

Molecular diagnosis in food allergy – when to ask for it?

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Declarations

- I have no conflicts of interest.
- I would like to thank my colleagues who have shared some of these slides with me – Professor George du Toit, Professor Alexandra Santos, Rebecca Batt (Nurse consultant), James Gardner (Nurse consultant)

Outline

- Molecular diagnostics for food allergies
- When are allergy tests useful?
- Skin prick tests
- Specific-IgE tests
- Component testing
 - Peanut
 - Egg
 - Milk
 - Wheat

What is allergy testing?

- Looking for evidence of allergy vs tolerance
- Thorough allergy focused history paramount
- Allergy results should only be interpreted in light of the clinical history
- Aim of any allergy test is to guide future care and advice
- Improve quality of life and safety
- Don't test unless it will change management
- Avoid indiscriminate panel testing



IgE vs non-IgE mediated food allergy

IgE mediated

- Reactions usually happen within 1-2 hours of eating a food
- Common symptoms include: rash/hives/urticaria, angioedema of the lips/face/around the eyes, nausea/vomiting, diarrhoea
- Risk of anaphylaxis – wheeze, stridor, hoarse voice, difficulty breathing, dizziness/collapse
- SPT and specific IgE testing is needed to help confirm the diagnosis

Non-IgE mediated

- Reactions often appear 24-48 hours later
- Symptoms tend to appear via skin (eczema flare) or are gastrointestinal in nature (diarrhea, constipation, reflux)
- If longer term – can be seen as failure to thrive especially in younger children
- These reactions are not caused by IgE antibodies so SPT and specific IgE testing is not helpful/not recommended

GUIDELINE

EAACI guidelines on the diagnosis of IgE-mediated food allergy

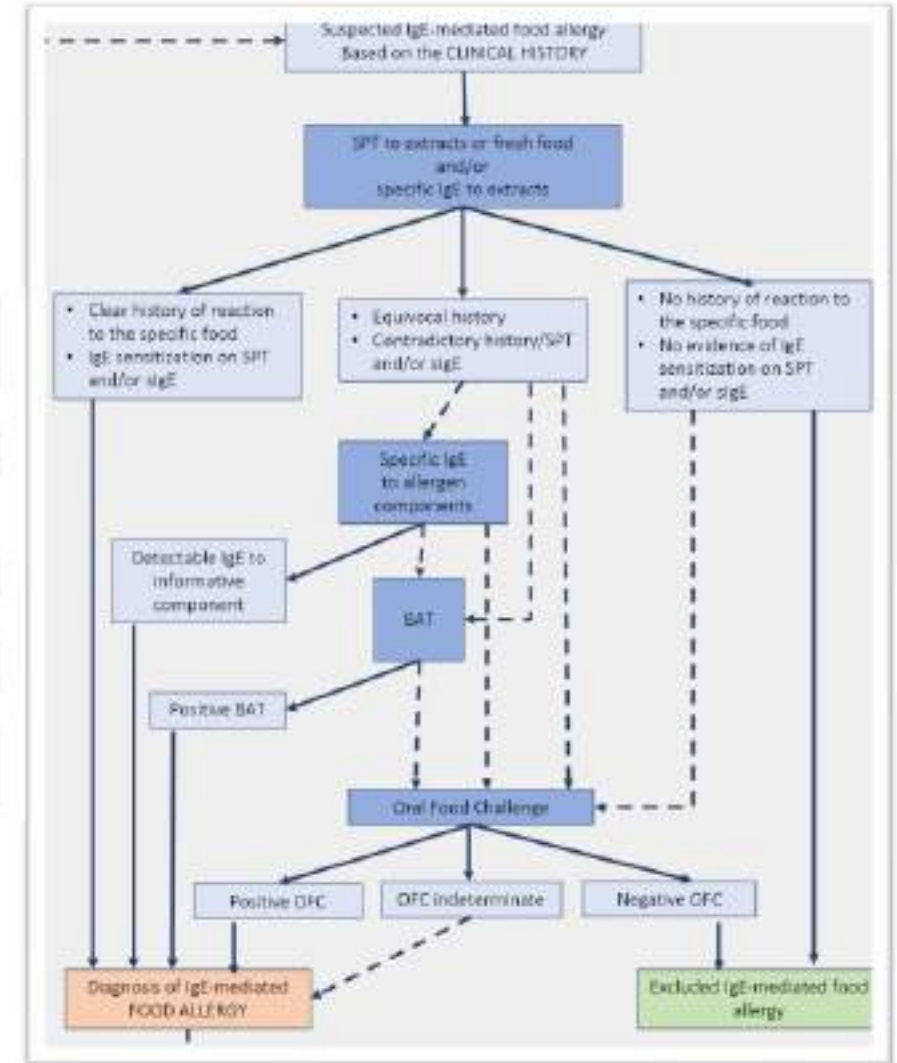
Alexandra F. Santos^{1,2,3} | Carmen Riggioni⁴ | Ioana Agache⁵ | Cezmi A. Akdis⁶ |
Mubeccel Akdis⁶ | Alberto Alvarez-Perea^{7,8} | Montserrat Alvaro-Lozano^{9,10} |
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Carina Venter⁷⁰ | Brian P. Vickery⁷¹ | Berber Vlieg-Boerstra^{72,73,74} | Thomas Werfel⁷⁵ |
Margitta Worm⁷⁶ | George Du Toit^{1,3} | Isabel Skypala^{77,78}



Diagnostic approach according to the EAACI Guidelines

Key recommendations	Certainty of evidence	Strength of recommendation
1. Start with a detailed allergy-focused clinical history	Low	Strong
2. Perform skin prick test and/or specific IgE to allergen extracts (or fresh foods in case of SPT)	High	Strong
3. Use molecular allergology—specific IgE to Ara h 2, Cor a 14, Ana o 3, respectively for peanut, hazelnut, cashew nut allergies, respectively	High	Strong
4. Use BAT to peanut or sesame, if available	High	Conditional
5. Use only recommended tests above	Very low	Strong
6. Reassess over time for possible resolution of food allergy	Moderate	Conditional
7. Oral food challenge is the reference standard test and should be done in equivocal cases	High	Strong
8. Open oral food challenge are acceptable for most clinical cases and double-blind challenge should be reserved for patients with an inconclusive open challenge or for research	Low	Conditional

GOLD STANDARD = ORAL FOOD CHALLENGE



Step 1: Take an allergy focused history/physical examination

EXAMPLES on areas to focus on



Which end organ is affected and what symptoms are experienced?



What food or foods are causing concern?

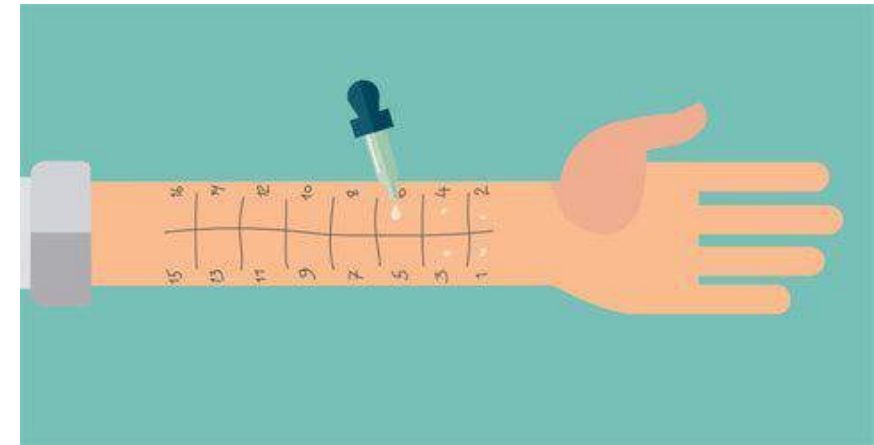


What is the time course between the onset of symptoms and the intake of the food?

