your reference)

- 1980-85 Allergen extract standardization at Hollister-Stier
- Molecular Biology (PhD) , 1990, with 7 years work in immunology Cornell and the Univ. of Michigan
- Research Scientist at Monsanto (1997 - 2004)
  - Allergenicity studies on GM crops
  - Is a GM protein an allergen? Is it potentially cross-reactive?
- **Research Professor at UNL (2004 - 2021)**
  - Food Allergy and the Allergenicity Assessment of GMOs and new or novel food sources
  - Manager www.AllergenOnline.org
  - Chair WHO/IUIS Allergen Nomenclature Committee
  - Fellow AAAAI, member EAACI and American College of Allergy

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Reality of GM and Novel foods

- GM events >>100 approved in the US
- Each took ~ 13 years / event to develop & approve
- Each took ~ $140 million USD regulatory studies and global approvals
- US, Canada, Australia…relatively clear processes
- China & Europe…hesitant, process is not as clear
- **IN 25 YEARS…there is NO EVIDENCE of new Allergy risks from any approved GM event**
- **AND consider NOVEL FOODS…whole new foods…algae, fungus, new grain?**

CODEX 2003 & 2009 for GMOs Assessment for IgE - mediated allergies (and celiac disease and nutrients and toxins) Guideline

1. **Is the protein a known allergen?** (Bioinformatics and literature searches…if indicated test serum IgE
2. **Is the protein nearly identical to an allergen & potentially cross-reactive?** Bioinformatics and literature searches…if indicated, test serum IgE
3. **Will consumption of the protein lead to elicitation or sensitization? (If not known to be an allergen or potentially cross-reactive, other tests?)**
    1. **Stability in pepsin & abundance in food fractions**
    2. **For some products effects of thermal processing impact**

METHODS NOT proven & PREDICTIVE should not be used: Computer epitope predictions, complex cell assays and animal models

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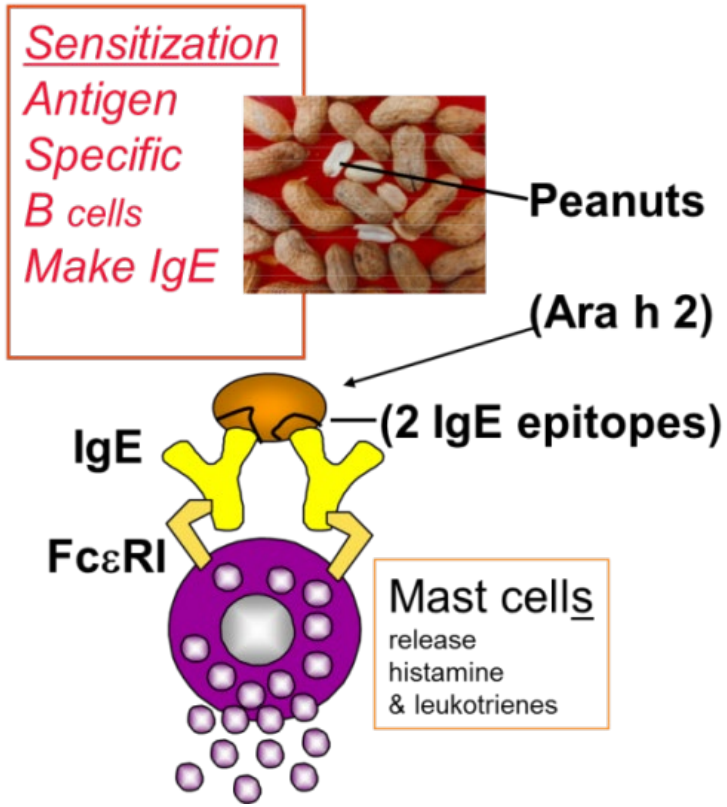
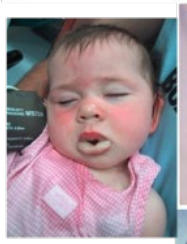
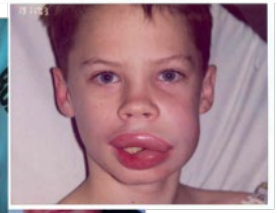
ELICITATION after sensitization: multiple IgE epitopes per protein variable doses per person and per protein!



IgE mediated food allergy affects between 2% and 8% of consumers – Reactions are to specific food proteins: reactions are usually mild, but can be quite severe





Angioedema
Asthma
Hives
Vomit
Diarrhea
Hypotension

Sometimes mixed IgE, T- cell and eosinophil reactions

RARELY Systemic Anaphylaxis, which can result in death

Oral Allergy Syndrome, or mild skin reactions, are more common…. Systemic anaphylaxis is relatively rare

