Diagnostic Testing in Food Allergy

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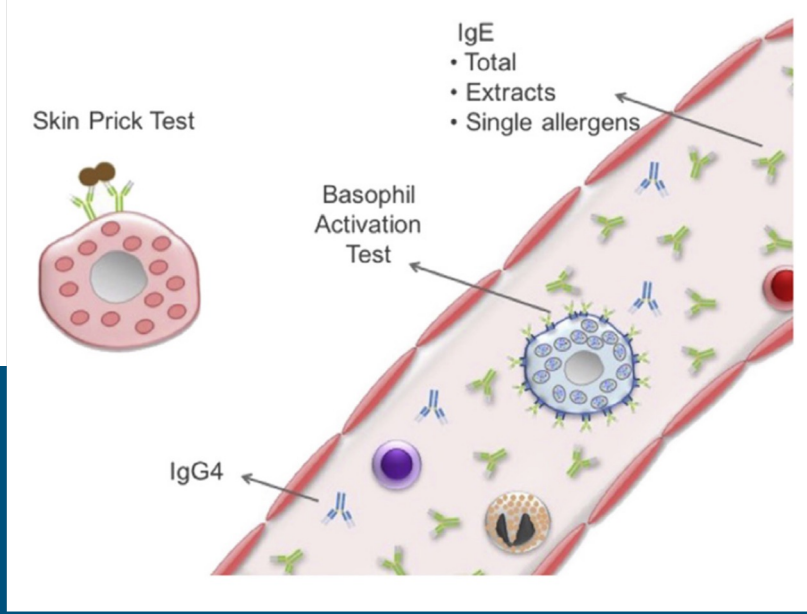
Outline

- Testing Goals
- SPT
- IgE and CRD
- BAT
- Epitope Testing
- OFC



Food Allergy Testing Goals

For ***Diagnosis***

History
(Route of exposure, Timing, Reaction, Severity, Age, Duration of Reaction, Treatment)

+

Testings

↓

Likelihood Ratio

For ***Treatment**** (SLIT/OIT, etc)

History

+

Burden of avoidance

+

Risk of treatment vs **Risk of anaphylaxis**

↓

Treatment Plan

*It would be helpful to estimate the ***threshold*** of reaction

Food Allergy Diagnosis

Skin prick test

Skin patch test

Food-specific IgE

Oral food challenge

Food-specific serum IgE (sIgE)

Utility of food-specific IgE concentrations in predicting symptomatic food allergy

J ALLERGY CLIN IMMUNOL
MAY 2001

Hugh A. Sampson, MD *New York, NY*

Improved screening for peanut allergy by the combined use of skin prick tests and specific IgE assays

J ALLERGY CLIN IMMUNOL
JUNE 2002

Fabienne Rancé, MD, Michel Abbal, MD, and Valérie Lauwers-Cancès, MD *Toulouse, France*

J Allergy Clin Immunol Pract. 2017 Mar-Apr; 5(2): 237–248.
doi: 10.1016/j.jaip.2016.12.003

Making the Most of *In Vitro* Tests to Diagnose Food Allergy

Alexandra F. Santos, MD, PhD[a,b,*] and Helen A. Brough, MBBS, PhD[a,b]

Table I

Examples of diagnostic cutoffs with 95% PPV and 50% NPV for specific IgE to food allergen extracts[14, 107, 125]

Approximate predictive value	Cow's milk	Egg	Peanut	Fish
95% PPV	32 kU/L	7 kU/L	15 kU/L	20 kU/L
50% NPV	2 kU/L	2 kU/L	2 kU/L*	–
			5 kU/L*	

NPV, Negative predictive value; *PPV*, positive predictive value.

*The 50% NPV cutoff is different depending on the previous history of reaction: 2 kU/L if the patient reports a reaction and 5 kU/L if the patient has never had an allergic reaction to peanut in the past.

Diagnostic cut-off varies in studies, and countries, based on patient population and OFC protocols.

sIgE / Total IgE Ratio.,

Does this ratio improve prediction of OFC outcome?

Some studies ++ and some studies -- (no change vs sIgE alone)

- **Discrepancy due to to the foods studied**
  - **Gupta et al -- useful for persistent food allergies eg, peanut, tree nuts, shellfish, and seeds**
  - **Mehl et al -- evaluated foods that often have transient allergy, cow's milk, egg, and wheat**

- **A multicenter study of children with suspected PN or hazelnut allergies evaluated Ara h2, PN, and hazelnut IgE ratios** ***(Grabenhenrich L. et al. JACI 2016)***
  - **Peanut-specific/total IgE was also not better than Ara h 2 sIgE in diagnosing PA**
  - **Similar results were reported for hazelnut allergy**

sIgG4 / sIGE Ratio

Does this ratio improve prediction of OFC outcome?