Based on the clinical history, physically examine the child or young person, in particular look for: growth and physical signs of malnutrition signs indicating allergy-related comorbidities (atopic eczema, asthma and allergic rhinitis)

Skin prick tests

- Sensitivity and specificity of SPT can be variable – 30-90% depending on:
 - type and form of allergen
 - Prick to prick tests
 - Commercial extracts
 - prick technique
- Conventional positive result is $SPT \geq 3\text{mm}$
- Many studies have been done with thresholds with 95% positive predictive values (PPV)
- Sporik et al 2000 – reported these cut offs as having 100% PPV for diagnosing food allergy
 - $\geq 8\text{mm}$ for cow's milk
 - $\geq 7\text{mm}$ for egg
 - $\geq 8\text{mm}$ for peanut



Advantages and disadvantages of SPT

Advantages

Quick

Immediate results

Cost effective

Visual representation to patient



Disadvantages

Variable technique

Need to stop antihistamines

Irritant reactions

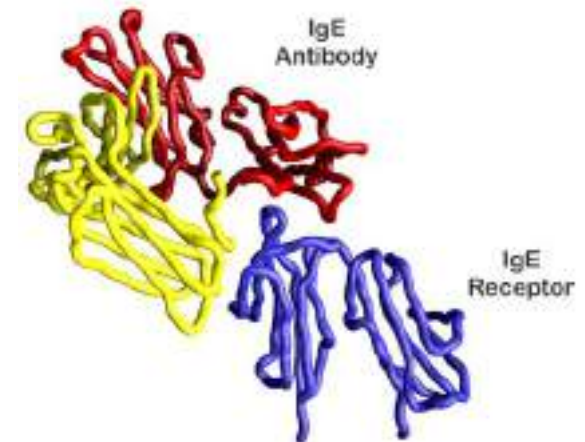
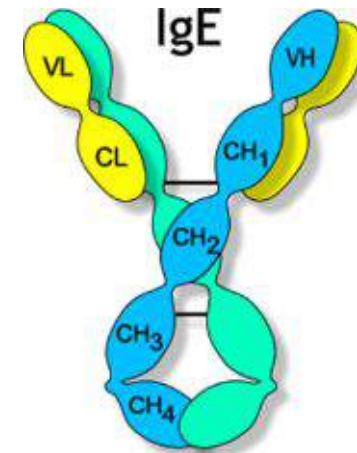
Relies on skin condition & health

Potential risk of allergic reaction

Avoid if recent anaphylaxis

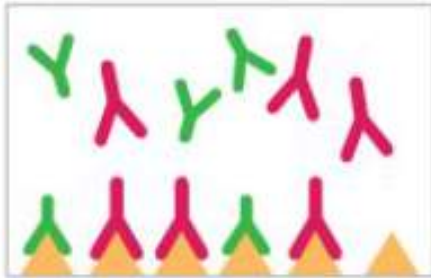
In vitro IgE testing

- Total IgE indicating atopic status
- Specific IgE (RAST)
- >450 allergens
- indicate sensitisation
- Multiple allergy screening assays e.g. ISAC and ALEX
- Recombinant testing (CRD)



Detection of specific IgE

Step 1



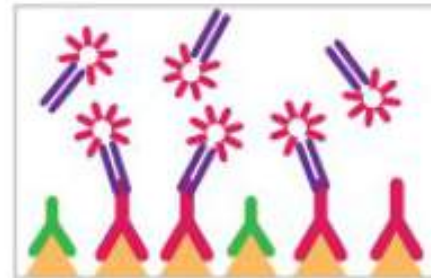
Allergens are coated onto the well and patient serum is added.

Step 2



Allergen-specific IgE and IgG bind to the allergens on the well. Unbound antibodies and other serum proteins are removed by washing.

Step 3



Affinity purified, enzyme-labeled anti-IgE antibody is added, and binds to allergen-specific IgE only. It does not bind to IgG. Unbound anti-IgE reporter antibody is removed by washing.

Step 4



Chromogenic enzyme substrate solution is added, producing color at bound anti-IgE reporter sites. Color intensity is proportional to IgE concentration in the serum, indicating a positive reaction.

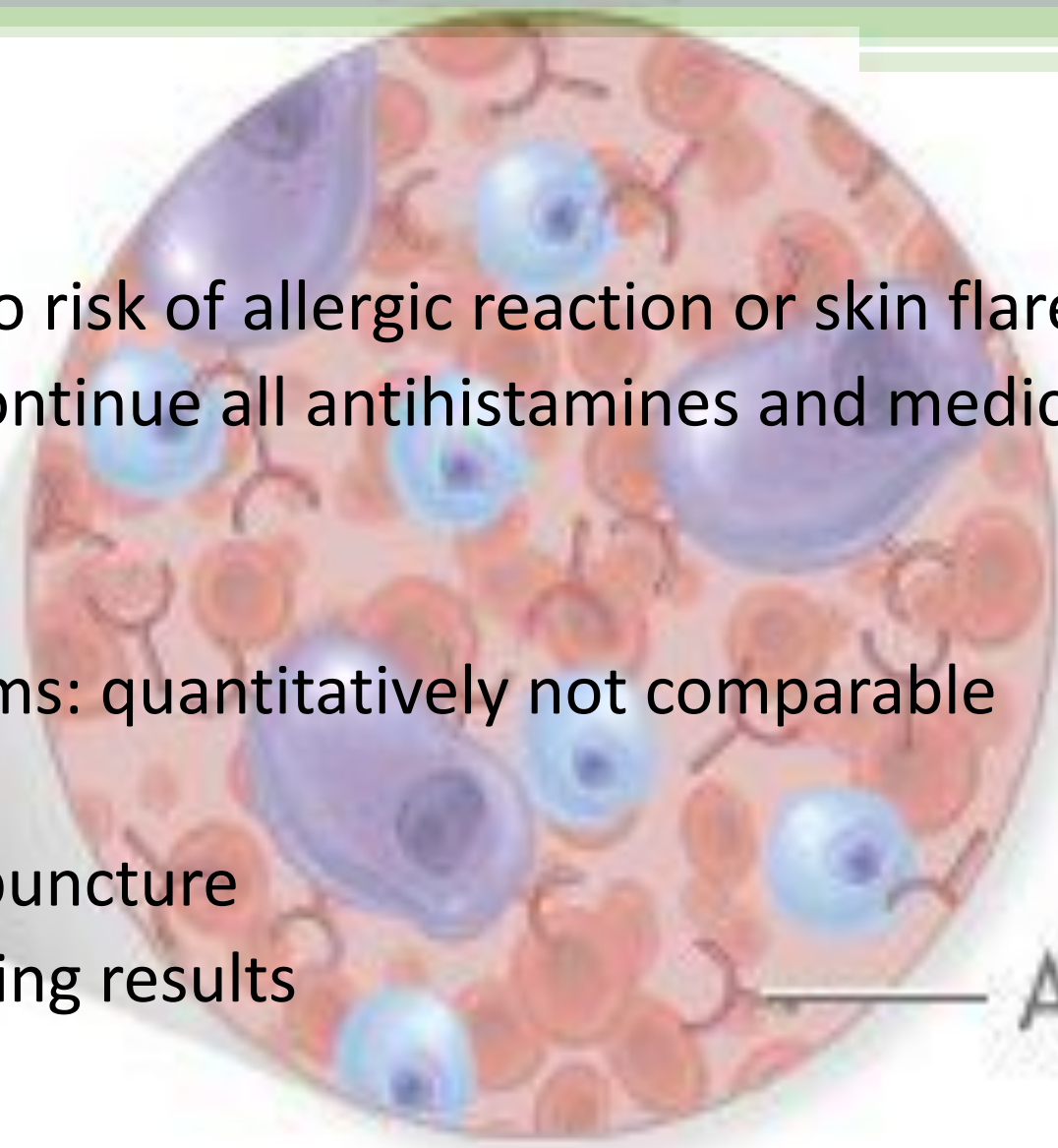
▲ Allergen Y IgE Y IgG * Anti-IgE Reporter

