Clinical applications of the basophil activation test in food allergy

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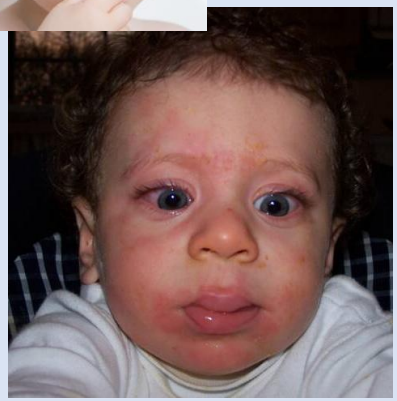
MRC Medical Research Council | KING'S College LONDON | Imperial College London | asthma UK

Outline

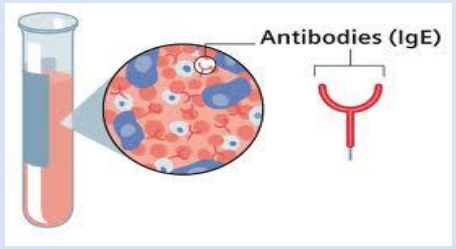
- Diagnosis of food allergy
- Utility of BAT in food allergy
- Bringing BAT to clinical practice

Diagnosis of IgE-mediated food allergy

Clinical History



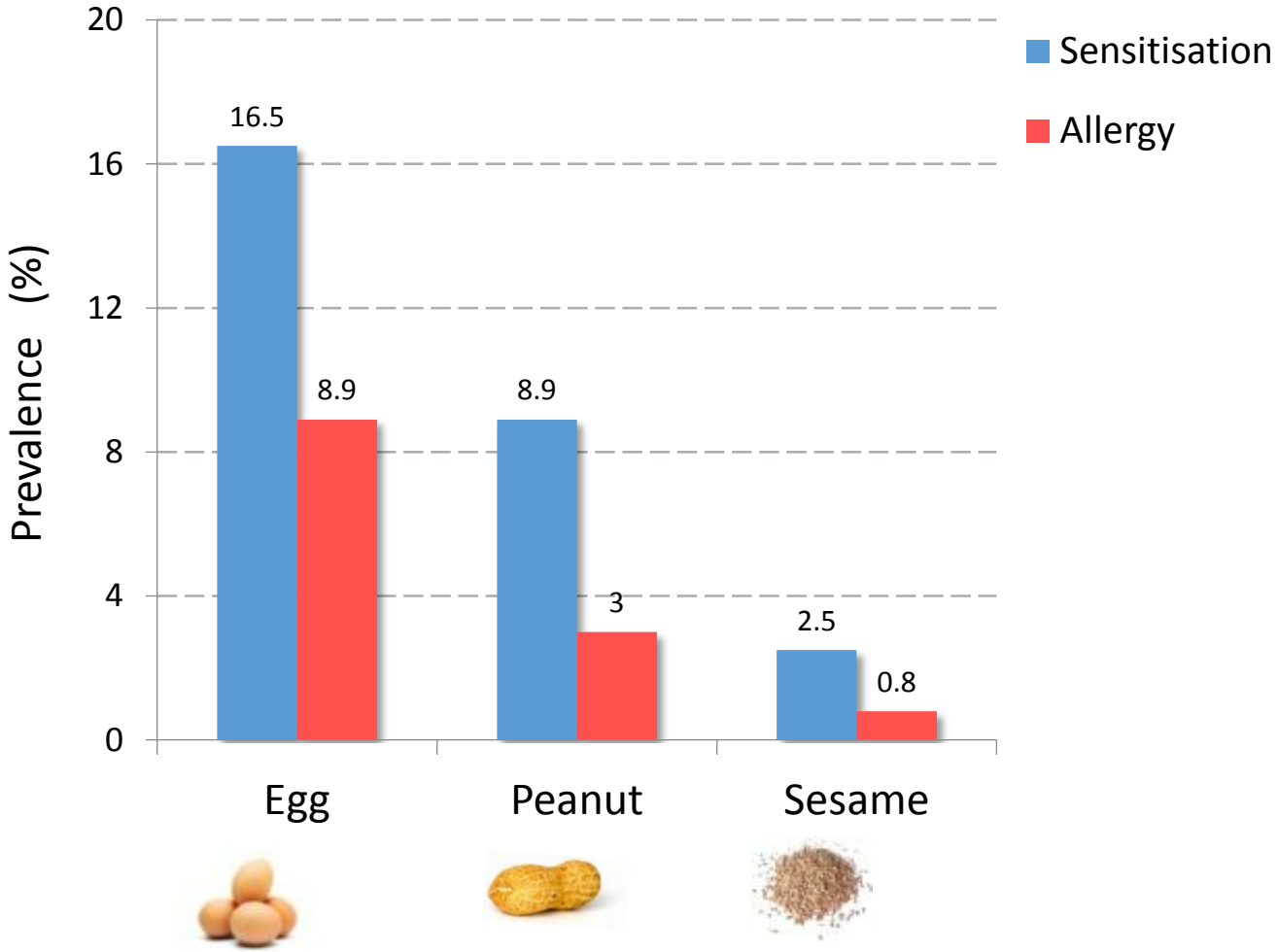
Allergy tests



Oral food challenge

Gold Standard

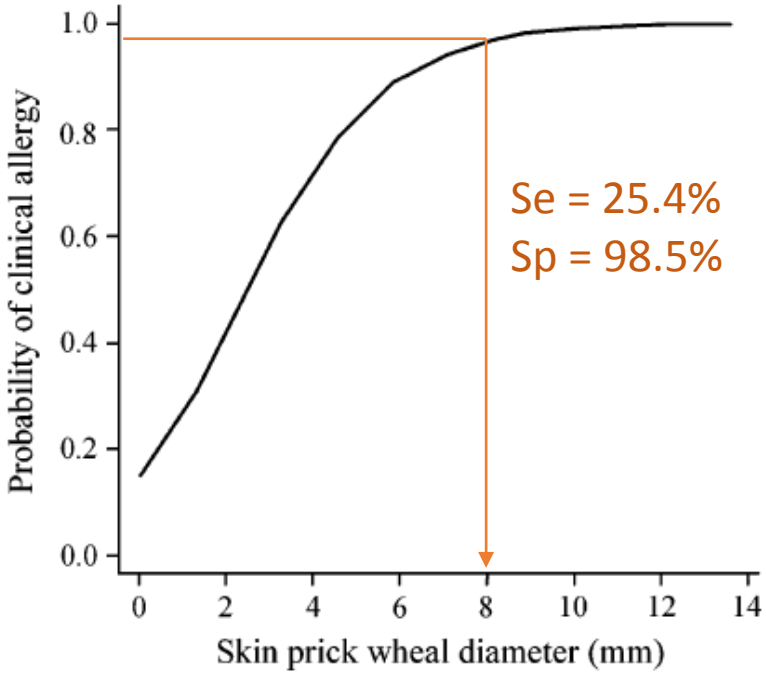
The majority of IgE-sensitised children are not allergic



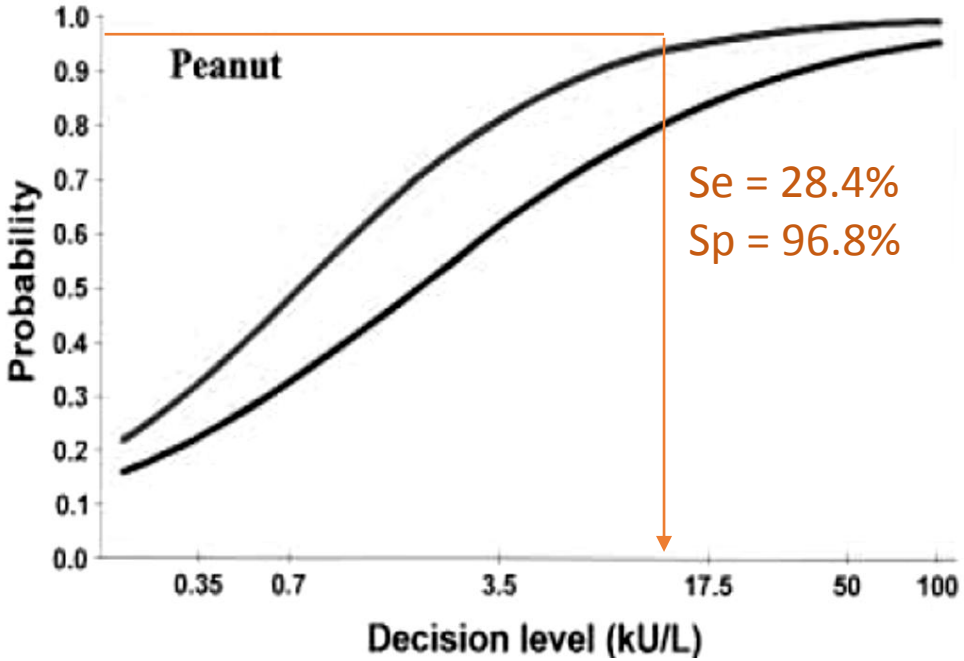
Osborne NJ et al J Allergy Clin Immunol 2011