- **Diagnostic utility has not been established**
- **Sensitized/tolerant children tend to have higher allergen-specific IgG4/IgE ratios than allergic children**
  - **Higher ratio in children who do not knowingly eat peanut**
  - **Increases over time in patients undergoing OIT and other forms of food immunotherapy**

Component Resolved Diagnostics (CRD)

"component testing"

Definition: Component testing in food allergies is an approach utilized to ***characterize the molecular components*** of each allergen involved in a specific IgE (sIgE)-mediated response, in order to ***improve diagnostic accuracy***.

Different proteins may cause variable reactions.

Food

604783 Allergen Profile, Food IgE II With Component Reflexes*

Method: Thermo Fisher ImmunoCAP® Allergen-specific IgE test

Almond	Codfish	Macadamia Nut	Pistachio	Soybean
Brazil Nut	Corn	Milk	Scallop	Walnut
Cashew Nut	Egg White	Peanut (Whole)	Sesame Seed	Wheat
Clam	Hazelnut (Filbert)	Pecan	Shrimp	

If milk IgE ≥ 0.35 kU/L, reflex tests α-lactalbumin, β-lactoglobulin, and casein will be added.
If egg white IgE ≥ 0.35 kU/L, reflex tests ovalbumin and ovomucoid will be added.
If IgE to Brazil nut, cashew nut, hazelnut (filbert), peanut (whole), and/or walnut is 0.10 kU/L, reflex testing will be completed as follows:
Brazil nut: Ber e 1 / cashew nut: Ana o 3 / hazelnut (filbert): Cor a 1, Cor a 8, Cor a 9, and Cor a 14 /
peanut (whole): Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9 / walnut: Jug r 1 and Jug r 3

LabCorp

ImmunoCAP® Nut Components

	CCD	Profilin	PR-10	LTP	Storage Proteins
Peanut	MUXF3	Bet v 2*	Ara h 8	Ara h 9	Ara h 1 Ara h 2 Ara h 3 Ara h 6
Hazelnut	MUXF3	Bet v 2*	Cor a 1	Cor a 8	Cor a 9 Cor a 14
Walnut	MUXF3	Bet v 2*		Jug r 3	Jug r 1
Brazil nut	MUXF3	Bet v 2*			Ber e 1
Cashew nut	MUXF3	Bet v 2*			Ana o 3

*Surrogate markers for profilin Phl p 12, Bet v 2 or Pru p 4.

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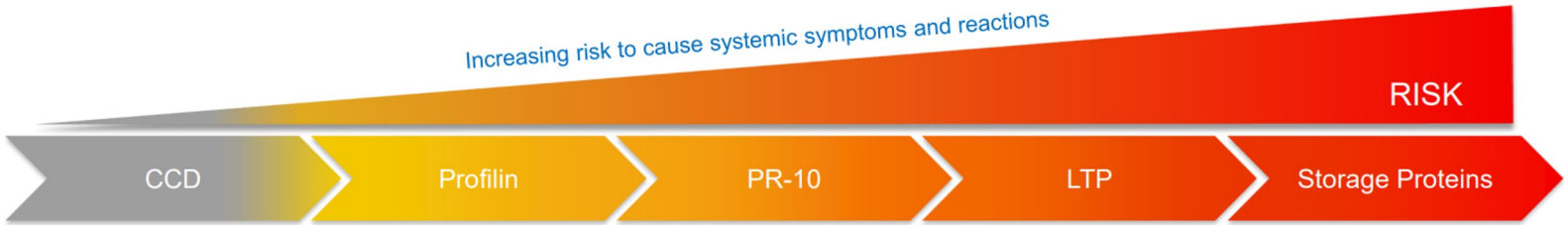
Peanut Components



- Usually does not provoke any clinical reactions[1]
- Highly cross-reactive (same structure in pollen, plant food, and venoms) [1]

- Sensitization is usually asymptomatic[1,2]
- Abundant in nature[1,2]

- Labile to heat and digestion[3]
- Mainly local reactions[4]
- Associated with birch pollen allergy (cross-reactivity)[5]

- Stable to heat and digestion[6]
- Associated with local and systemic reactions[6,7]
- Associated with allergy to stone fruits (cross-reactivity) [7]