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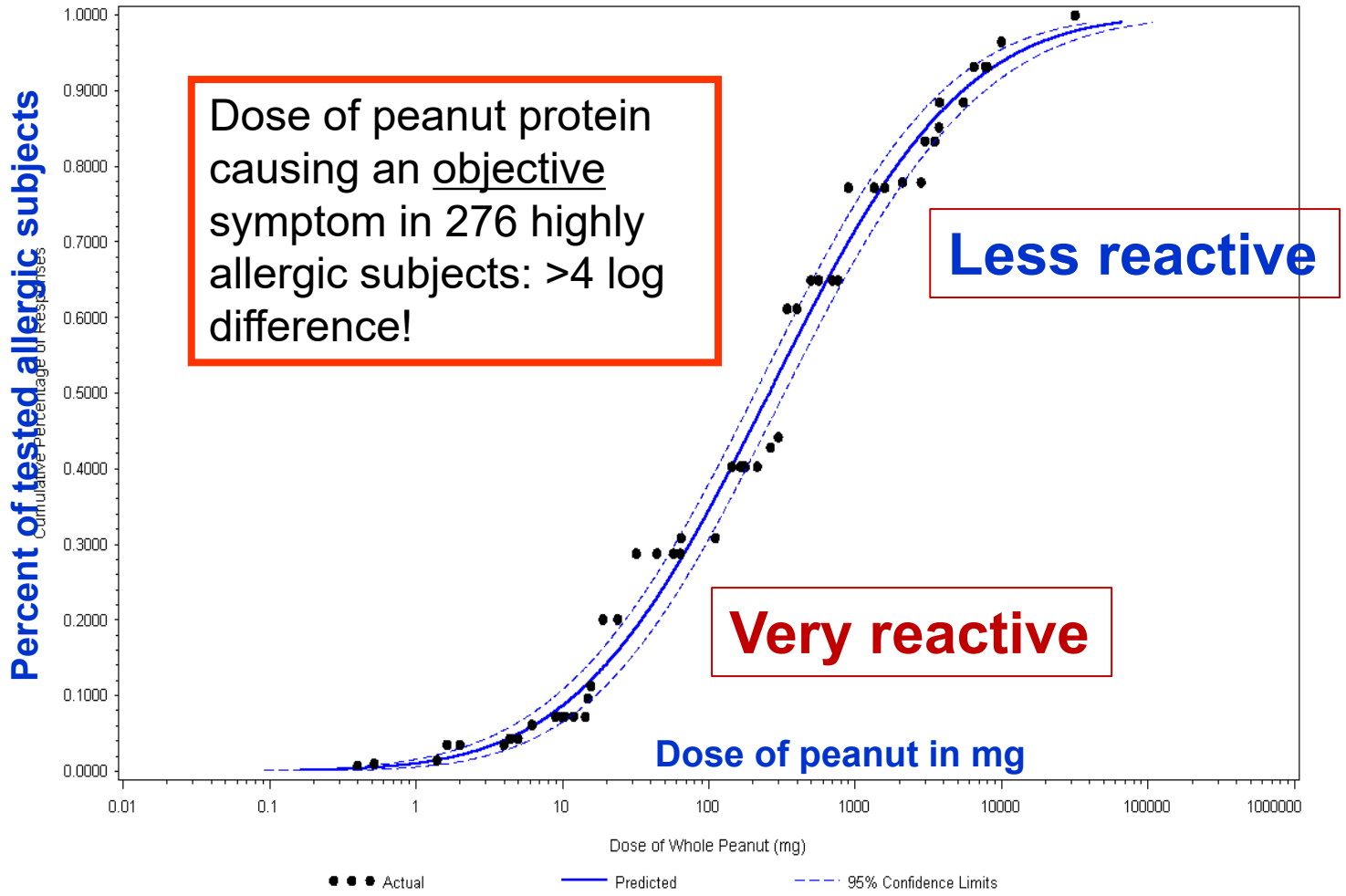
Individual Peanut Allergic Subject's threshold doses range more than 10,000-fold between people based on Food Challenges (first objective symptom, whole peanut allergic subjects) DOSE is important!

276 Peanut Allergic Subjects
Dose to cause SOME objective Symptoms



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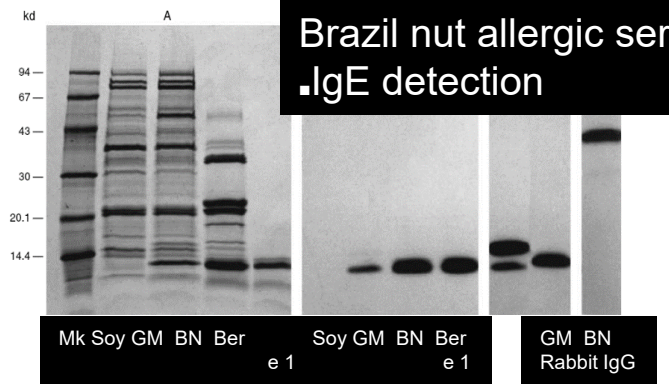
Can a GM trait increase food allergy risk? Pre-market tests 1995 GM Soybean with 2S albumin from Brazil nut (not a known allergen). Tests by Nordlee/Taylor showed RISK Pioneer HiBred STOPPED in Development without government intervention (NE J Med Nordlee et al. 334:688)



Immunoblot
Brazil nut allergic sera
▪ IgE detection

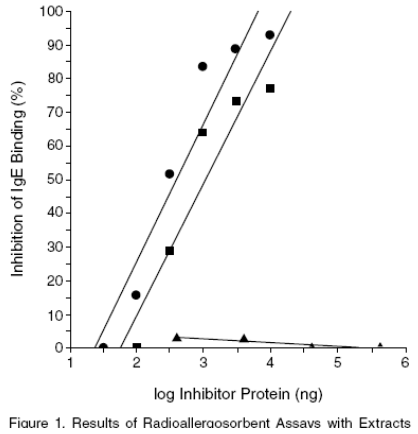


Figure 1. Results of Radioallergosorbent Assays with Extracts

RAST Inhibition
Brazil nut protein solid
▪ GM soy inhibits
• Brazil nut inhibits
▸ Non-GM soy does not

Skin Prick Tests
Brazil nut allergic patient

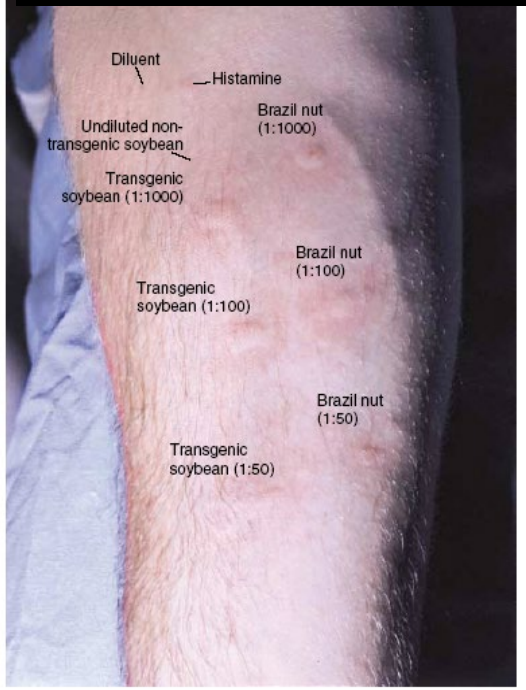


Figure 3. Reactivity on Skin-Prick Testing to Extracts of Transgenic Soybean, Nontransgenic Soybean, and Brazil Nut in a Subject Allergic to Brazil Nuts.
The dilutions are given in parentheses.