Advantages

- In Vitro test: no risk of allergic reaction or skin flare
- Patients can continue all antihistamines and medications

Disadvantages

- Different systems: quantitatively not comparable
- Cost
- Need for venepuncture
- Delay in obtaining results

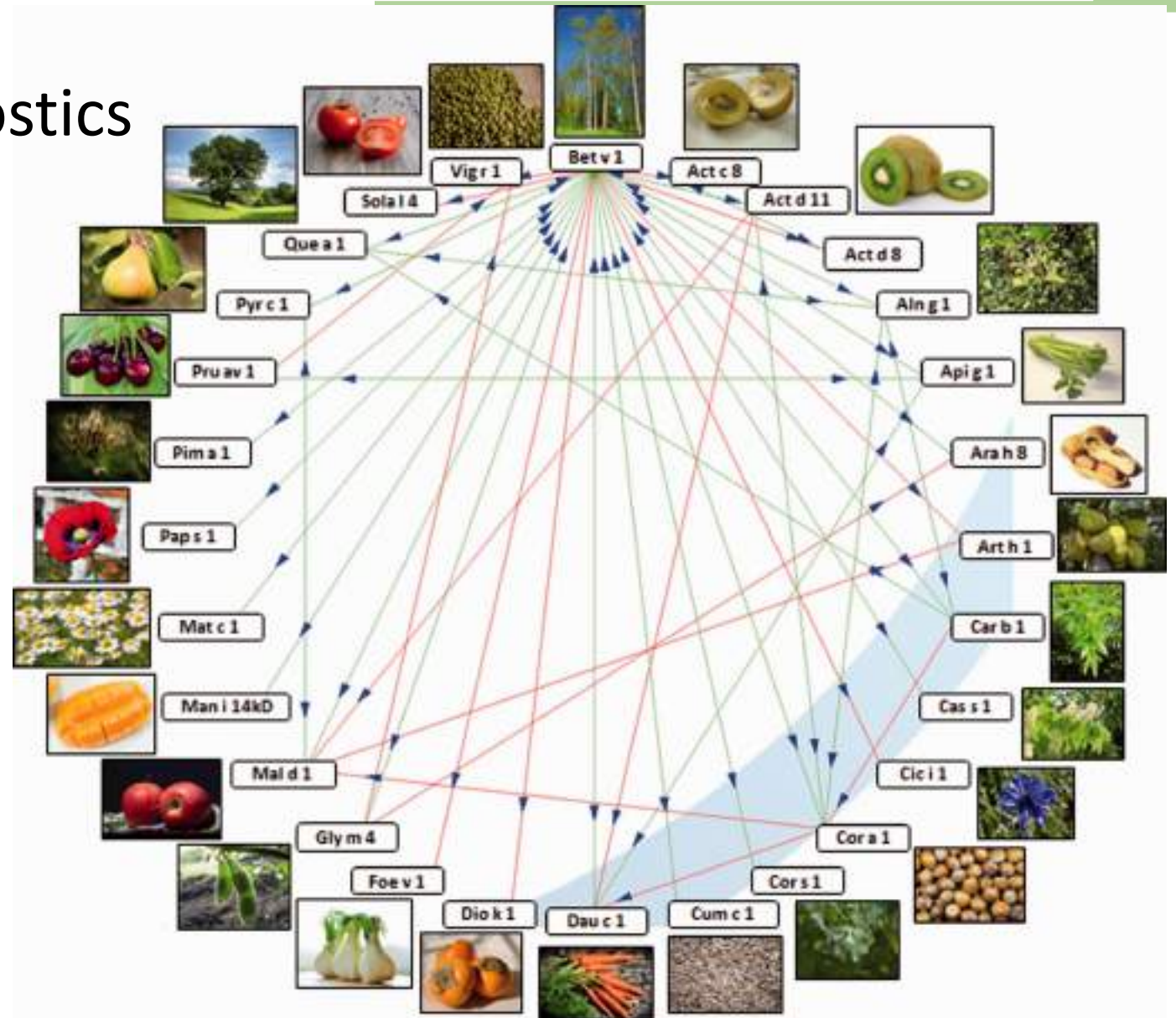


Antibodies (IgE)

Foods	SPT \geq 3 mm		Specific IgE \geq 0.35 kUA/L	
	Sensitivity	Specificity	Sensitivity	Specificity
Cow's milk	88% (95% CI: 76%-94%)	68% (95% CI: 56%-77%)	87% (95% CI: 75%-94%)	48% (95% CI: 36%-59%)
Egg	92% (95% CI: 80%-97%)	58% (95% CI: 49%-67%)	93% (95% CI: 82%-98%)	49% (95% CI: 40%-58%)
Wheat	73% (95% CI: 56%-85%)	73% (95% CI: 48%-89%)	83% (95% CI: 69%-92%)	43% (95% CI: 20%-69%)
Peanut	95% (95% CI: 88%-98%)	61% (95% CI: 47%-74%)	96% (95% CI: 92%-98%)	59% (95% CI: 45%-72%)

Component Resolved Diagnostics

- The importance of interpreting results in the context of the clinical history is paramount
- Component resolved diagnostic (CRD) further clarifies the diagnosis
- Informs us what type of sensitisation and allergy a patient has