95% PPV cut-offs increase specificity

Skin prick test



Serum specific IgE

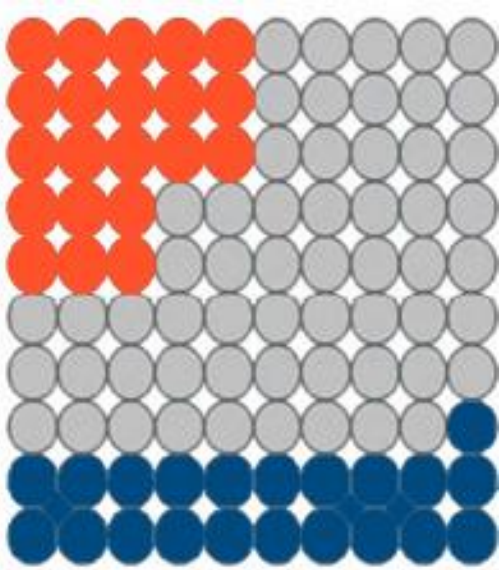


Roberts & Lack J Allergy Clin Immunol 2005
Sampson H & Ho J Allergy Clin Immunol 1997
Sampson H J Allergy Clin Immunol 2001

The majority of sensitised patients fall into the 'grey area'

SPT ≥8 mm

Likely to be peanut allergic



SPT 3 to 7 mm

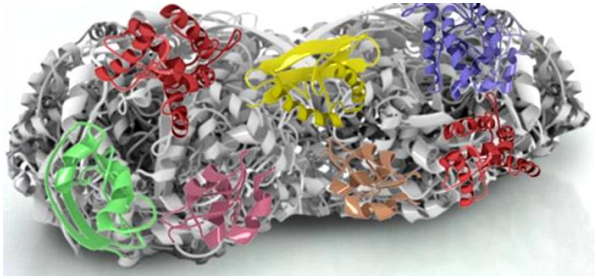
Immunological grey area

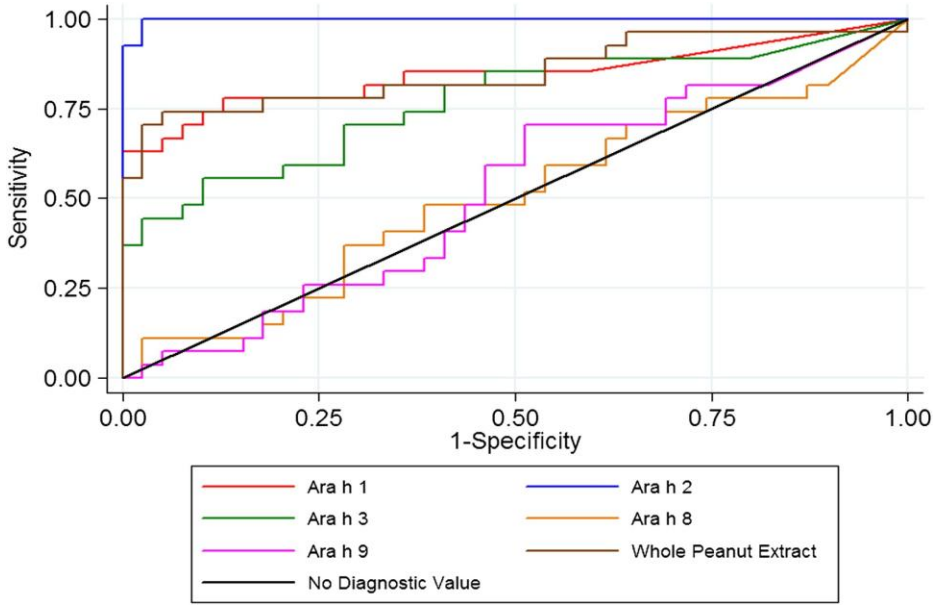
SPT <3 mm

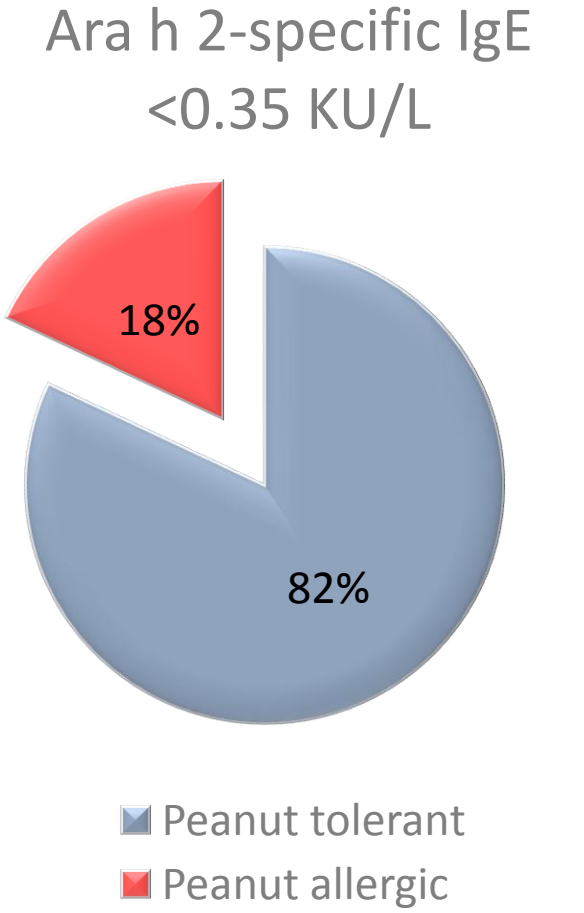
Very unlikely to be peanut allergic

Adapted from: Roberts & Lack Clin Exp Allergy 2000

A considerable proportion of allergic patients have equivocal allergy test results







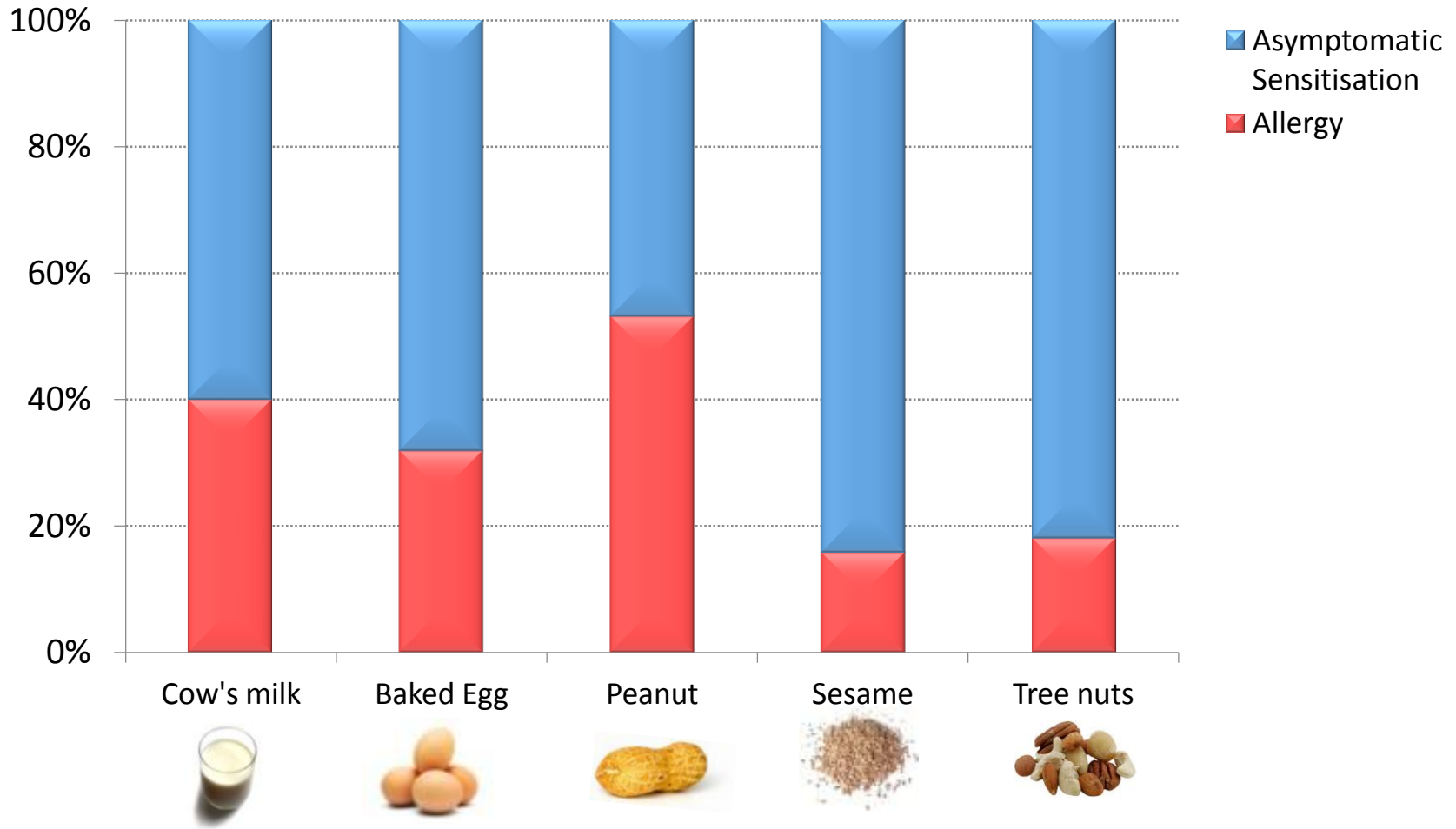
Nicolaou N et al. J Allergy Clin Immunol 2011
Dang TD et al. J Allergy Clin Immunol 2012