- Stable to heat and digestion[8,9]
- Associated with systemic reactions[6]
- Indicates primary sensitization[10]

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2. Katelaris CH: Food allergy and oral allergy or pollen-food syndrome. Curr Opin Allergy Clin Immunol 2010, 10:246–251.20.
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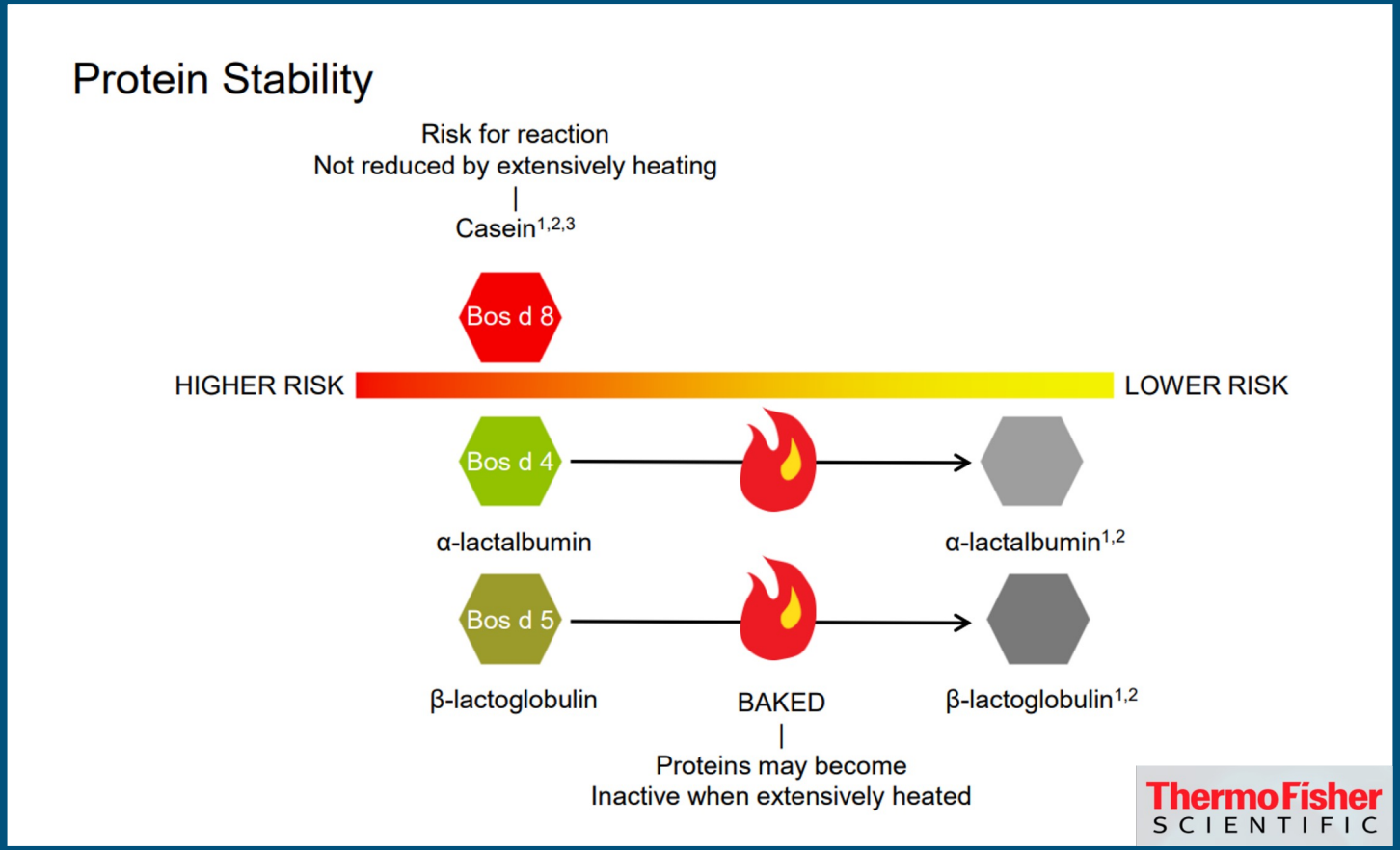
Table III