Over 25 years, No Commercial GMO crop has Caused new allergic Food reactions!

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Bioinformatics: Allergen database comparisons

AllergenOnline Homepage started in 2004, version 21 released 14 Feb 2021

http://www.allergenonline.org

AllergenOnline
HOME OF THE FARRP ALLERGEN PROTEIN DATABASE

AllergenOnline provides access to a peer reviewed allergen list and sequence searchable database intended for the identification of proteins that may present a potential risk of allergenic cross-reactivity.

This website was designed to help in assessing the safety of proteins that may be introduced into foods through genetic engineering or through food processing methods. The objective is to identify proteins that may require additional tests, such as serum IgE binding, basophil histamine release or in vivo challenge to evaluate potential cross-reactivity.

The database is updated annually. Version 4 was released on a public website in 2004. Version 19 was released on 10 February 2019. The database is freely accessible with the intent of providing a simple and useful tool that may be useful in food safety evaluations.

Features and Tools Available

Sequence search routines for food safety

We continue provide simple amino acid search routines to allow you to compare a protein sequence with the sequences in the current AllergenOnline database, which is updated on an annual basis. This is intended primarily for evaluating new proteins in Genetically Modified crops or in Novel Foods.

Search for full-length alignments by FASTA: The most predictive search is the overall FASTA alignment (see FASTA Help Page), with identity matches greater than 50% indicating possible cross-reactivity (Aalberse, 2000).

Search for 80 amino acid alignments by FASTA: A precautionary search using a sliding window of 80 amino acid segments of each protein to find identities greater than 35% (according to CODEX Alimentarius guidelines, 2003).

Search for 8 amino acid exact match: An 8-amino acid short-sequence identity search is provided since some regulatory authorities demand results of this extremely precautionary search. Our scientific opinion is that there is no evidence that an 8 amino acid match will identify a protein that is likely to be cross-reactive and could be missed by the conservative 80 amino acid match (35%). In our experience, isolated identity matches of 8 contiguous amino acids occur by chance alone at some modest rate, matches of 7 and 6 occur more commonly. Experience (published and unpublished) demonstrates that two proteins sharing only a single short identity match of from 6 to 8 contiguous amino acids do not share IgE binding in the absence of more extensive identity alignments (at least >35% identity over 80 or more amino acids). And that sequences sharing less than 50% identity over their full-lengths are rarely cross-reactive. Thus we recommend not using these short identity matches as there is no scientific evidence that they predict IgE cross-reactivity and they do not predict shared clinical activities.

New Celiac Disease Protein Database Risk Assessment Tool

In 2012 FARRP added a new bioinformatics tool in response to the CODEX Alimentarius Guidelines approved in 2003, to compare query sequences to peptides and proteins known to elicit celiac disease in some people. It was updated to version 2 in January, 2018 and now includes 1013 peptides, 72 proteins and 72 references. This tool meets the new European Food Safety Authority guideline for evaluating proteins from wheat and wheat-relatives for risks of celiac disease (June, 2017). A manuscript has been submitted for publication describing the methods, the comparisons that include identity matches to peptides, or high scoring FASTAs alignments to any of the 72 proteins. The database provides an exact peptide query and FASTAs search algorithms to evaluate whether a novel

Version information

CURRENT VERSION	
Version №:	21
Peer Reviewed Sequences:	2233
taxonomic-protein groups:	912
Released On:	February 14, 2021
Download:	V21.pdf

RECENT OPEN ACCESS PUBLICATIONS

- Defeating Late Blight disease of potato open Access 480 483 March 2021
  Defeating Late Blight disease of potato Open Access 480 483 March 2021.pdf
- Fall Armyworm in Africa and Asia
  2021_ African and Asian agriculture.pdf
- Predicting possible cross-reactivity, Food and Chemical Toxicology 2021
  Whole proteomes from Genomes vs Allergenonline 2021 FCT.pdf
- Scientia Global--Discussion 2020
  Genetic Engineering--future food security.pdf

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Bioinformatic searches - AllergenOnline

- **Overall FASTA3 New protein vs. AOL (<50% low chance cross-reactivity)**
- **CODEX (>35% ID over 80 AA…needs adjustment)**
- **Why use adjustments for Sliding 80mer with adjustments for sequences < 80 AA?**
- Exact peptide 8 AA, useless

Ara h 2, if a 71 AA segment is transferred to rice: Possible death of someone allergic to peanut if transferred into rice

>Ara h 2 is 150 AA, but 71 AA of full-length protein
RQQWELQGDRRCQSQLERANLRPCEQHLMQKIQRDEDSYGRDPYSPSQD[illegible]PSQDPDRR DPYSPSPYD[illegible]

Hit #	Defline	Species	Best %ID	# Hits > 35%	Full Alignment			Links	
					E-val	%ID	length	NCBI	Details
1	gi|31322017|gid|290|allergen Ara h 2 isoform [Arachis hyp	Arachis hypogaea	88.75%	1of1	1.5e-026	100.00%	71	gi|31322017	GO!
2	gi|26245447|gid|290|allergen Ara h 2.02 [Arachis hypogaea	Arachis hypogaea	88.75%	1of1	1.6e-026	100.00%	71	gi|26245447	GO!
3	gi|224747150|gid|290|Ara h 2.01 allergen [Arachis hypogae	Arachis hypogaea	72.54%	1of1	6.5e-019	82.90%	70	gi|224747150	GO!
4	gi|15418705|gid|290|allergen II [Arachis hypogaea]	Arachis hypogaea	72.54%	1of1	6.5e-019	82.90%	70	gi|15418705	GO!