Protein Groups

PR-10 proteins, Bet v 1 homologue

- Heat labile protein

Profilins

- Highly cross-reactive, present in most plants

Storage proteins

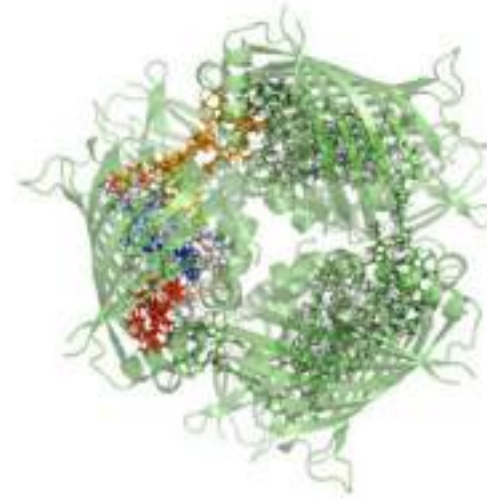
- Proteins found in seeds
- Often stable and heat resistant
- Often associated with systemic and severe reactions

LTP, lipid transfer protein

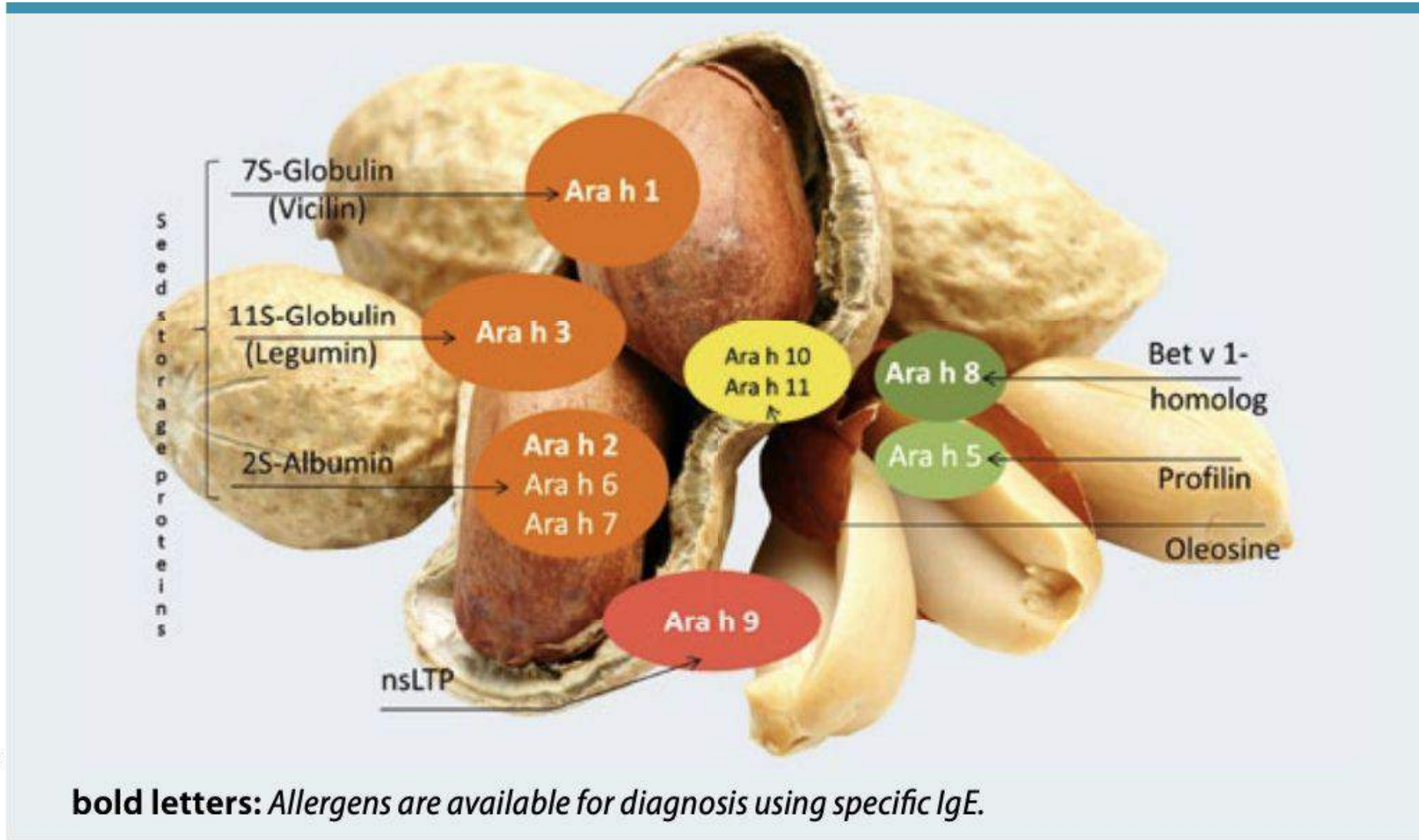
- Stable to digestion and heat
- Often associated with systemic and severe reactions in addition to OAS

CCD, cross-reactive carbohydrates

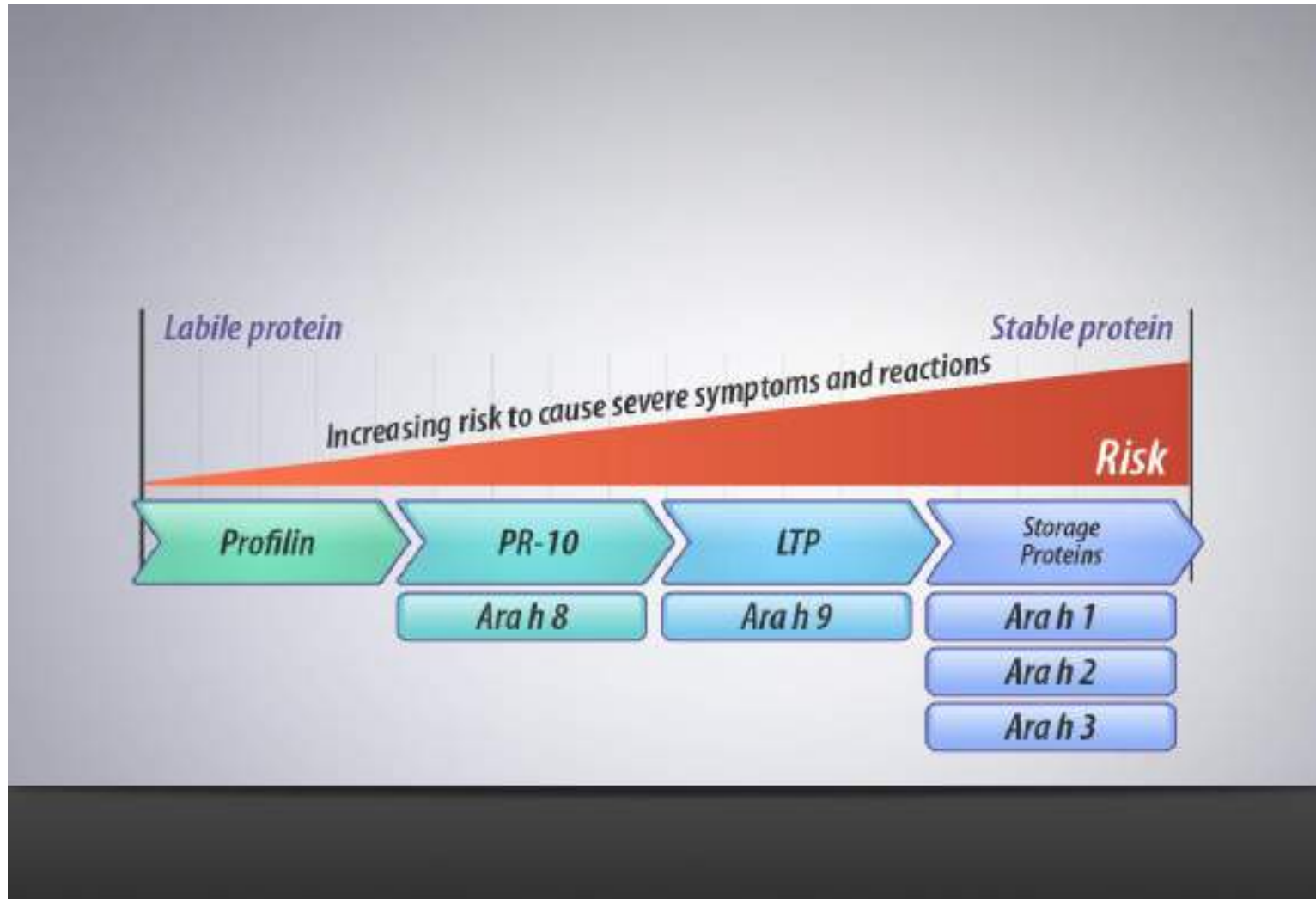
- Highly cross-reactive, present in most plants
- Seldom associated with clinical symptoms