Oral food challenge to diagnose food allergy

- Risk of immediate-type allergic reaction
- Risk of late reactions
- Resource-intensive
- Increasing demand
- Multiple challenges per patient
- Not fool-proof!
  - 3% false-negatives
  - 3% false-positives
  - 2-9% indeterminate

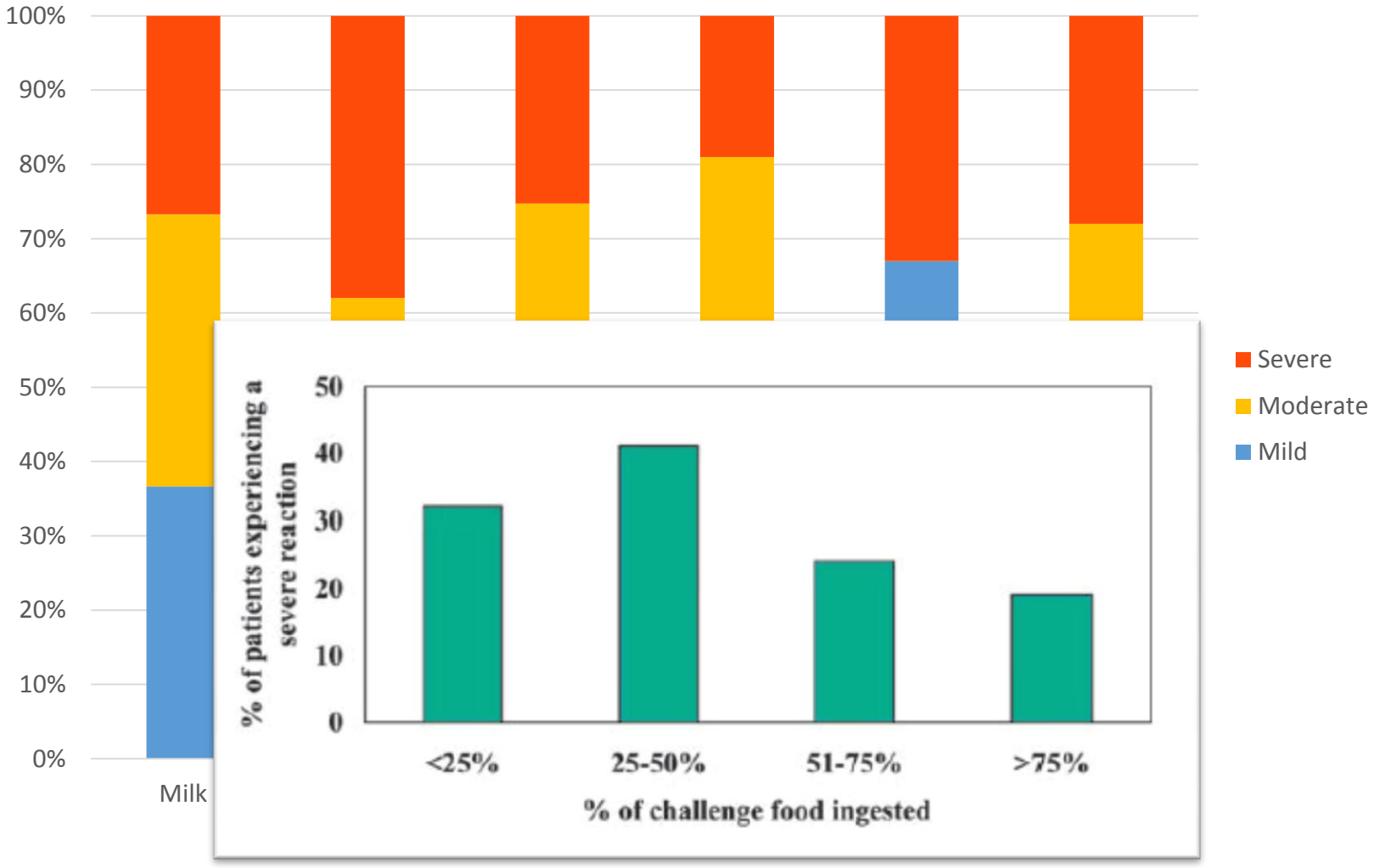
Perry TT et al JACI 2004; Saleh-Langenberg Allergy 2016
Caffarelli C et al Lancet 2001; Ludman S et al PAI 2013
Nolan RC et al PAI 2007; Niggemann B et al JACI 2012

Up to about 50% of oral food challenges are positive



Lieberman JA et al J Allergy Clin Immunol 2011
Novak-Wegrzyn A et al J Allergy Clin Immunol 2012
Santos AF et al J Allergy Clin Immunol 2014

Severity of allergic reactions during challenges



Perry TT et al J Allergy Clin Immunol 2004

Outline

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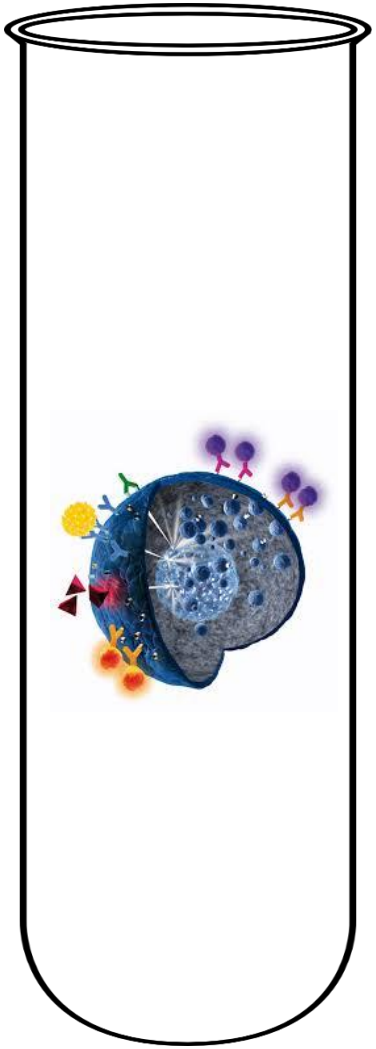


Food-induced anaphylaxis

occurs without elevated serum tryptase

Samples collected from patients with food-induced anaphylaxis:

- Fatal (patient 3)
- Near-fatal (patient 12)
- ICU (controls 1-3)