AllergenOnline Database v21 (February 14, 2021)

Major food allergen sources & proteins (out of hundreds of proteins / source)

- Peanut (**Ara h 1, 2, 3, 6**; Ara h 8, 9; others) **2S albumins, vicilin, glycinin**; 18 total types
- Tree nuts Almond, pecan & walnut, cashew & pistachio, hazelnut, Brazil nut (**2S albumins, vicilin, glycinin**; LTPs, PR10 proteins)
- Cow's milk (**multiple Caseins, beta Lactoglobulin, alpha-Lactalbumin**; LFN, BSA, IgG
- Chicken egg (**ovomucoid, ovalbumin**; ovotransferrin, lysozyme)
- Crustacean shellfish (**tropomyosins** (Pen m 1 maybe Arg kinase; others)
- Finned (boney) fish (**Parvalbumin**, aldolase, enolase; others)
- Soybean (**Gly m 5 (3 beta conglycinins), Gly m 6 (5 glycinins)**; Gly m 4, Gly m 8)
- Wheat (**Tri a 14 (LTP), Tri a 19 (omega-5 gliadin), Tri a 40 (CM alpha-amylase inhibitor**, many others)
- Sesame (**Ses i 1 and Ses i 2, 2S albumins; Ses i 6, Ses i 7 11S globulins**)

Open Reading Frames? (ORF)

- Define ORF (stop to stop standard or start to stop) using Expasy Tools, **UNLIKELY to be Expressed w/o Txn start**
- All six reading frames from DNA sequence
- LENGTH of predicted ORF (Some countries **30 AA** or more is sensible (**2 epitopes?**). Some countries want 8 AA length (or less)
- Compare **predicted ORF AA segments to AllergenOnline**.
- Goodman evaluated 62,000 BP of DNA insert for an Omega 3 Fatty Acid canola…. 7 expressed proteins, and many potential ORFs…but only at JUNCTIONS of genes for China and Japan
- **If matches; do appropriate serum IgE tests**.

Genome prediction of Proteomes + Bioinformatics

Abdelmoteleb M et al. Food and Chem Tox 2021 Jan;147:111888.

Evaluating potential risks of food allergy of novel food sources based on comparison of proteins predicted from genomes and compared to www.AllergenOnline.org ver: 18b

- **New foods**: Red Algae, Fusarium and Green Algae (Chlorella)
- **AND total proteomes predicted from genomes of 23 other species**: Almond, Apple, Arabidopsis, Baker's yeast, Bos, Candida, Chicken, Drosophila, Gadus, **Human**, Maize, Papaya, Peach, Peanut, Pecan, Phaseolus, Pistachio, Potato, Rice, Salmon, Soybean, Walnut, Wheat

Unique protein identity FASTA matches to AllergenOnline.org: >35% ID / 80 AA 2021

Species	*E* score 1e-7	*E* score 1e-30	*E* score 1e-100
Chlorella (green algae)	159	64	14
Galdieria (red algae)	73	39	8
Fusarium (F. flavolapis)	232	125	30

Species (examples)	*E* score 1e-7	*E* score 1e-30	*E* score 1e-100
Human	5565	2556	557
Cod fish	502	268	72
Bos (cow)	680	356	71
Peanut	2971	1506	218
Rice	981	523	63
Salmon	2892	1217	320
Potato	1374	723	86

Judging possible risks by identity matches to HUMAN proteins – Tolerance is LIKELY

HUMAN protein	Overall % identity	Length aligned	Allergenic source	others	Importance
Tropomyosin	**94%**	**284 AA**	**Tilapia**	**Salmon 85% and others**	**Low level risk ?**
Tubulin	**84%**	**295**	**House Dust mite**	**3 HDM**	**Low level risk ?**
Trypsin	42%	250	House Dust mite	Other mite allergens	Very low risk
Myosin	40%	171	Shrimp	HDM	Very Low risk
Collagen	43%	1308	Fish	Cow	Very Low risk
Troponin C	40%	147	Storage mite	Cockroach	Very Low risk
Enolase	**88%**	**432**	**Tuna fish**	**chicken**	**Low level risk ?**
Parvalbumin	**79%**	**110**	**Chicken**	**Frog, fish**	**Low level risk?**
Aldolase	**81%**	**364**	**Catfish**	**Fish**	**Low level risk?**

Species with clinically known Allergens

Sicherer & Sampson 2018 JACI

Species	# Identified allergens	Frequency (Cross-reactivity)	Important allergens	Other less important
Peanut Arachis hypogaea	18 allergenic proteins with multiple isoforms	**1 to 2 % of US** (diagnosed with low rate with lupin, pea, soybean, & SOME tree nuts)	**2S albumins Vicilins Glycinins**	LTP, Bet v 1 like Oleosins defensins
Tree nuts	2 to 8 proteins per species	**0.5 to 1.2% of US and EU** ~ 95% pecan and walnut; less than 90% for almond, hazelnut; cashew, pistachio	**2S albumins, vicilins, glycinins**	Bet v 1 like, LTPs, oleosins
Cow - milk Bos taurus	Caseins (4), beta-lac, alpha lac and total 12	**0.5% to 3% kids** at 1 year of age US	**Caseins Beta-lactoglobulin Alpha-lactalbumin**	Lactoferrin, lysozyme, transferrin, IgG, BSA, lipocalin
Chicken - egg Gallus gallus	2 major a few minor	**1.2 to 2% of children** with allergy to hen's egg	**Egg ovomucoid Egg ovalbumin**	
Shrimp Penaeus sp.	7 allergens from multiple sp.	**0.6 to 2% allergy** IgE to tropomyosin often x-react House dust mite TM not	**Tropomyosin, Arginine kinase,**	Myosin, sarcoplasmic Ca+ binding ptn Myosin, troponin C, Triosephosphate Isom