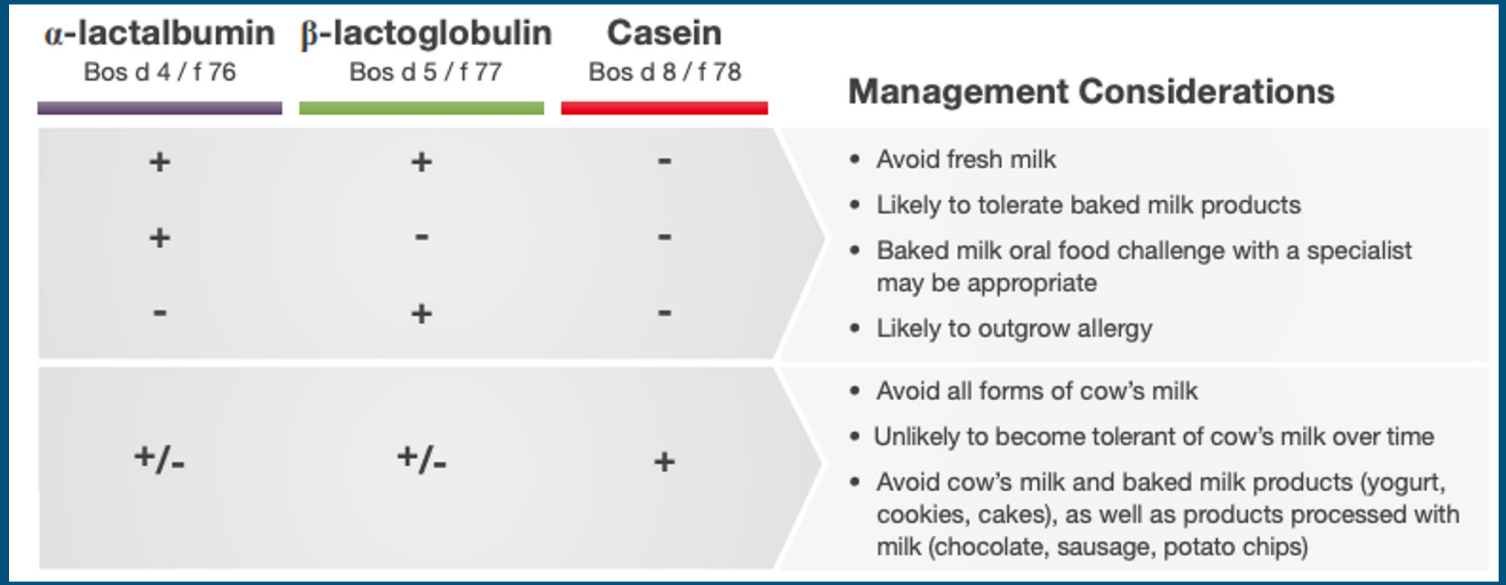
Allergen components associated with clinical allergy and examples of cutoffs for specific IgE testing to main allergen components

Foods	Components associated with clinical allergy	Cutoffs for specific IgE to main components
Peanut	Ara h 1	Ara h 2 sIgE: 0.35 to 42.2 kU/L had 90%-95% PPV[16], [24], [27]
	Ara h 2	
	Ara h 3	
	Ara h 9 (in Southern Europe)	
Hazelnut	Cor a 9	Cor a 9 sIgE: 1 kU/L had 83% accuracy[28]
	Cor a 14	Cor a 14 sIgE: 0.72 to 47.8 kU/L had 87%-90% accuracy[27], [31]
	Cor a 8 (in Southern Europe)	
Cashew, Pistachio	Ana o 3	Ana o 3 sIgE: 0.16 kU/L had 97.1% accuracy for cashew and/or pistachio nut allergy[127]
Brazil nut	Ber e 1	Ber e 1 sIgE: 0.25 kU/L had 94% PPV[128]
Walnut	Jug r 1	Jug r 1 sIgE: 0.1 kU/L had 91% PPV[129]
	Jug r 3	
Soya	Gly m 5	Gly m 8 sIgE: 1 kU/L had 89% PPV[56]
	Gly m 6	Gly m 8 sIgE: 0.1 kU/L had 83% NPV[56]
	Gly m 8	
Wheat	Tri a 19 (IgE-mediated wheat allergy and WDEIA)	Tri a 19 sIgE: 0.04 AU had 100% PPV and 88% NPV for IgE-mediated wheat allergy[51], [52]

Milk Components



Milk Components



α-lactalbumin Bos d 4 / f 76	β-lactoglobulin Bos d 5 / f 77	Casein Bos d 8 / f 78	Management Considerations
+	+	-	• Avoid fresh milk • Likely to tolerate baked milk products • Baked milk oral food challenge with a specialist may be appropriate • Likely to outgrow allergy
+	-	-	
-	+	-	
+/-	+/-	+	• Avoid all forms of cow's milk • Unlikely to become tolerant of cow's milk over time • Avoid cow's milk and baked milk products (yogurt, cookies, cakes), as well as products processed with milk (chocolate, sausage, potato chips)