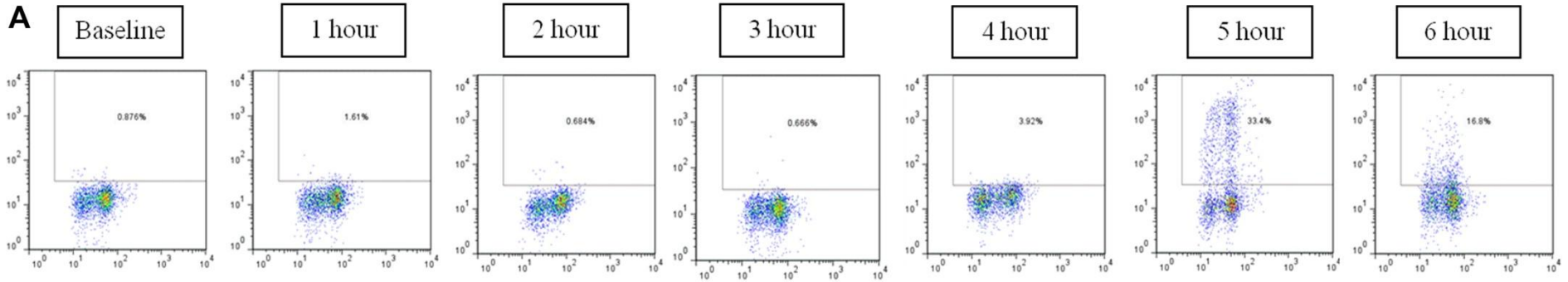
Normal range of serum tryptase < 2.5-5 ng/ml

Anaphylaxis usually serum tryptase >10 ng/ml

Serum sample	Hours after ingestion	Tryptase (ng/ml)
Patient 3	Post-mortem	2.14
Patient 12	0.75	1.2
	5	<1
	8	<1
	24	<1
	48	<1
	72	1.5
Control 1	1	<1
	5	<1
	24	<1
Control 2	0.5	<1
	1	<1
	5	<1
Control 3	2	<1
	2.5	<1
	4	3.4
	24	<1

Sampson HA et al. New Engl J Medicine 1992

Basophil activation concomitant with delayed anaphylaxis to mammalian meat



Commins SC et al. J Allergy Clin Immunol 2014

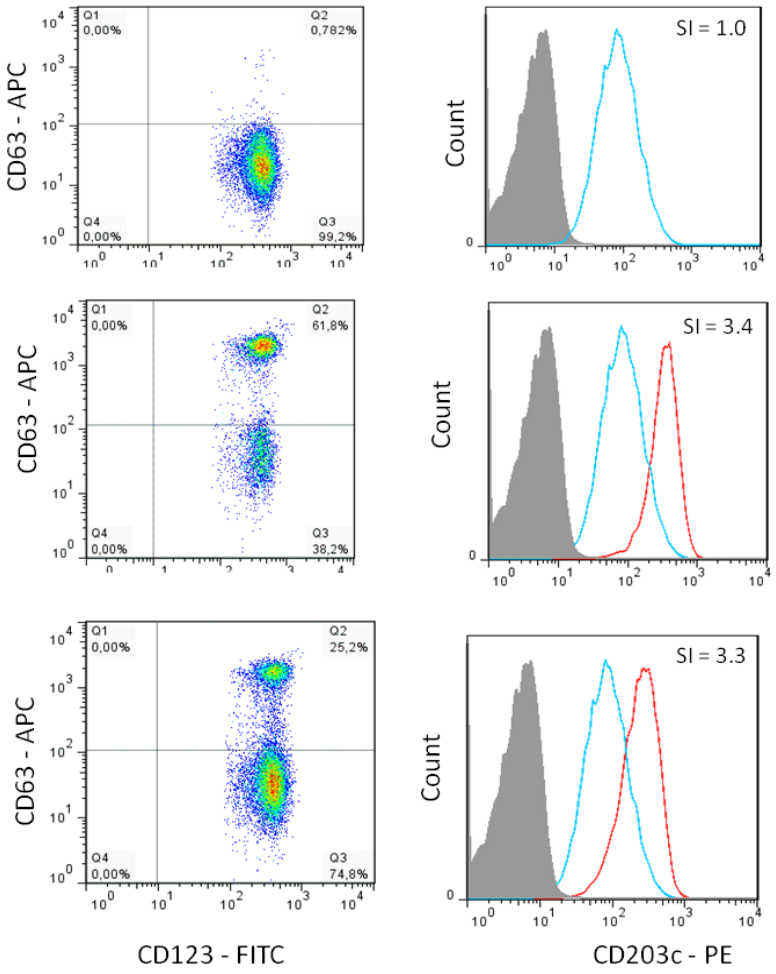
Basophil activation testing using flow cytometry

Basophil identification markers

- CD123/HLA-DR
- CD203c
- CCR3
- CRTH2 / CD3
- IgE or FceRI

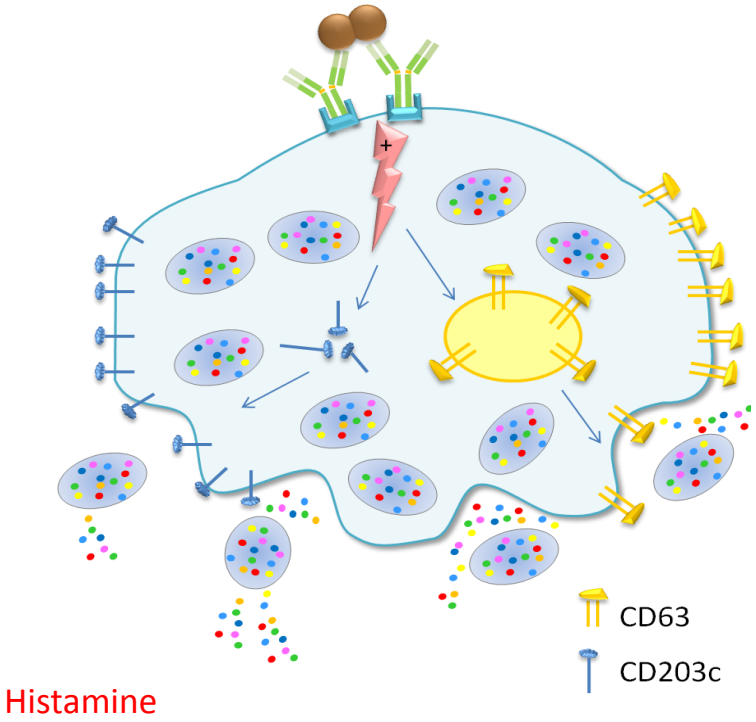
Basophil activation markers

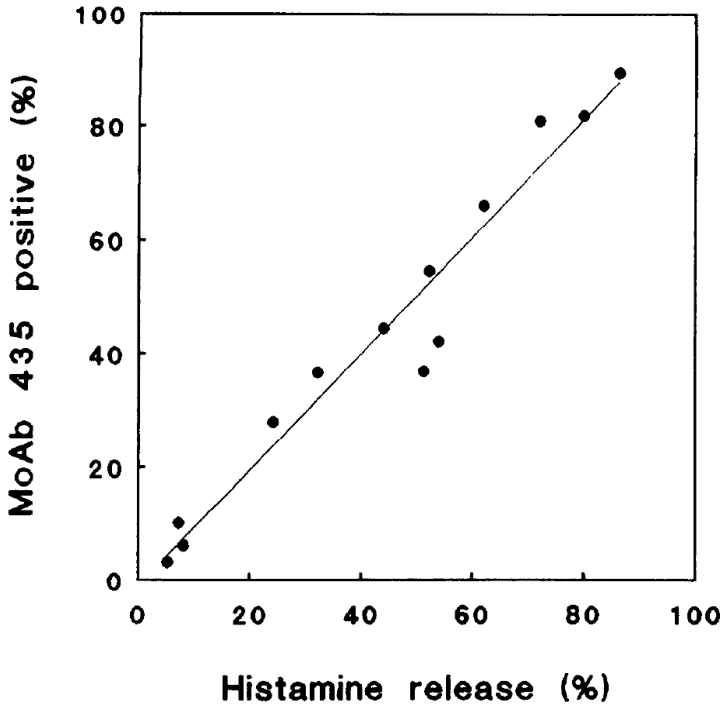
- CD63
- CD107a
- CD203c
- CD164
- CD11b
- CD13
- CD69



MacGlashan DW Jr, J Allergy Clin Immunol 2013
Santos AF & Lack G. Clin Transl Allergy 2016
Santos AF & Shreffler WG. Clin Exp Allergy 2017