Peanut components



Risk assessment of peanut components



Ara h 8

- PR-10 protein homologous to the birch component Bet v 1
- Associated with allergy to birch and birch-related tree pollen
- Labile protein
- Mainly causing local symptoms (Pollen Food Syndrome)
- Common in Northern Europe



Ara h 9- Lipid Transfer Protein

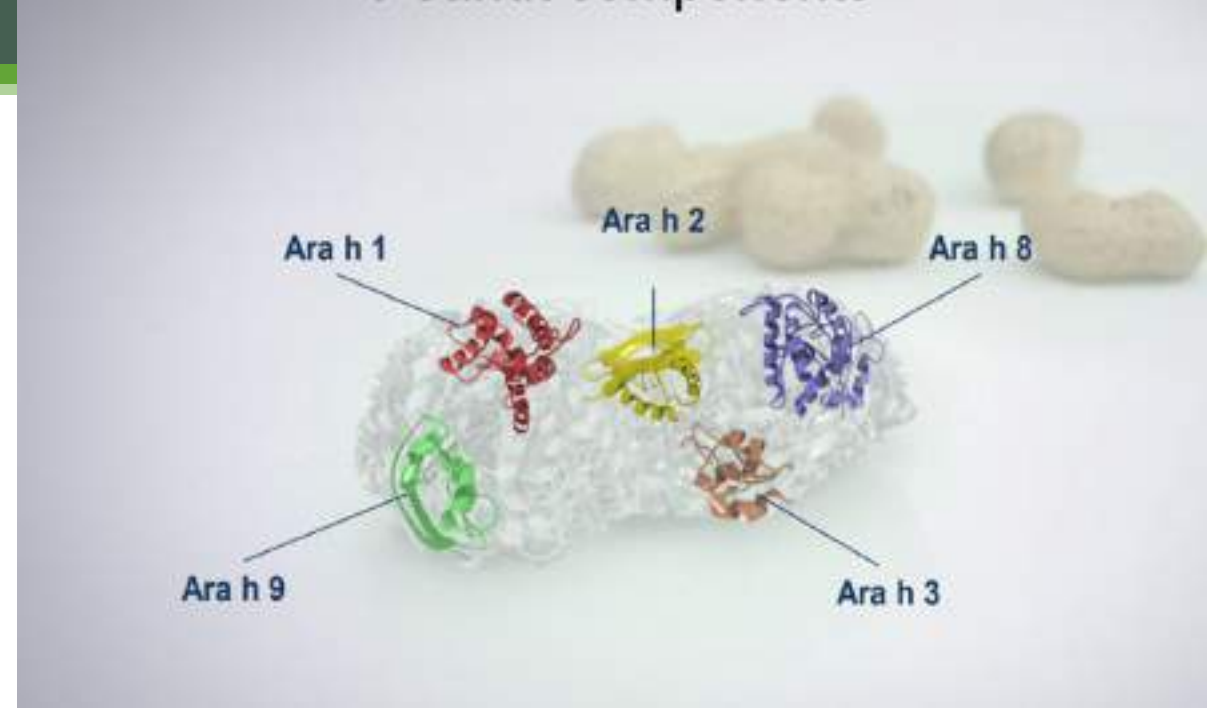
- Cross reactivity marker
- Associated with allergy to peach and peach related fruits (Pru p 3)
- Stable and heat resistant
- High likelihood of systemic reactions
- Common in Mediterranean populations



PEANUT - Ara h 1, 2, 3, 6

- Storage proteins
- Stable and heat resistant
- High likelihood of systemic reactions
- **Ara h 2 being the most accurate predictor of true allergy and severe reactions**

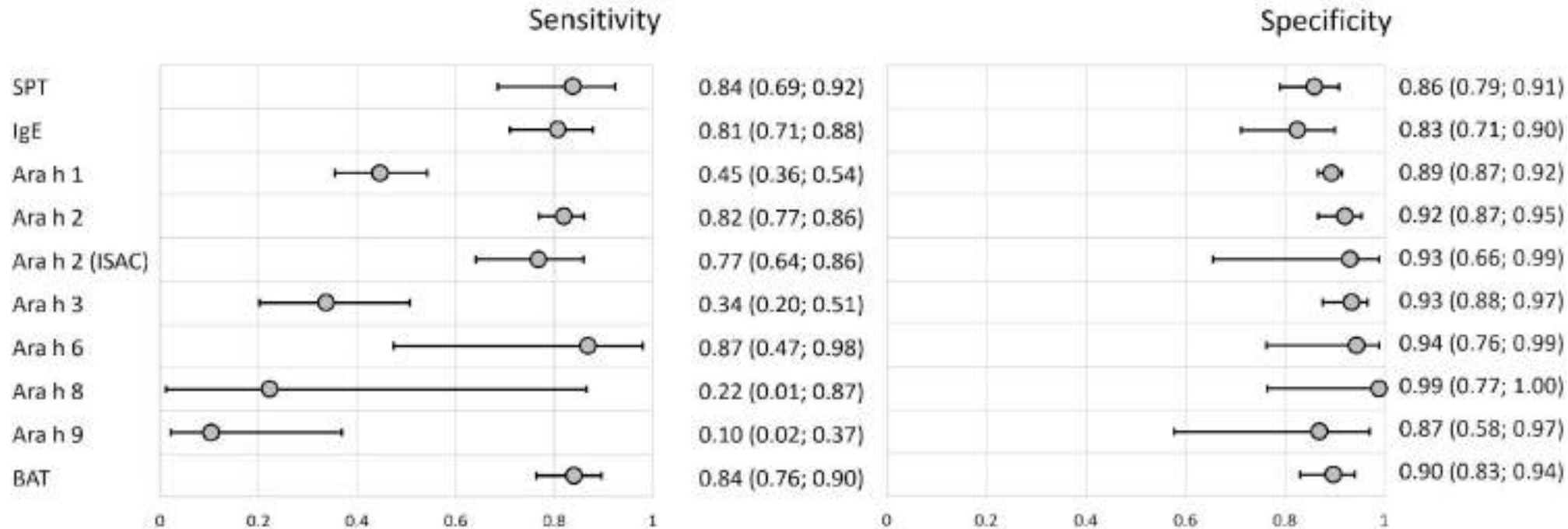
Ara h 2 cutoffs vary widely by study: 0.35-42.2kU/L with 90-95% PPV