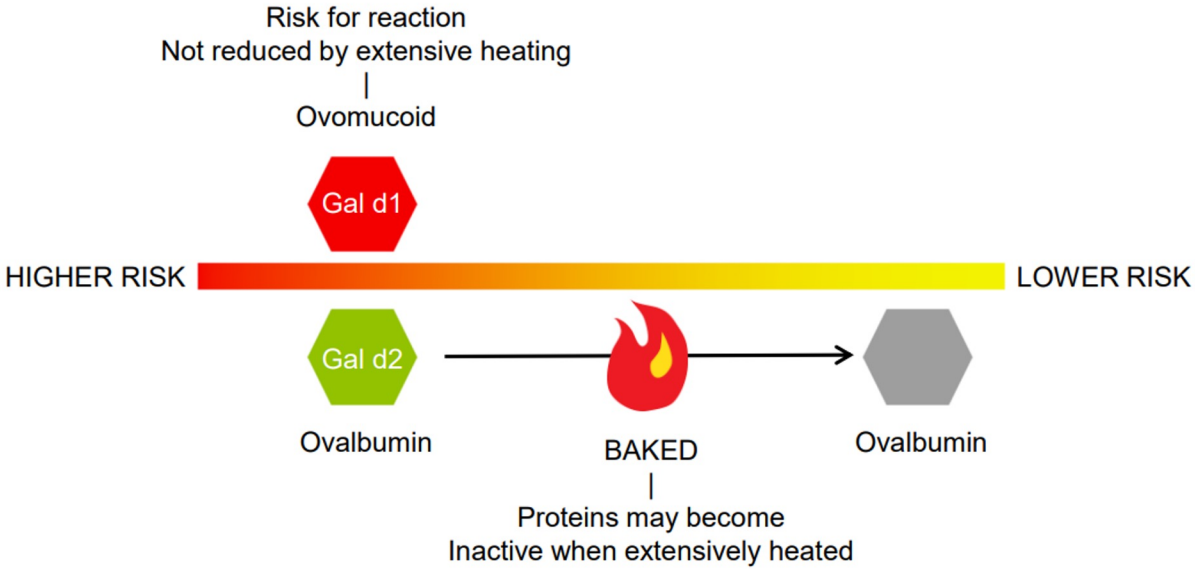
Boiled Milk IgE, Anyone?

Test Name	In Range	Out Of Range	Reference Range
ALLERGEN SPECIFIC IGE			
MILK (BOILED)	<0.10		<0.35 kU/L
CLASS	0		

The test method is the Phadia ImmunoCAP allergen-specific IgE system. CLASS INTERPRETATION <0.10 kU/L= 0, Negative; 0.10 - 0.34 kU/L= 0/1, Equivocal/Borderline; 0.35 - 0.69 kU/L=1, Low Positive; 0.70 - 3.49 kU/L=2, Moderate Positive; 3.50 - 17.49 kU/L=3, High Positive; 17.50 - 49.99 kU/L= 4, Very High Positive; 50.00 - 99.99 kU/L= 5, Very High Positive; >99.99 kU/L=6, Very High Positive

Egg Components

Protein Stability



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Egg Components

Ovalbumin Gal d 2 / f 232	Ovomucoid Gal d 1 / f 233	Management Considerations
+	-	• Avoid uncooked eggs • Likely to tolerate baked egg • Baked egg oral food challenge with a specialist may be appropriate • Consider repeating IgE component test biennially during childhood to determine potential tolerance • May be transferred via breast milk, so mothers of infants with egg allergy should take caution when breast-feeding
+/-	+	• Avoid all forms of egg • Consider repeating IgE component test biennially during childhood to determine potential tolerance • Patients sensitized to ovalbumin with low levels of IgE to ovomucoid may react to egg that is not fully baked

Wheat Component

Cross reactivity with Grasses (timothy, orchard) and other Grains (rye, barley)

- Up to 65% of grass allergic patients have detectable wheat sIgE

Gliadin (alpha, beta, gamma, and omega)

- Unstable and may be underrepresented in whole-wheat tests
- Gliadin component mix does not contain grass cross-reacting IgEs
- Testing for ***gliadin component mix*** is recommended in addition to wheat IgE

Omega-5-gliadin (Tri a 19)