CD63 expression correlates with histamine release





Knol EF et al. J Allergy Clin Immunol 1991

BAT to diagnose food allergy

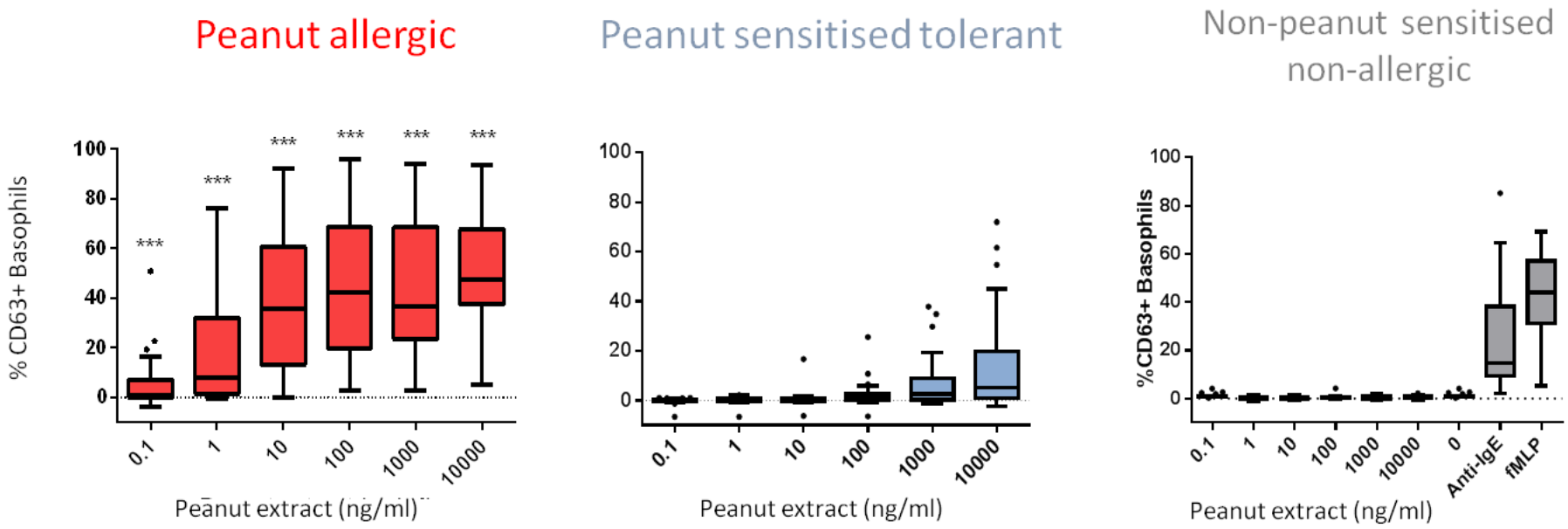
Food allergy	Food extract or allergen component	Study	Cut-offs	Sensitivity	Specificity
Cow's milk allergy	Cow's milk extract	Rubio (2011)[68]	>6% CD63+	91%	90%
		Sato (2010)[46]	SI CD203c ≥1.9	89%	83%
	Casein	Sato (2010)[46]	SI CD203c ≥1.3[46]	67%	71%
Egg allergy	Ovalbumin	Ocmant (2009)[48]	≥5% CD63+	77% for CD63	100% for CD63
			SI CD203c ≥1.6	63% for CD203c	96% for CD203c
Baked egg allergy	Egg white extract	Sato (2010)[46]	SI CD203c ≥2.4	74%	62%
	Ovomucoid		SI CD203c ≥1.7	80%	73%
Raw egg allergy	Egg white extract	Sato (2010)[46]	SI CD203c ≥1.7	77%	63%
	Ovomucoid		SI CD203c ≥1.6	83%	83%
Wheat allergy	Wheat extract	Tokuda (2009)[50]	>11.1% CD203c+	86%	58%
	Omega-5 gliadin (nTri a 19)		>14.4% CD203c+	86%	58%
	Omega-5 gliadin (rTri a 19)		>7.9% CD203c+	83%	63%
Peanut allergy	Peanut extract	Santos (2014)[4]	≥4.78% CD63+	98%	96%
	Ara h 2	Glaumann (2012)[22]	ND	92%	77%
Hazelnut allergy	Hazelnut extract	Brandstrom (2015)[58]	CD-sens >1.7	100%	97%
PFAS to hazelnut		Erdmann (2003)[65]	≥6.7% CD63+	85%	80%
Peach allergy	Peach extract	Gamboa (2007)[62]	>20% CD63+SI CD63 >2	87%	69%
	Pru p 3		>20% CD63+SI CD63 >2	77%	97%
PFAS to apple	Apple extract	Ebo (2005)[64]	≥17% CD63+	88%	75%
PFAS to carrot	Carrot	Erdmann (2003)[65]	≥8.9% CD63+[65]	85%	85%
PFAS to celery	Celery	Erdmann (2003)[65]	≥6.3% CD63+[65]	85%	80%

SI, stimulation index; PFAS, pollen-food syndrome; ND, not determined.

Santos AF & Shreffler WG. Clin Exp Allergy 2017

Santos AF & Brough HA. J Allergy Clin Immunol 2017

Basophil activation test discriminates between peanut allergy and tolerance



N=104 (12 NR)

*** p<0.001

** p<0.05

Santos AF et al J Allergy Clin Immunol 2014

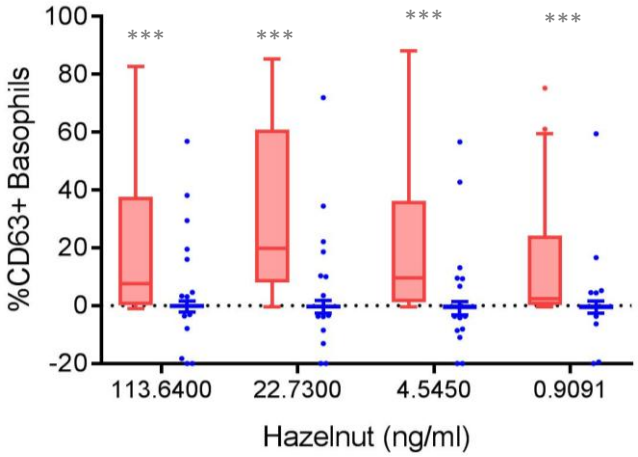
Basophil activation test discriminates between peanut allergy and tolerance

Optimal cut-off	Primary population n=104 (43 PA, 36 PS, 25 NA)	Validation population n=65 (25 PA, 24 PS, 16 NA)
Sensitivity (%)	97.6% (87.4; 99.9)	83.3% (74.0; 92.7)
Specificity (%)	96.0% (86.3; 99.5)	100.0% (100.0; 100.0)
PPV (%)	95.3% (84.2; 99.4)	100.0% (100.0; 100.0)
NPV (%)	98.0% (89.1; 99.9)	90.2% (82.8; 97.7)
Accuracy (%)	96.7% (93.1; 100)	93.4% (87.2; 99.7)

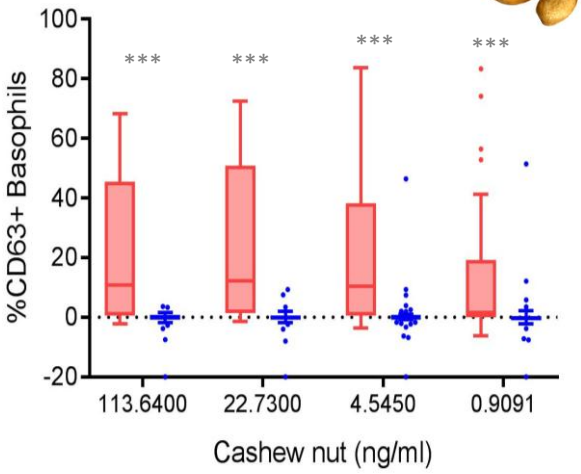
Data taken from: Santos AF et al J Allergy Clin Immunol 2014