Evidence	Methods	Findings
Dang et al 2012	Abstract – 200 infants (Health Nuts cohort)	Ara h 2-sIgE cut off 0.46kUA/L – 73% sensitivity, 95% specificity Peanut IgE 6.2kUA/L – 44% sensitivity, 95% specificity
Nilsson et al 2019	Systematic review	Ara h 2-sIgE 0.35kUA/L – 83.3% sensitivity, 83.6% specificity
Kansen et al 2022	150 children	Ara h 2-sIgE ≤0.1kUA/L 100% NPV ≥5.0kUA/L 89-97% PPV
Riggoni et al 2023	Systematic review	Ara h 2-sIgE 0.44kUA/L – 82% sensitivity, 92% specificity

Ara h 2 can enhance certainty of diagnosis and reduce the number of OFC needed to rule out peanut allergy in unclear cases

Peanut allergy



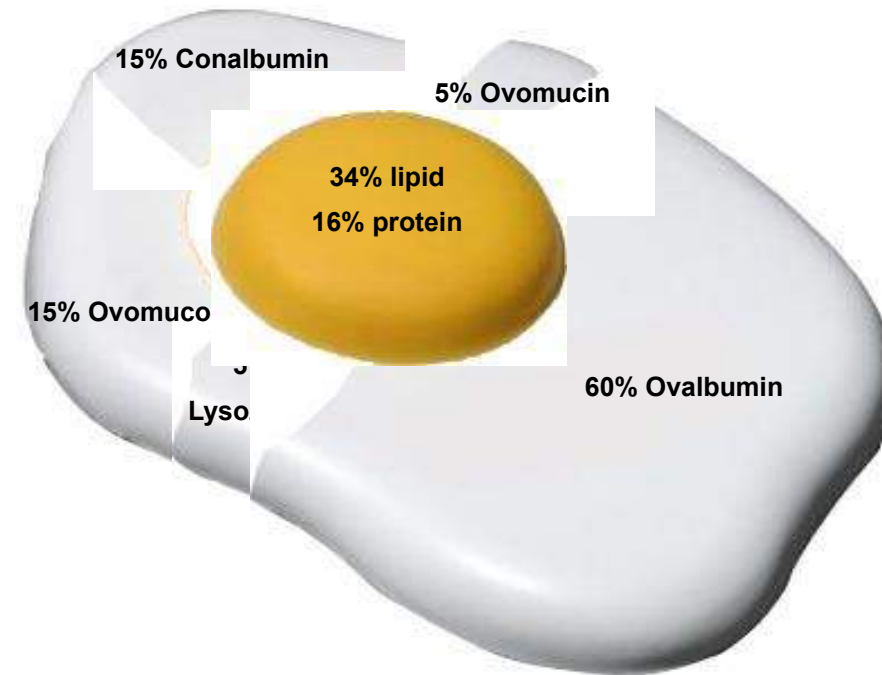
Diagnostic test	Cut-off		Subjects included	Nr of studies
	Median	IQ range		
Ara h 2-sIgE	0.44 kU _A /L	0.3 – 1.3	2924	27
BAT	5.0 %	4.7 - 7.1	308	4

What's in Egg?

Major Egg Allergen Components³

Gal d 1	Ovomucoid
Gal d 2	Ovalbumin
Gal d 3	Conalbumin
Gal d 4	Lysozyme

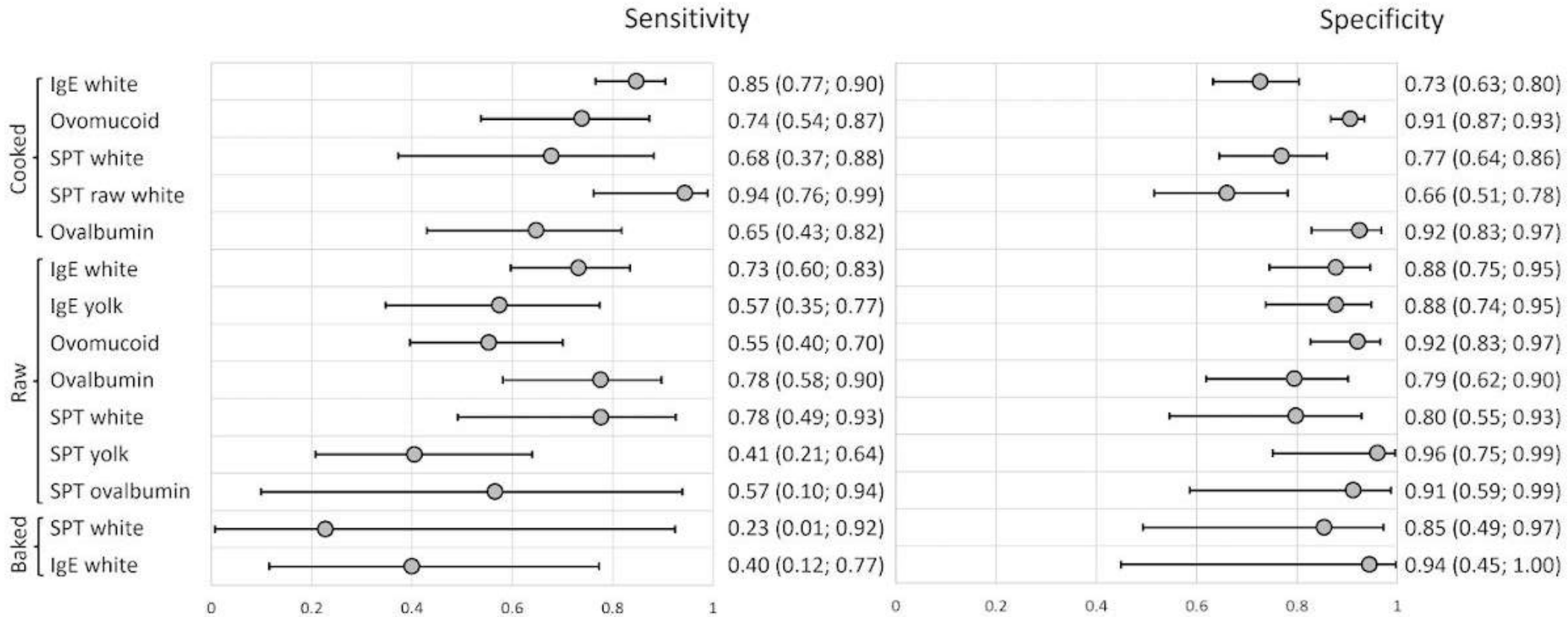
Heat resistant but OVM less heat resistant