- Associated with wheat-dependent exercise-induced anaphylaxis (WDEIA)

Basophil Activation Test (BAT)

1. Stimulation

Whole blood + Stimulants
- Allergen
- Negative control
- Positive controls

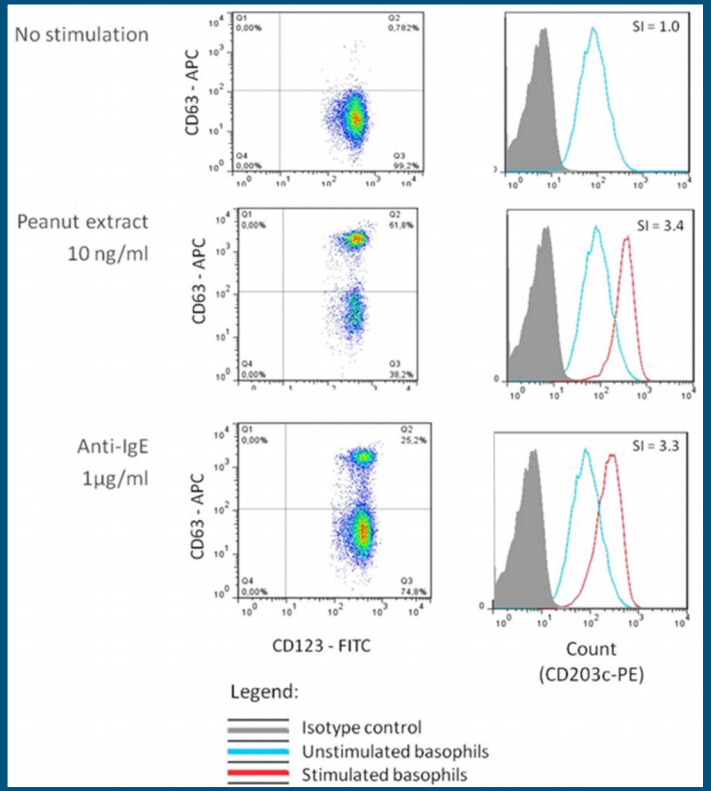
2. Staining

- Stop degranulation
  (EDTA + variation in temperature to 4°C)
- Staining
  (with antibodies conjugated to fluorochromes)

3. Red blood cell lysis

- Red blood cell lysis
- Cell suspension ready for analysis

4. Flow cytometry



Santos and Lack. Clin Transl Allergy (2016)

Basophil Activation Testing (BAT)

Requires fresh whole blood

5–10% non-responsive rate to IgE-mediated stimulation

Test ordering?

Issues with insurance coverage?

Epitope Testing: Background

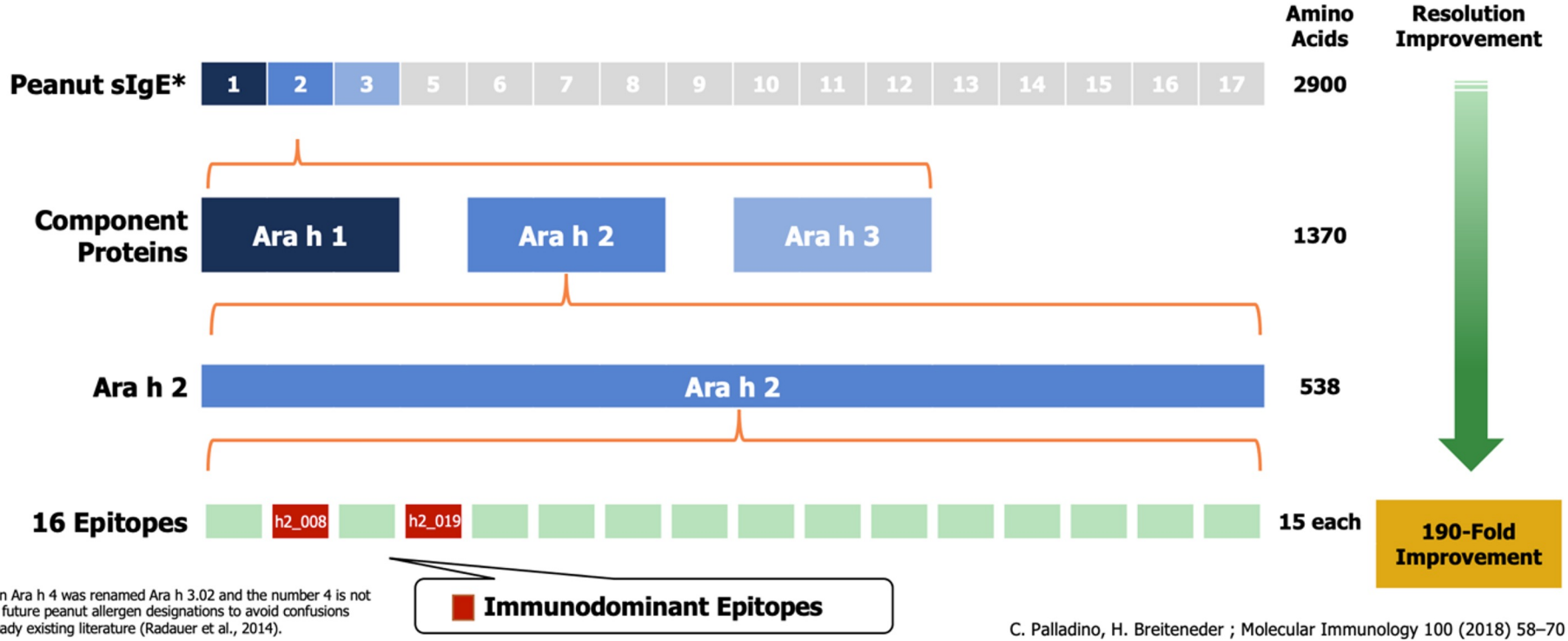
Accurate and reproducible diagnosis of peanut allergy using epitope mapping.
Suárez-Fariñas, M, Suprun, M, Kearney, P, et al. Allergy. 2021; 76: 3789– 3797.