BAT to tree nuts

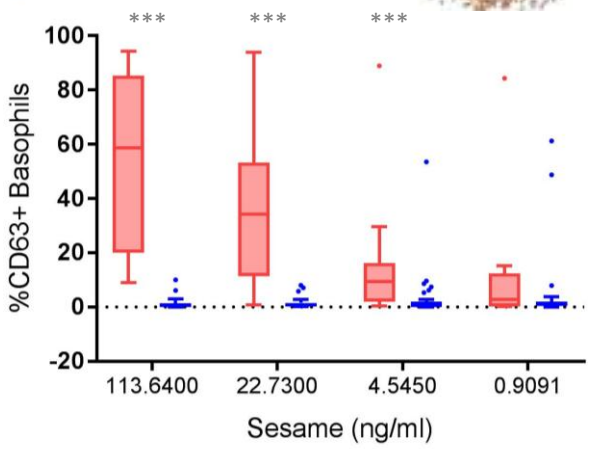
Hazelnut



Cashew nut



Sesame



Allergic
Non allergic

***p<0.001

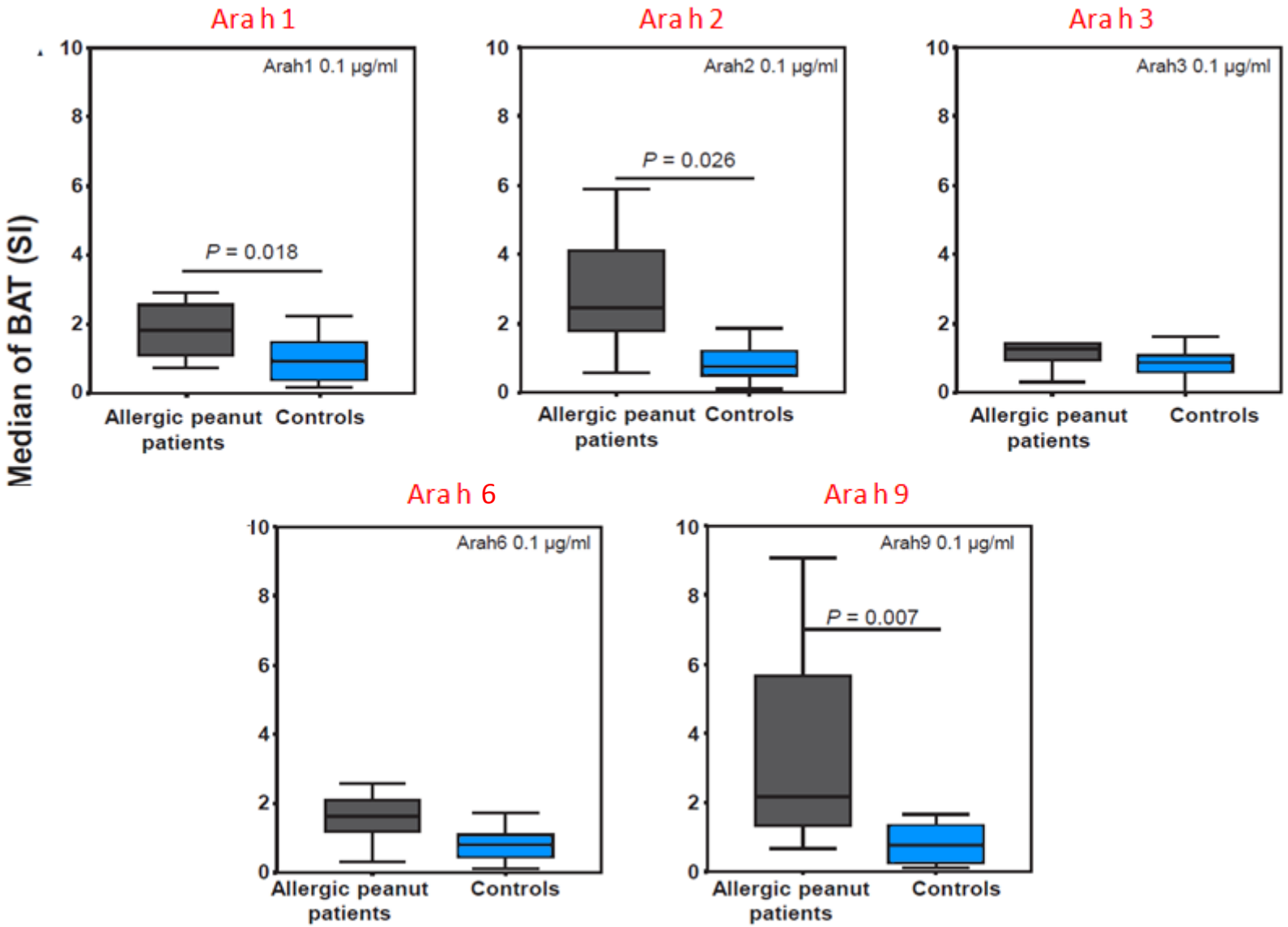
Santos AF et al, unpublished

BAT using extracts or single allergens to diagnose food allergy

Extract or allergen	Author, year	Cut-offs	Sensitivity	Specificity	PPV	NPV
Cow's milk	Sato 2010	SI CD203c ≥1.9	89%	83%	86%	86%
	Rubio 2011	>6% CD63+ (resolution)	91%	90%	81%	96%
Casein	Sato 2010	SI CD203c ≥1.3	67%	71%	74%	63%
Egg white	Sato 2010	SI CD203c ≥2.4 (baked egg allergy)	74%	62%	85%	44%
	Sato 2010	SI CD203c ≥1.7 (raw egg allergy)	77%	63%	92%	33%
Ovomucoid	Sato 2010	SI CD203c ≥1.7 (baked egg allergy)	80%	73%	90%	53%
	Sato 2010	SI CD203c ≥1.6 (raw egg allergy)	83%	83%	97%	42%
Ovalbumin	Ocmant 2009	≥5% CD63+ or SI CD203c ≥1.6 (egg allergy)	77% (CD63) 63% (CD203c)	100% (CD63) 96% (CD203c)	-	-
Wheat	Tokuda 2009	>11.1% CD203c+ (wheat allergy)	86%	58%	77%	71%
Omega-5 gliadin	Tokuda 2009	nTri a 19: >14.4% CD203c+ (wheat allergy)	86%	58%	77%	71%
	Tokuda 2009	rTri a 19: >7.9% CD203c+ (wheat allergy)	83%	63%	81%	67%
Peach	Gamboa 2007	>20% CD63+ and SI CD63 >2	87%	69%	-	-
Pru p 3	Gamboa 2007	>20% CD63+ and SI CD63 >2	77%	97%	-	-

Santos AF et al J Allergy Clin Immunol In Pract 2017

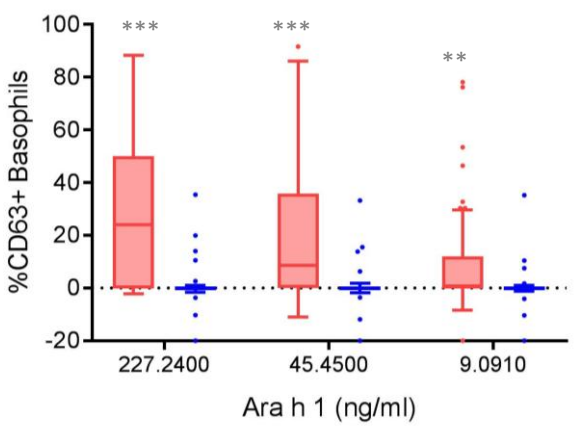
BAT using single allergens



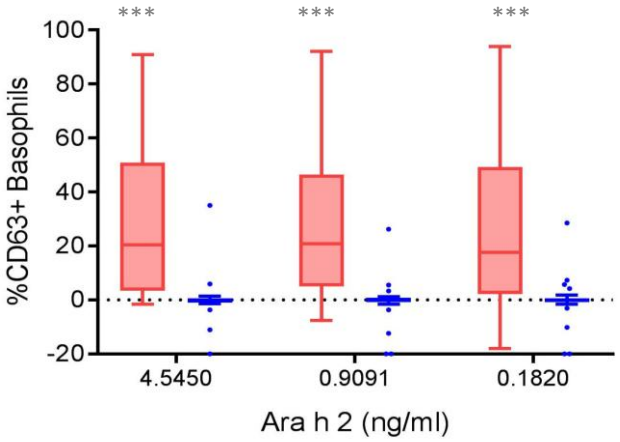
Mayorga C et al, Allergy 2014

BAT using single peanut allergens

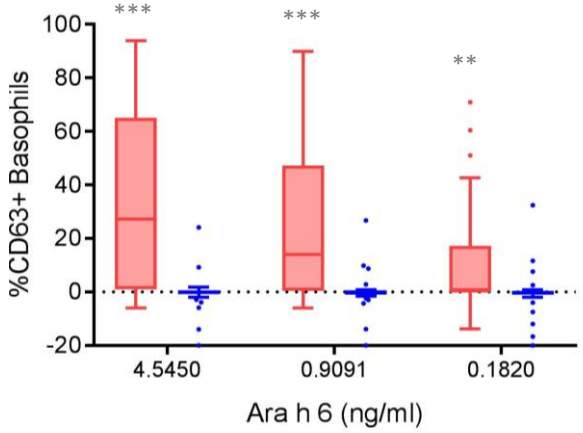
Ara h 1



Ara h 2



Ara h 6



Allergic
Non allergic

***p<0.001

Santos AF et al, unpublished

Patient candidates for BAT to foods

Likelihood of clinical allergy from history		Likelihood of clinical allergy from specific IgE or SPT results		
		Low (<0.35 KU/L or <3 mm)	Intermediate (0.35 to <15 KU/L or 3 to <8 mm)	**High** (≥15 KU/L or ≥8 mm)
	High	Possible allergy	Probable allergy	Allergy
	Intermediate	Possible allergy	Possible allergy	Probable allergy
	Low	No allergy	Possible allergy	Possible allergy