Interpretation of Codex: Goodman et al., 2008 Shades of Grey

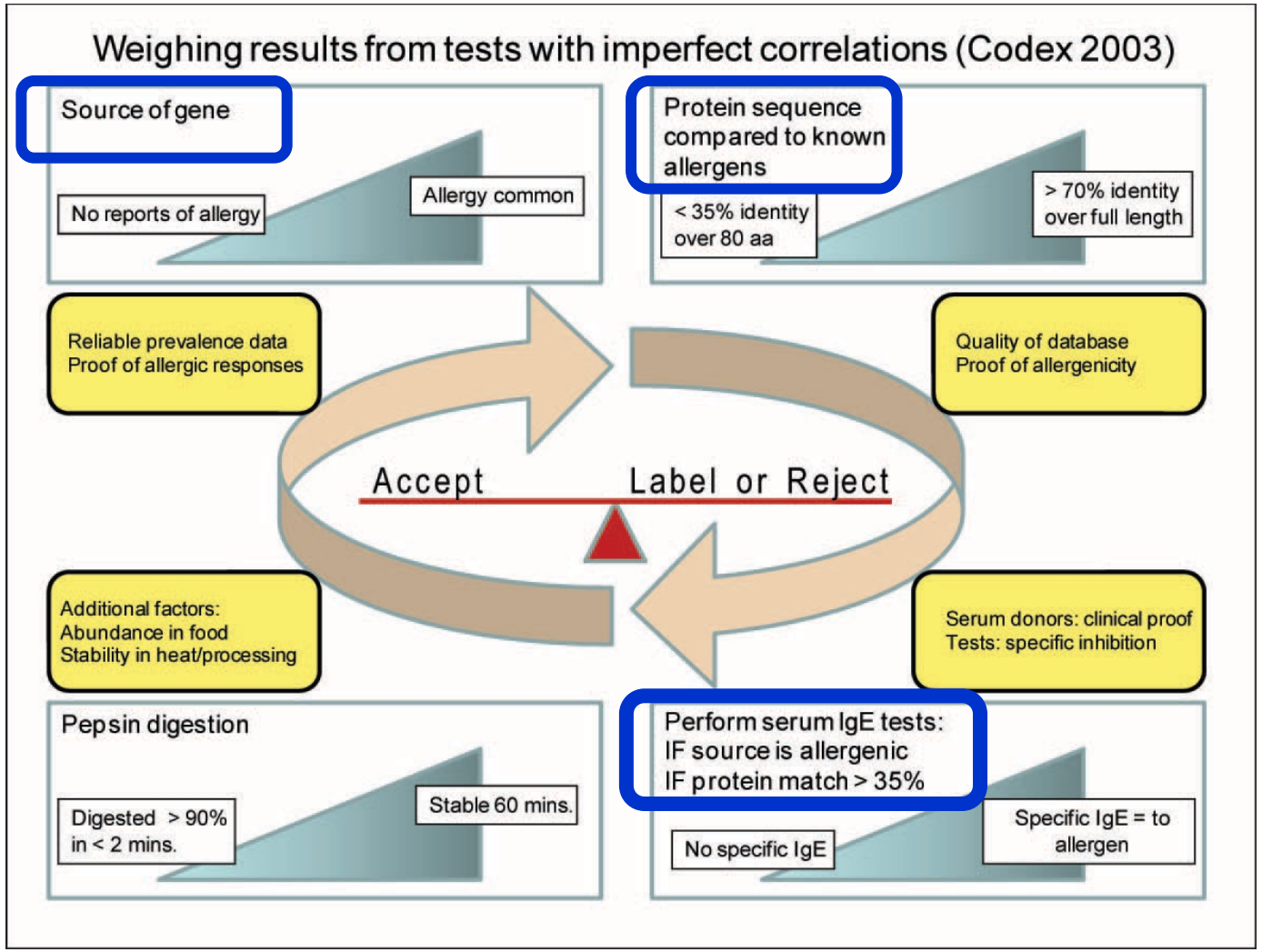
Allergenicity assessment of genetically modified crops—what makes sense?

NATURE BIOTECHNOLOGY VOLUME 26 NUMBER 1 JANUARY 2008

"Weight of Evidence" EXPERT Opinion and interpretation

Intent, prevent the transfer of an allergen or highly cross-reactive protein into a new source

But 35% ID is TOO conservative!



Clinical Experience: Species with known Allergens

Sicherer & Sampson 2010 JACI 125(2):S116-125

TABLE I. Estimated food allergy rates in North America

Prevalence	Infant/child	Adult
Milk	2.5%	0.3%
Egg	1.5%	0.2%
Peanut	1%	0.6%
Tree nuts	0.5%	0.6%
Fish	0.1%	0.4%
Shellfish	0.1%	2%
Wheat, soy	0.4%	0.3%
Sesame	0.1%	0.1%
Overall	5%	3% to 4%

Rates of clinical cross reactivity:

Allergy to:	Related food	Approximate clinical reaction rate
Peanut	Most beans	5%
A tree nut	Other tree nut	35% Higher for: walnut-pecan, almond-hazel, cashew-pistachio
A fish	Other fish	50%
Shellfish	Another shellfish	75%
Grain	Another grain	20%
Cow's milk	Goat/sheep milk Mare's milk Beef	>90% 5% 10%