- Developed utilizing the LEAP cohort, then validated using two independent cohorts
  - 133 subjects from the non-interventional arm of the LEAP trial
  - CoFAR2 (82 subjects) & POISED (84 subjects)
- Measured Ara h2 ses-IgE in combination
- PN allergy status confirmed on DBPCFC
- Validation using CoFAR2 and POISED cohorts
  - test correctly diagnosed 93% of the subjects, with a sensitivity of 92%, specificity of 94%, a PPV of 91%, and NPV of 95%
- Overall accuracy was superior to existing diagnostic tests for peanut allergy including skin prick testing, peanut sIgE, and peanut component sIgE testing

EPITOPE MAPPING

The resolution of Epitope Mapping eliminates the biological noise associated with sIgE and Component-resolved Diagnostics

Testing at the epitope level **improves resolution by 190-fold** by allowing the measurement of epitope level antibody binding (1 Ab: 1 epitope)



*The allergen Ara h 4 was renamed Ara h 3.02 and the number 4 is not available for future peanut allergen designations to avoid confusions with the already existing literature (Radauer et al., 2014).

C. Palladino, H. Breiteneder ; Molecular Immunology 100 (2018) 58–70

Epitope Testing: Details

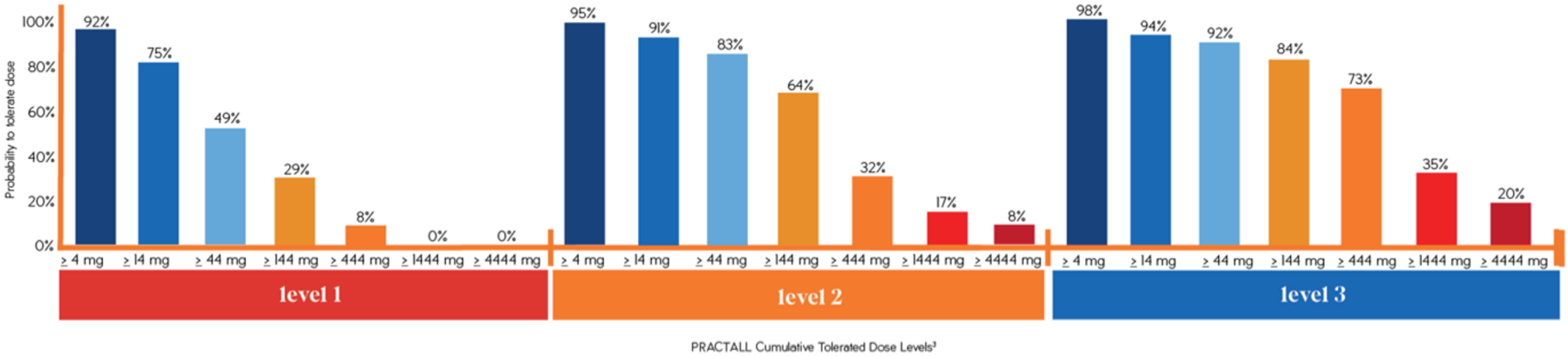
Details:

- Requires 2ml in a EDTA Lavendar Top
- Spin down to yield 200mcl of plasma
- Transfer to microcentrifuge tube for transport
- Refrigerate or store room temp.
- Send by FedEx within 2 days of draw.