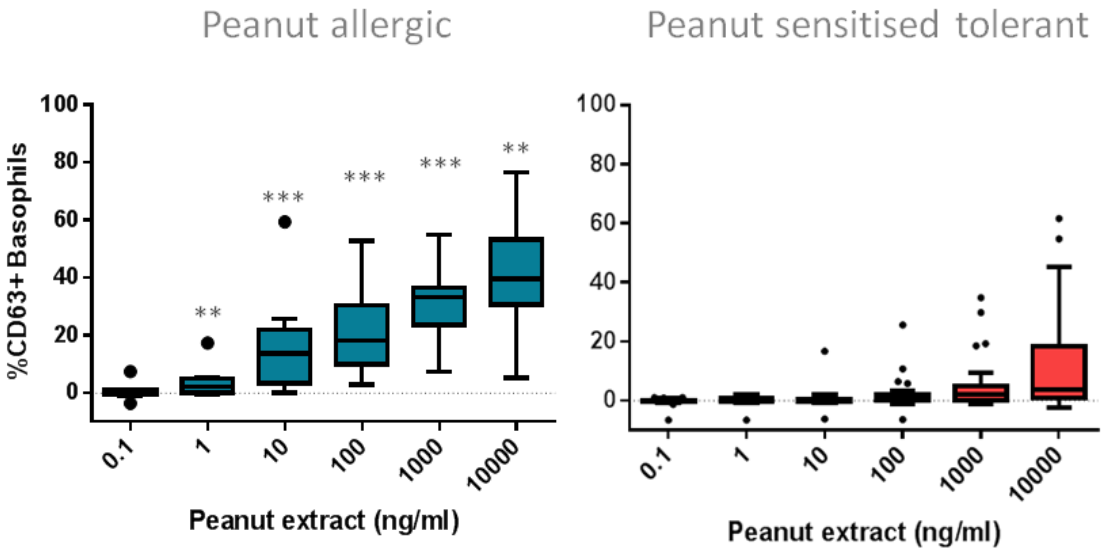
Stiefel G & Roberts G. Arch Dis Child Educ Pract Ed 2012

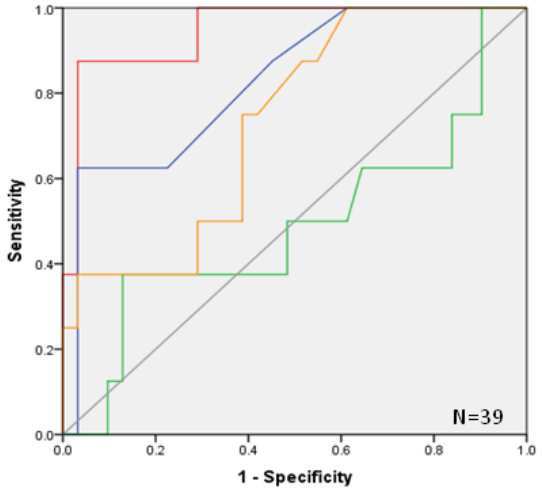
Sensitised patients with equivocal allergy test results

	Peanut allergic	Peanut sensitized but tolerant	p value
Age (years)	5 (2-6)	4 (0.5-13)	0.964
Oral exposure to peanut - n (%)	0 (0%)	7 (19.5%)	0.618
SPT to peanut (mm)	7 (2-9)	2 (0-12)	0.002
Peanut-sIgE (KU_A/L)	0.94 (0.14, 14.5)	0.81 (0.01, 35.7)	0.964
Ara h 1-sIgE (KU_A/L)	0.03 (0.01, 8.67)	0.06 (0, 3.79)	0.622
Ara h 2-sIgE (KU_A/L)	0.15 (0.05, 8.95)	0.06 (0.01, 1.84)	0.023
Ara h 3-sIgE (KU_A/L)	0.01 (0.01, 1.62)	0.05 (0, 1.36)	0.189
Ara h 8-sIgE (KU_A/L)	0.01 (0.01, 4.66)	0.01 (0, 35.8)	0.893
Ara h 9-sIgE (KU_A/L)	0.01 (0.01, 0.28)	0.02 (0, 11.0)	0.823
	N=8	N=36	

Santos AF et al, J Allergy Clin Immunol 2014

BAT discriminates between peanut allergy and tolerance in peanut-sensitised patients with equivocal allergy tests





	AUC	Accuracy
BAT	0.95 (0.87, 1.0)	92%
SPT	0.83 (0.67, 0.98)	82%
PsIgE	0.49 (0.24, 0.74)	63%
Ara h 2	0.73 (0.55, 0.91)	76%