In the United States there are 3 major test manufacturers

References

1. Sicherer SH. (2001). Clinical implications of cross-reactive food allergens. JACI 108(6):881-890.
2. Smeekens JM, Bagley K, Kulis M. (2018). Tree nut allergies: Allergen homology, cross-reactivity, and implications for therapy. Clin Exp Allergy 48(7):762-772.
3. Faber MA, van Gasse AL, Decuyper II, et al., (2018). Cross-reactive aeroallergens: Which need to cross our mind in food allergy diasnosis" J Allergy Clin Immunol Pract 6(6):1813-1823.
4. Cox AL, Eigenmann PA, Sicherer SH. (2021. Clinical relevance of cross-reactivity in food allergy. JACI Pract. 9(1):82-99.
5. Carlson G, Coop C. (2019). Pollen food allergy syndrome (PFAS): a review of current available literature. Ann Allergy Asthma Immunol 123:359-365. (PFAS).
6. Cabanillas B, Jappe U, Novak N. (2018). Allergy to peanut, soybean, and other legumes: recent advances in allegen characterization, stability to processing and IgE cross-reactivity. Mol Nutr Food Res 62(1): doi:10. 1002/mnfr.201700445.
7. Bastiaan-Net S, Reitsma M, Cordewener JHG, et al., (2019). IgE cross-reactivity of cashew nut allergens. Int Arch Allergy Immunol 178(1):19-32.
8. Foong RX, Dantzer JA, Wood RA, Santos AF. (2021). Improving Diagnostic Accuracy in food allergy. JACI P 9(1):71-80
9. de Gier S, Verhoeckx K. (2018). Insect (food) allergy and allergens. Mol Immunol 100:82-106.
10. Werfel T, Asero R, Ballmer-Weber BK, et al. 2015. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 70(9):1079-1090.
11. Goodman RE, Vieths S, Sampson HA, et al. (2008). Allergenicity assessment of genetically modified crops—What makes Sense? Nature Biotech 26(1):73-81.
12. Goodman RE, Ebisawa M, Ferreira F, et al, (2016). AllergenOnline: a peer-reviewed, curated allergen database to assess noel food proteins for potential cross-reactivity. Mol Nutr Food Res 60(5):1183-1198.
13. Goodman RE Tetteh AO. (2011). Suggested improvements for the allergenicity assessment of genetically modified plants used in foods. Curr Allergy Asthma Rep. 11(4):317-324.
14. Goodman RE, Chapman MD, Slater JE. (2020). The Allergen: Sources, extracts, and moledulcs for diagnosis of allergic disease. JACI in Practice 8:2506-2514.
15. Bernhisel-Broadbent J, Sampson HA. (1989) Cross-allergenicity in the legume botanical family in children with food hypersensitivity. JACI 83:435-440
16. Bernhisel-Broadbent J, Taylor S, Sampson HA. (1989). Cross-allergenicity in the legume botanical family in children with food hypersensitivity. JACI 84:701-709.

Allergenic protein characteristics

- **Abundance** is important in food allergen sources
- Allergens are NOT highly identical to human proteins
- Food allergens are highly diverse (plants, animals, crustaceans, fish, fungus)
- Airway allergens (pollen, molds, animal dander)
- Dermal or injected allergens (mosquito salivary proteins, wasp stings)
- Allergens that cause allergy **usually are Not highly identical to many common food proteins from diverse sources, except minor allergens**
- Stability of a dietary protein in **PEPSIN may** CORRELATE **with allergy IF it is also an abundant protein**.
- Aalberse 2000, proteins sharing <50% identity are rarely cross-reactive, proteins sharing >70% identity full-length are often cross-reactive

Limitations of knowledge of allergens

- People react to ingestion or contact exposure of PROTEIN SOURCES
- People can be Sensitized or Tolerized: (**IgE / or IgG/IgA or ignored)**
- IgE binding to proteins often **CORRELATES** with allergy, but is not proof
- Allergy needs Basophil and Mast cell activation with IgE binding multiple epitopes
- Diagnostic criteria **HISTORY of allergy to the specific SOURCE**
  - SPT **(skin prick test EXTRACTS, rarely proteins)**
  - Laboratory IgE binding to extracts **& to Proteins**
  - **Basophil or Mast cell activation** with Serum IgE + Allergenic protein
  - **Oral Food Challenge tests** is with extracts, RARELY using **PROTEINS**

Primary Food Allergy	Cross Reactive Food	Risk (varies with region)
Crustacean Shellfish 	Other Crustaceans	~75%
	Mollusks/Bivalves (Clam, Mussel, Oyster, Squid) 	<50%
Mollusks/Bivalves 	Crustaceans (Crab, Shrimp, Lobster) 	>70%
Finned Bony Fish 	Other Finned Bony Fish	~50%
	Cartilaginous Fish (Dogfish, Ray, Shark) 	<5%
Peanut 	Tree Nuts (co-allergy)	~33%
	Lupine 	~20%
	Sesame (co-allergy)	10-15%
	Green Bean, Pea, Soy 	5-20%
Other Legumes		
If Soy 	Peanut	>75%
If Chick Pea 	Lentil, Pea 	>50%
Tree Nuts 	Other Tree Nuts	15-33%
	Sesame (co-allergy)	10-15%
If Walnut	Pecan 	~66-75%
If Pecan	Walnut	>95%
If Cashew	Pistachio 	~66-83%
If Pistachio	Cashew 	>95%
If Peanut and Tree Nut	Sesame (co-allergy) 	50%
Milk (Cow) 	Milk (Sheep, Goat) 	>90%
	Milk (Camel, Mare) 	<5%
	Beef 	~10-20%

Major and Minor IgE allergen sources & defining likely Cross-reactivity

Cox, Eigenmann, Sicherer, JACI Practice 2021

Peanut = Co-allergy to "tree-nuts" at 30%;
"Lupine" at 20%
"sesame" at 20%
Green bean, soybean at 5 to 20%

Genetic and Protein complexity of allergens
Which proteins are the Actual allergens, and dose

- Whole grass genomes by additions
- Wheat is Diploid, Tetraploid and **Hexaploid - IgE and celiac disease**

Wheat grasses **Celiac disease (many glutens) & IgE Allergy (28 ptns in WHO/IUIS, mostly airway allergens)**

Triticinae
- Aegilops sp
- Thinopyrum sp
- Triticum sp
- Hybrids of these

Hordeinae
- Agrpyron sp
- Hordeum sp – barley
- Secale sp – rye

Aveneae
- Avena sp. - Oats

- **Peanut** is a tetraploid hybrid of two diploid peanuts
- Allergens were duplicated and mutated so there are isoforms
- Major Allergens (18 different proteins in WHO IUIS Primary allergens:
  - Ara h 1 (multiple vicillins)
  - Ara h 2 (two 2S albumins)
  - Ara h 3 (multiple glycinins)
  - Ara h 6 (One isoform 2S albumin)