Ovomucoid

Evidence	Methods	Findings
Hameda Y 2012 JACI 2012	100 children	OVM better than Egg White sIgE for diagnosis
Ando H JACI 2008	108 Children	Combined results OVM and egg white IgE guides diagnosis especially regarding tolerance of heated egg white
Lemon-Mule JACI 2008	117 Children	OVM>50kU/L highly predictive of heated egg reactivity so most still need OFC
Riggoni et al 2023	Systematic review	Cooked egg: - OVM-sIgE cut off 0.8kU/L (sensitivity 74%, specificity 91%) -Egg white-sIgE cut off 3.5kU/L (sensitivity 85%, specificity 73%)

Egg allergy



Diagnostic test	Cut-off		Subjects included	Num of studies
	Median	IQ range		
Egg white-sIgE	3.5	1.7-5.5	1687	13

Assessment of egg allergy

Egg white (Specific IgE) + Ovomuroid

Egg white: neg
Ovomucoid: neg

Low risk for clinical
reactions to egg

Egg white: pos
Ovomucoid: neg

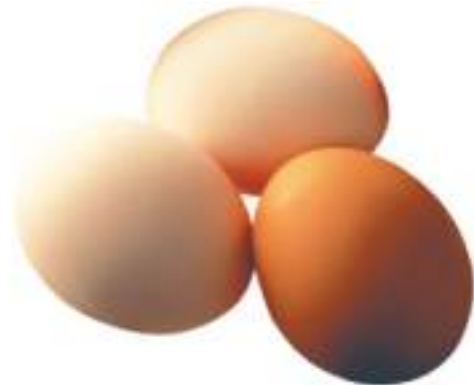
Risk for clinical
reactions to egg

Absence of
IgE antibodies to
ovomucoid indicates
tolerance to
ingestion to baked
egg

Egg white: pos
Ovomucoid: pos

High risk for clinical
reactions to egg

Increased risk for
persistent egg
allergy



slgE to Gal d 1 is a good predictor of clinical reactions

Egg white positive + Gal d 1 negative

Risk for clinical reactions to raw and slightly heated egg
Indicates tolerance to extensively heated egg

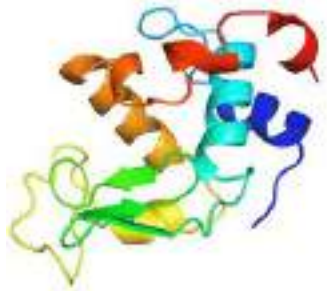
Egg white positive + Gal d 1 positive

High risk for clinical reactions to all forms of egg

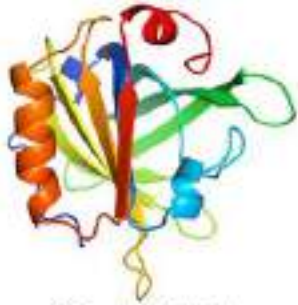
Low probability

High probability

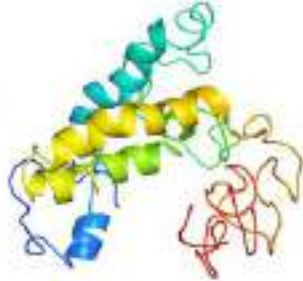
Milk



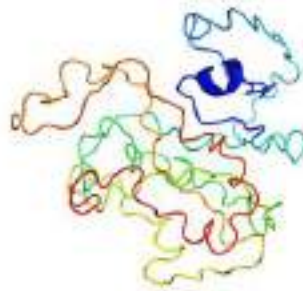
α -Lactalbumin



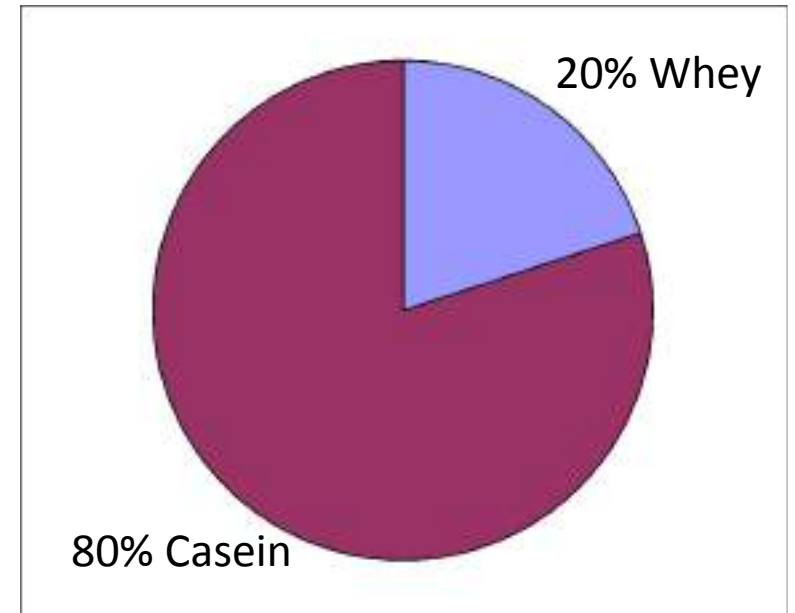
β -Lactoglobulin



α_{1} -Casein



β -Casein



Casein (Bos d 8)

- Heat stable protein
- Proteins remain stable even after 120mins boiling at 100 °c
- α -lactalbumin disappeared after 30mins
- β -lactoglobulin after 15mins
- Lactoferrin after 10mins



Recommended test profile

ImmunoCAP[®]
COMPLETE EXTRACT

Milk (f2)

ImmunoCAP[®]
COMPONENTS

Bos d 4 (f76) Bos d 5 (f77) Bos d 6 (e204) Bos d 8 (f73) Bos d lactoferrin (f334)

**Bos d 4,
α-lactalbumin**

- Risk for reactions to fresh milk
- IgE levels fall as tolerance develops
- Heat labile protein

**Bos d 5,
β-lactoglobulin**

- Risk for reactions to fresh milk
- IgE levels fall as tolerance develops
- Heat labile protein

**Bos d 6,
BSA**

- Risk for reactions to fresh milk
- The main allergen in beef
- Heat labile protein

**Bos d 8,
Casein**

- Risk for reactions to all forms of milk
- High levels are connected with persistent milk allergy
- IgE levels fall as tolerance develops
- Stable to heat

Bos d lactoferrin

- Risk for reactions to fresh milk
- Heat labile protein

Trace Exposure Risk

Accidental allergic reactions in children allergic to cow's milk proteins

Teresa Boyano-Martinez, MD,^a Carmen Garcia-Ara, MD, PhD,^a Maria Pedrosa, MD,^a José Maria Díaz-Pena, MD, PhD,^a and Santiago Quirce, MD, PhD^{a,b} *Madrid, Spain*

- Specific IgE levels against cow's milk and casein were greater than 24 KUA/L in 5 of the 6 children with severe reactions.

Boyano-Martinez, T., C. Garcia-Ara, et al. (2009). "Accidental allergic reactions in children allergic to cow's milk proteins." *J Allergy Clin Immunol* 123(4): 883–888



Casein

Tolerance to extensively heated milk in children with cow's milk allergy

Anna Nowak-Wegrzyn, MD, Katherine A. Bloom, MD, Scott. H. Sicherer, MD, Wayne G. Shreffler, MD, PhD, Sally Noone, RN, Niya Wanich, MD, and Hugh A. Sampson, MD *New York, NY*

- Low levels of Casein can indicate tolerance to baked milk products
- 75 % of children with a recent diagnosis of CMPA tolerated baked milk



Why baked milk?