Results:

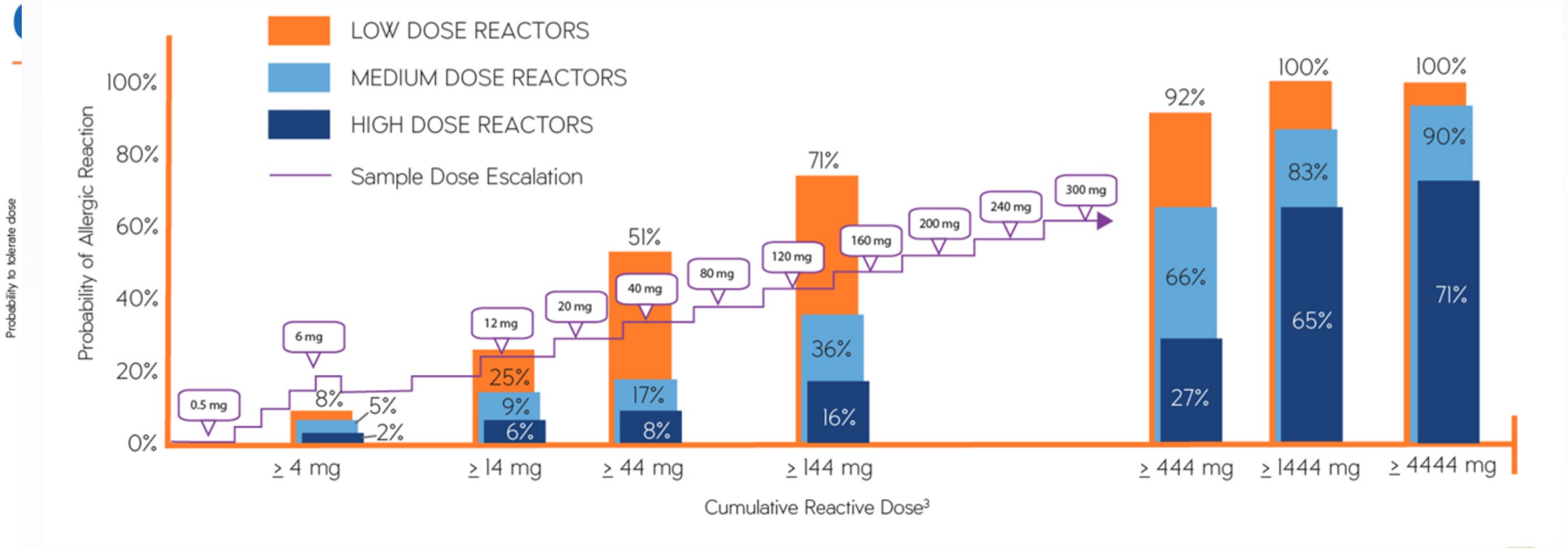
- Not allergic
- Allergic
  - Level 1 Very sensitive, high reactivity
  - Level 2 Moderately sensitive, may be able to tolerate low levels of food that has been “cross-contacted” with peanut
  - Level 3 Sensitive, may be able to tolerate low levels, OFC recommended

CLINICAL CONSIDERATIONS BY LEVEL



PRACTALL Cumulative Tolerated Dose Levels[3]

Cumulative reactive dose by reactor type



Epitope Testing: Pros / Cons of APD

- High Spec, Sens, PPV and NPV
- Provides a probability that a patient can tolerate specific amounts of PN
- Results can be superimposed with OIT dosing schedule / Palforzia ladder
- Information will evolve with continued research and data
- Applicable to peanut cultivars world-wide
- Accessible due to mobile phlebotomy service

- Does not predict the severity of reaction
- May result in a false negative if no serum IgE is detectable, but this is validated in LEAP PN tolerant as well to low IGE threshholds
- Not recommended for patients on omalizumab or OIT currently
- Requires phlebotomy

Oral Food Challenge (OFC)

Gold standard for diagnosis of food allergy

- Double-blind placebo-controlled food challenge (DBPCFC)

Time-intensive

Space-intensive

Resource-intensive

Risk of anaphylaxis

Best practice with consistent and strict protocols

Thank You