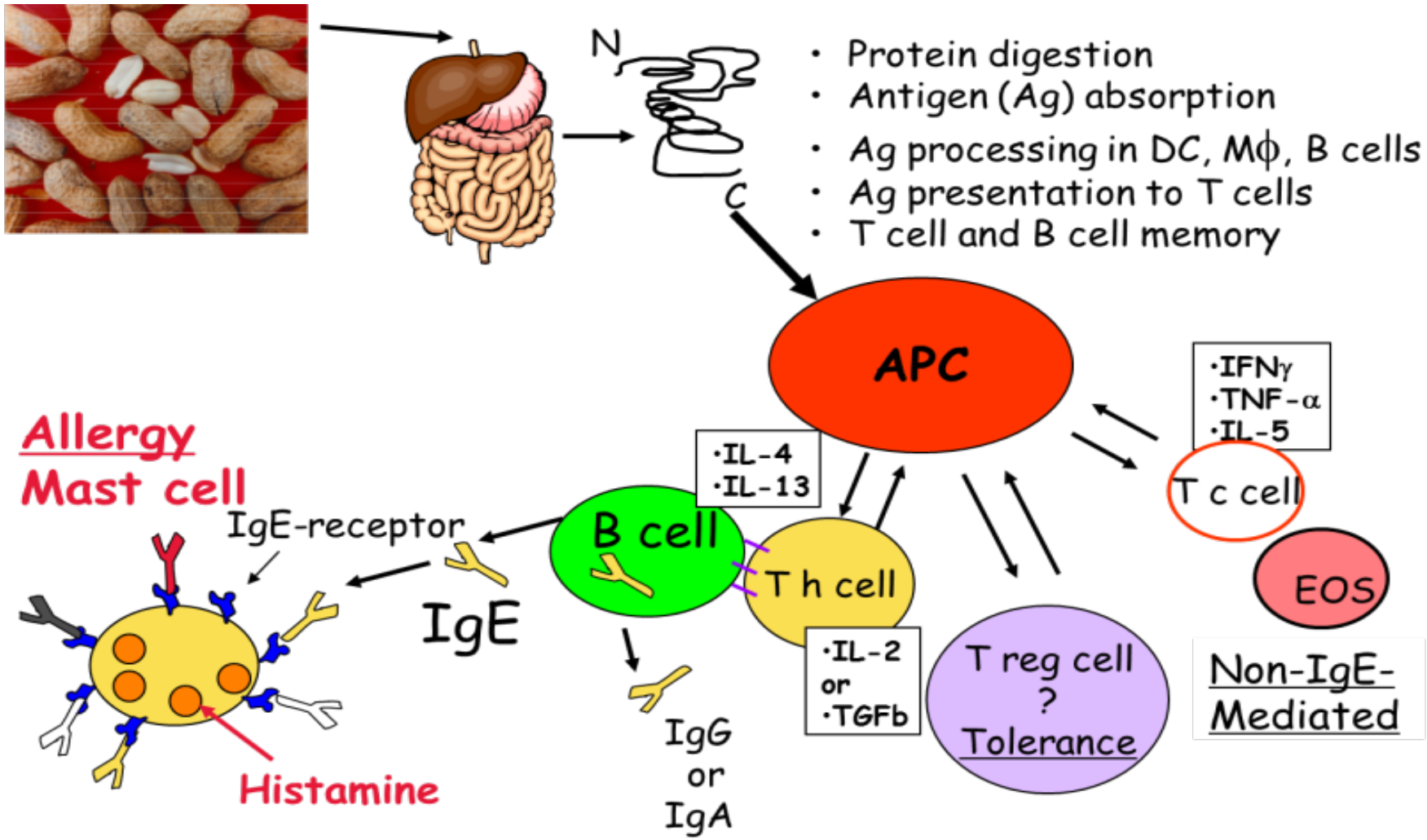
Novel foods vs GMO organisms allergy risks

- Full genome, full proteome, hundreds or thousands of proteins
- **vs**. one or few genes/proteins in a common food organism
- Is it different to consume a new food vs a GMO?

What are the potential risks, for the individual ?

- Prior allergy to the protein(s) or homologue
- Prior specific IgE that binds with some affinity / avidity
- Is the dose high of the new proteins (GM or Novel food) ?
- Is eating a GMO or Novel food different from risks of a European visiting China or India? Or citizens of China/India eating in the EU or US?
- **Can we predict future De Novo sensitization**? **(NOT AT THIS TIME)**