Dietary baked milk accelerates the resolution of cow's milk allergy in children

Jennifer S. Kim, MD,* Anna Nowak-Węgrzyn, MD,* Scott H. Sicherer, MD, Sally Noone, RN, Erin L. Moshier, MS, and Hugh A. Sampson, MD *New York, NY*

- Tolerance of baked milk is a good indicator for outgrowing allergy
- The addition of baked milk into the diet appears to accelerate tolerance

Casein

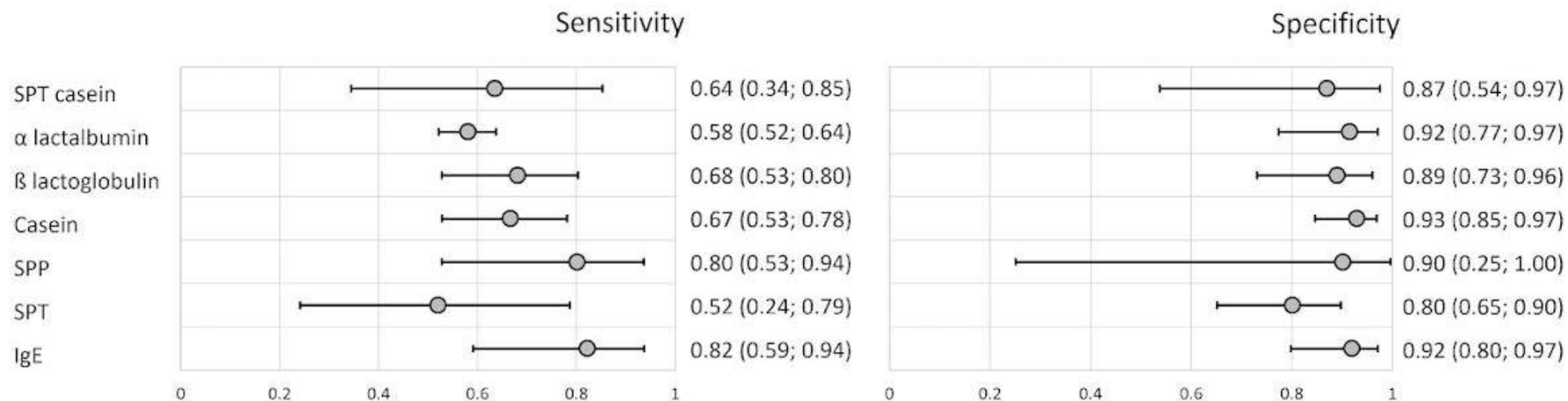


- Casein identified as major allergen – high levels often indicate allergy to both fresh and baked milk
- High levels of casein-sIgE antibodies are strongly associated with milk allergy in children and might be associated with prolonged allergy
- The casein test showed the best discriminating performance with a clinical decision point of 6.6 kUA/L corresponding to 100% specificity for milk allergy
- Children with sIgE to Casein above 1.8kUA had six-fold increase in risk of anaphylaxis

1. Ito et al. The usefulness of casein-specific IgE and IgG4 antibodies in cow's milk allergic children Clinical and Molecular Allergy 2012, 10:1
2. Cingolani et al. Usefulness of nBos d 4, 5 and nBos d 8 Specific IgE Antibodies in Cow's Milk Allergic Children. Allergy Asthma Immunol Res. 2014 March; 6(2): 121–125. Published online 2013 November

Evidence	Methods	Findings
Bartnikas Ann Allergy Asthma Immunol 2012	35	Milk IgE 1.0kUA/L, casein sIgE level 0.9kUA/L 90% predictive value for tolerating baked milk
Castro Allergol Immunopathol (Madr) 2015	184	Milk IgE 3.06kU/L – sensitivity 71%, specificity 98% Casein 1.47kU/L – sensitivity 66%, specificity 98%
Dominguez JACI 2025	50	Baked milk tolerance: Milk IgE >8.5kUA/L - sensitivity 72%, specificity 72% Casein >5.7kUA/L – sensitivity 72%, specificity 88%
Riggoni et al Allergy 2023	Systematic review	Cow's milk - Cow's milk IgE 3.5 kU/L – sensitivity 82%, specificity 92% - Casein sIgE 2.6kU/L – sensitivity 67%, specificity 93%

Cow's milk allergy



Diagnostic test	Cut-off		Subjects included	Num of studies
	Median	IQ range		
Cow's milk-sIgE	3.5	0.9-10.5	1965	16

slgE to Bos d 8 is a good predictor of reactions to milk

Milk positive + Bos d 8 negative

Risk for reaction to unheated milk products
Suggests tolerance to baked milk

Milk positive + Bos d 8 positive

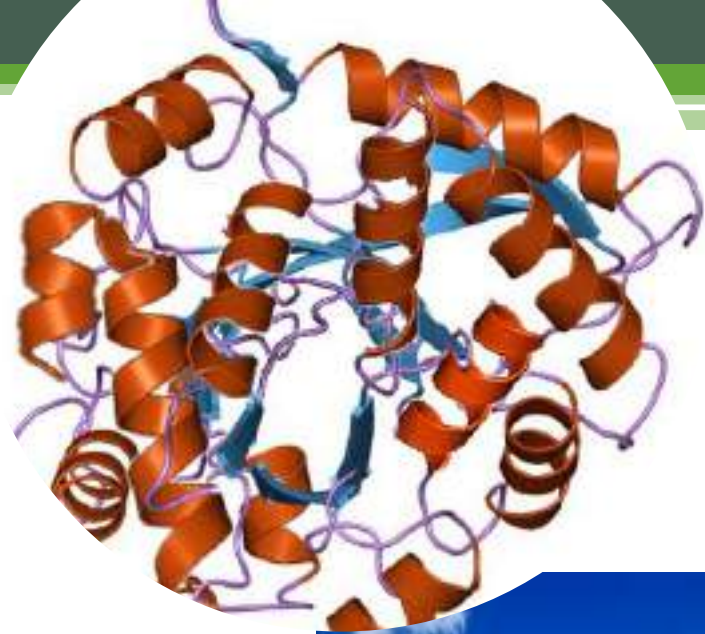
High risk for reaction to
milk in all forms

Low probability of reaction

High probability of reaction

Wheat

- Major wheat allergens – gliadins and glutenins
- Omega 5 gliadin (Tri a 19) – associated with WDEIA
 - Sensitivity indicates risk of WDEIA and correlated with OFC-proven IgE mediated wheat allergy
- No significant link in other studies



Wheat

- High levels of wheat sIgE levels predict slower resolution
- High IgE levels to gliadins have been shown to correlate with persistent wheat hypersensitivity
- Riggoni et al Allergy 2023 systematic review
 - sIgE wheat cut off 0.6kU/L has sensitivity 79%, specificity 78%
 - Wheat-5 gliadin sIgE 0.3kU/L has sensitivity 79%, specificity 78%

Conclusion

- Diagnostic allergy testing is an important part of the diagnostic work up
- Clinical history remains a key part in investigating allergies in the first instance
- Component testing gives us the ability to diagnose more accurately and perform a risk assessment
- Increased QOL for patients
- Health economic benefit

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