Food Sensitization vs. Tolerance IS COMPLEX



Overall Ideal Food Allergy Diagnostic tests Foong JACI P 9:77-80

J ALLERGY CLIN IMMUNOL PRACT
VOLUME 9, NUMBER 1

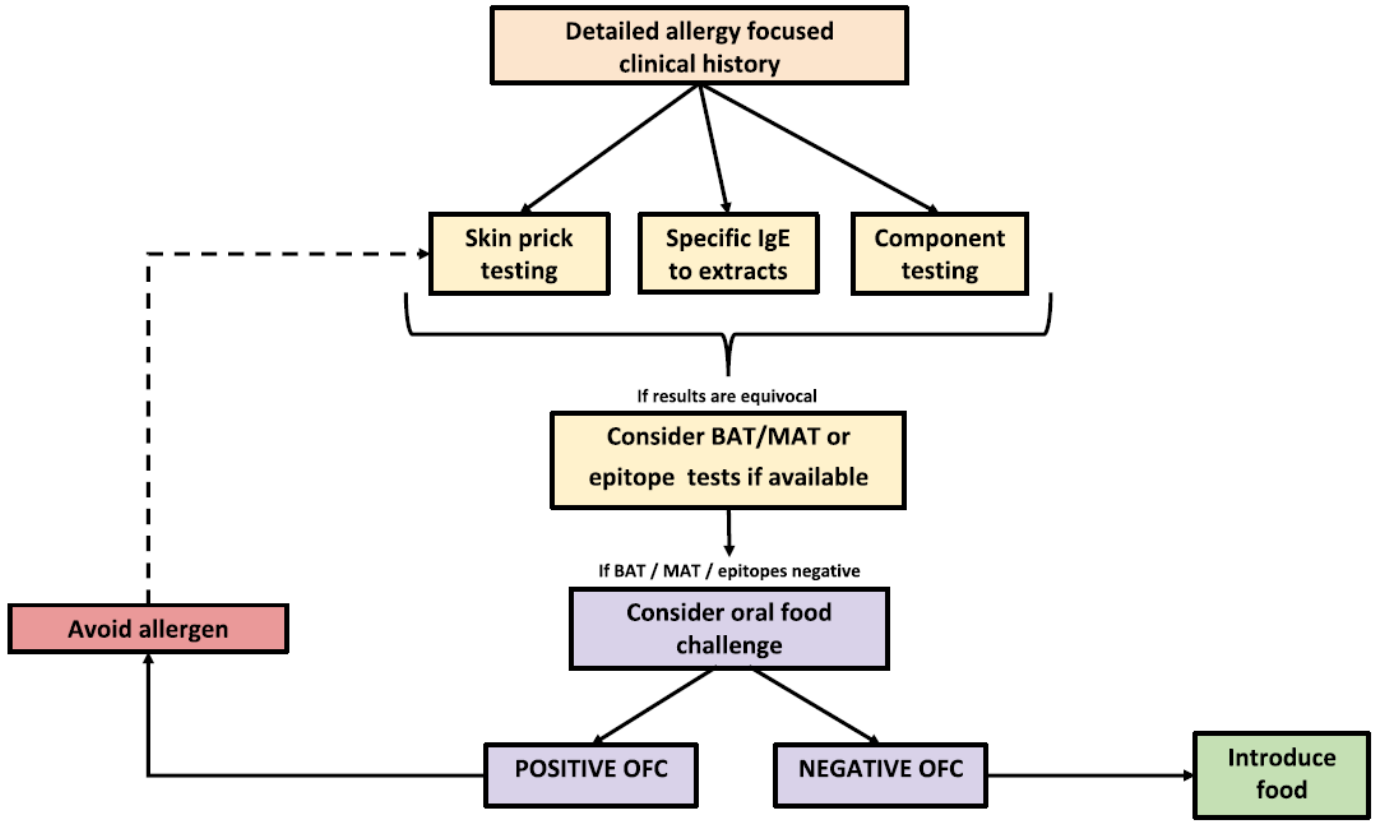


FIGURE 1. Proposed clinical approach to the diagnosis of IgE-mediated food allergy. *BAT*, Basophil activation test; *MAT*, mast cell activation test; *OFC*, oral food challenge.

Improving diagnostic accuracy food allergy

2021 Foong et al. JACI P 9:71-80

TABLE I. Diagnostic cutoffs for specific IgE and skin prick testing with 95% positive predictive

	Specific IgE[1,5-8]		Skin prick test[1,6,9]
Foods	**95% PPV**	**50% NPV**	**95% PPV**
Cow's milk*	15 kU/L (32 also reported) Infants ≤2 y: 5 kU/L	2 kU/L	≥8 mm Infants ≤2 y: 6 mm
Egg*	7 kU/L Infants ≤2 y: 2 kU/L	2 kU/L	≥7 mm Infants ≤2 y: 4-5 mm
Peanut	15-34 kU/L	2 kU/L if history of reaction 5 kU/L is no history of reaction	≥8 mm Infants ≤2 y: 4 mm
Fish	20 kU/L	—	
Tree nuts	20 kU/L		≥8 mm for walnut ≥12 mm for cashew
Sesame	50 kU/L (86% PPV)		≥8 mm

NPV, Negative predictive value; *PPV*, positive predictive value.
*These numbers were derived from uncooked milk and direct egg and do not apply to baked milk or baked egg.

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TABLE II. Proposed diagnostic cutoff levels and positive predictive value (PPV) for specific IgE to individual allergen components

IgE to individual allergen components	IgE (kU_A/L)	PPV
Milk casein to diagnose baked milk allergy[19]	20.2	69%
Egg ovomucoid		
to diagnose baked egg allergy[20]	50	95%
to diagnose cooked egg allergy[21]	26.6	95%
to diagnose raw egg allergy[22]	5.21	95%
Peanut Ara h 2[10,18,23]	0.35-42.2	90%-95%
Hazelnut Cor a 9[23-25]	1-2	79%-100% specificity
Hazelnut Cor a 14[23,26]	0.72-47.8	87%-90% specificity
Cashew Ana o 3[27,28]	0.16	98% specificity
	2	95%
Soya Gly m 8 [29]	1	89%
	3.55	74%
Wheat Tri a 19[30,31]	0.04	100%
	0.41	81%

Basophil assay cutoffs for PPV

TABLE III. Examples of diagnostic cutoffs for the basophil activation test (BAT) to extracts and component allergens and their positive predictive value (PPV)

BAT	Cutoff	PPV (%)
Cow's milk[80]	>6% %CD63+ basophils	81
Ovalbumin to diagnose egg allergy[81]	≥5% %CD63+ basophils	100
Peanut[10]	≥4.78% %CD63+ basophils	100
Ara h 2 to diagnose peanut allergy[73]	≥65.19% maximal %CD63 to Ara h 2/anti-IgE	100
Sesame[71]	≥10.9% %CD63+ basophils	94
Cashew nut[70]	≥5.5% %CD63+ basophils	91
Pistachio nut[70]	≥5.8% %CD63+ basophils	74
Walnut[70]	≥6.4% %CD63+ basophils	92
Pecan[70]	≥2.7% %CD63+ basophils	75
Hazelnut[72]	CD-sens>1.7	89
Pru p 3 to diagnose peach allergy[75]	>20% CD63+ basophils	96
rTri a 19 to diagnose IgE-mediated wheat allergy[82]	>7.9% CD203c+ basophils	81
Salmon[83]	0.54 ratio of %CD203c+ basophils to allergen and anti-IgE	85

PPV was estimated from prevalence and sensitivity when not reported in the respective study.