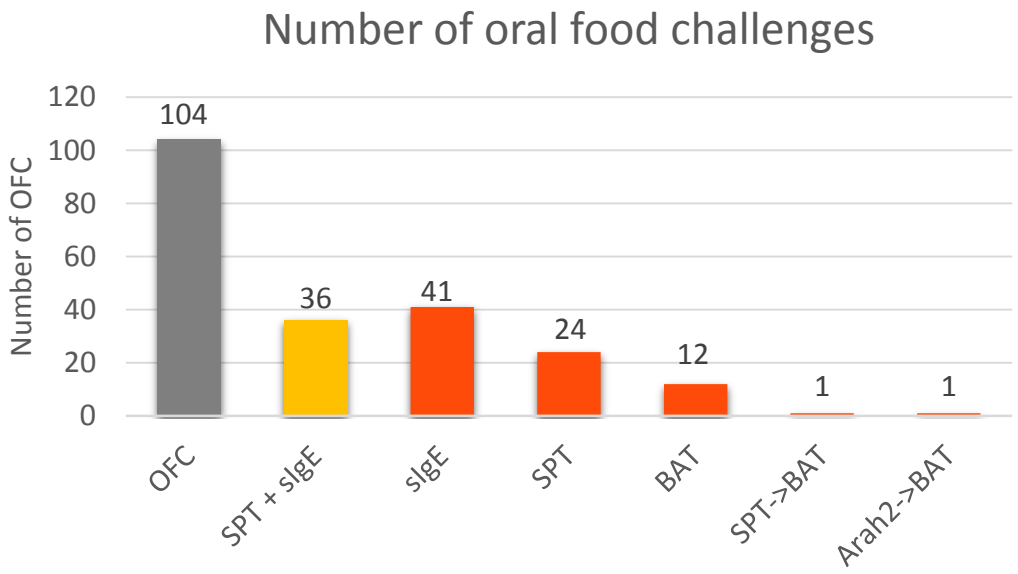
N=44

*** p<0.001

** p<0.01:

Santos AF et al. J Allergy Clin Immunol 2014

Sequential use of allergy tests to diagnose food allergy



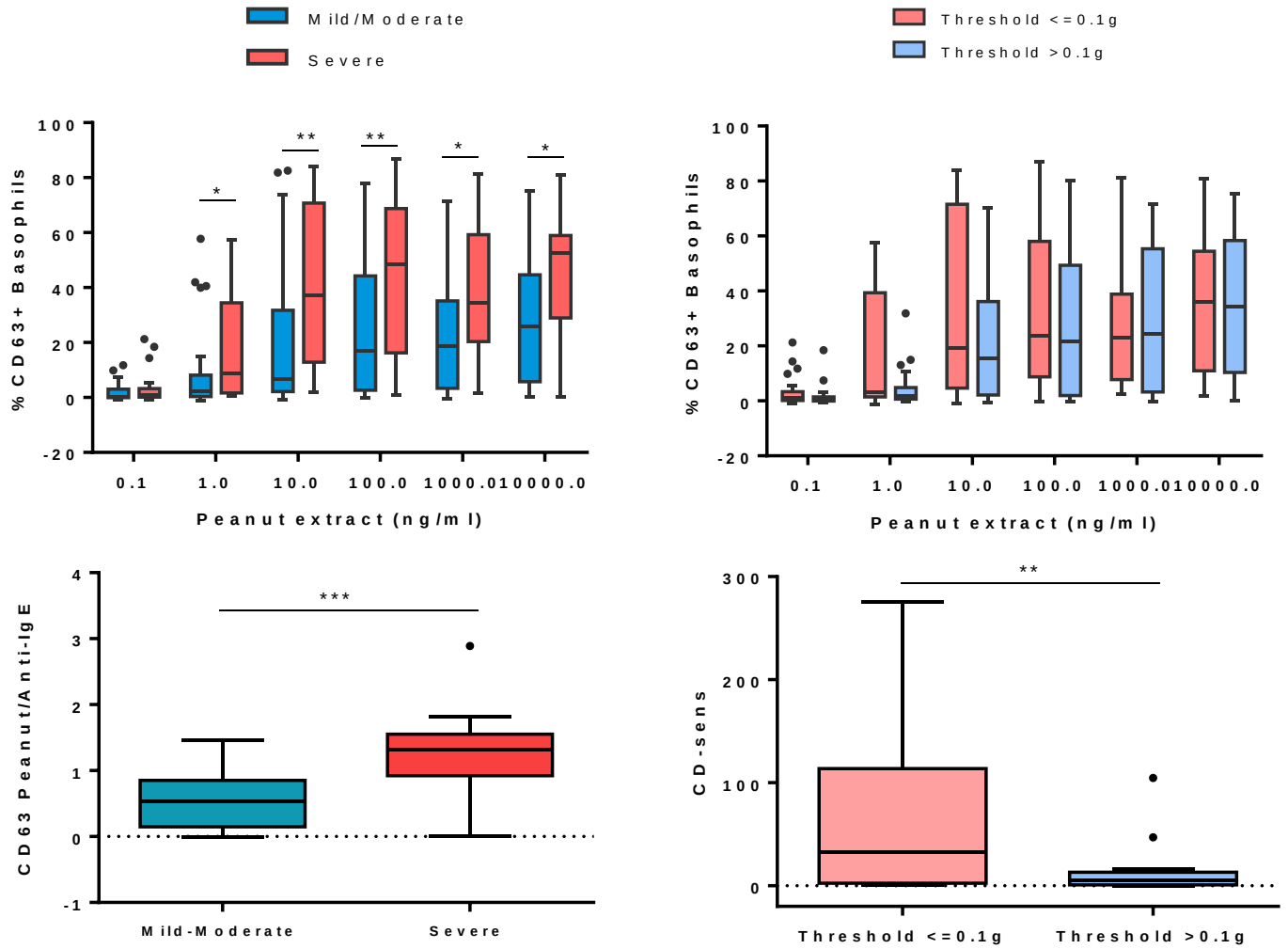
BAT as a second step in the diagnostic process	Correct Diagnoses	False positives	False negatives	Number of BAT	Change in OFC
SPT + Specific IgE	*67 (64%)*	*1 (1%)*	*0 (0%)*	*0 (0%)*	*0 (0%)*
SPT → BAT	98 (94%)	3 (3%)	2 (2%)	24 (23%)	-35 (-97%)
Specific IgE → BAT	93 (89%)	5 (5%)	3 (3%)	41 (39%)	-33 (-92%)
Ara h 2 → BAT	99 (95%)	2 (2%)	2 (2%)	19 (18%)	-35 (-97%)

Santos AF et al. J Allergy Clin Immunol 2014

Suggested approach for using BAT to diagnose food allergy

Santos AF & Lack G, Clin Transl Allergy 2016

BAT reflects the severity and the threshold of allergic reactions

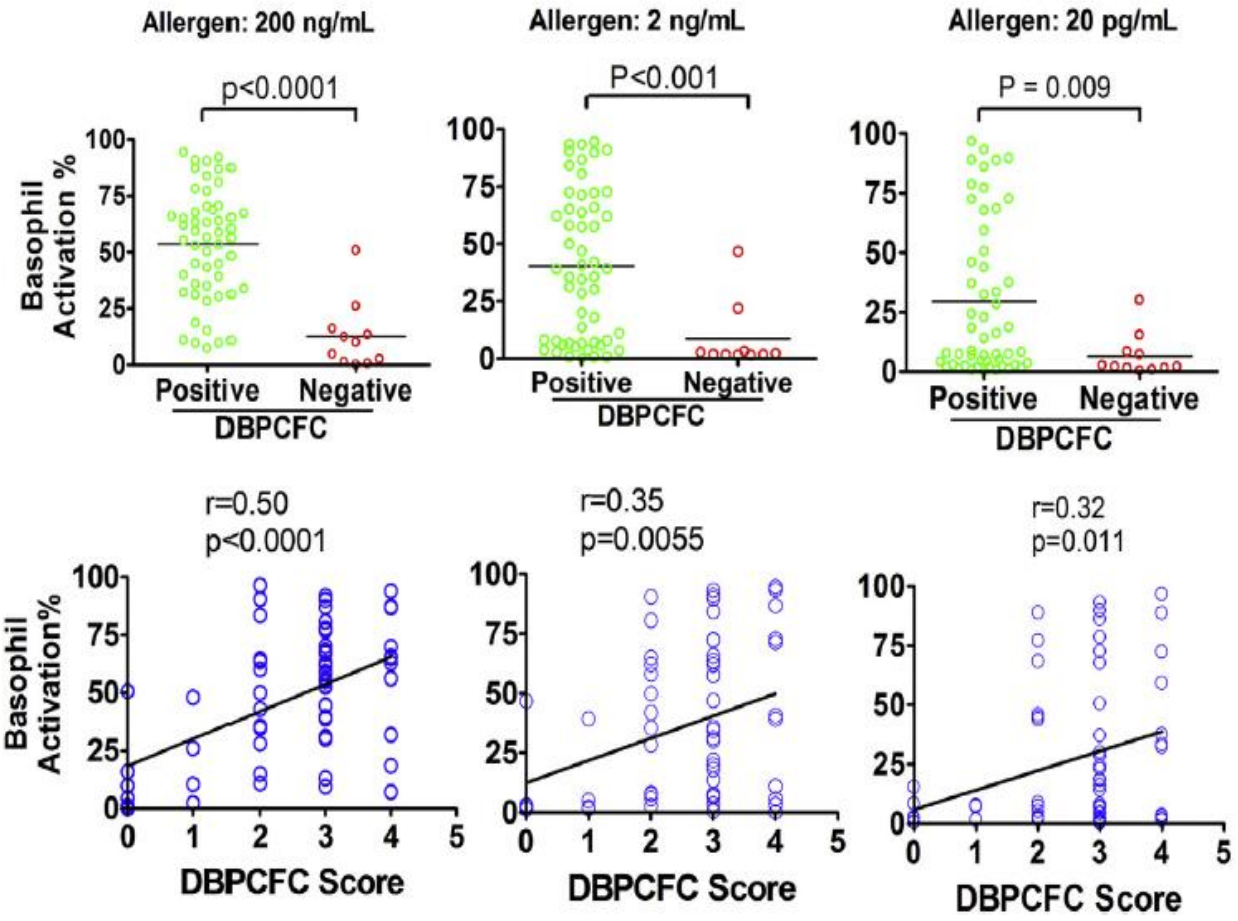


N=52 (3 NR)

***p<0.001 **p<0.01 * p<0.05

Santos AF et al, J Allergy Clin Immunol 2015

BAT reflects the severity and the threshold of allergic reactions

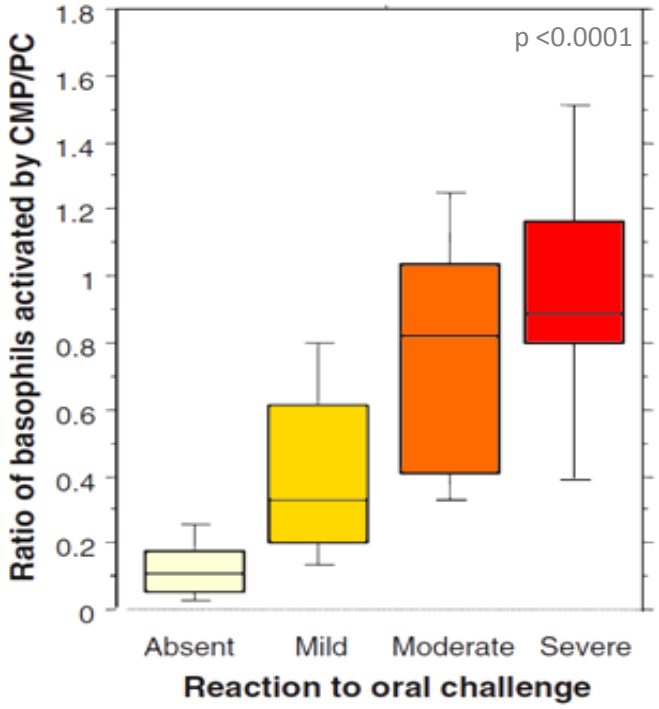


N=71

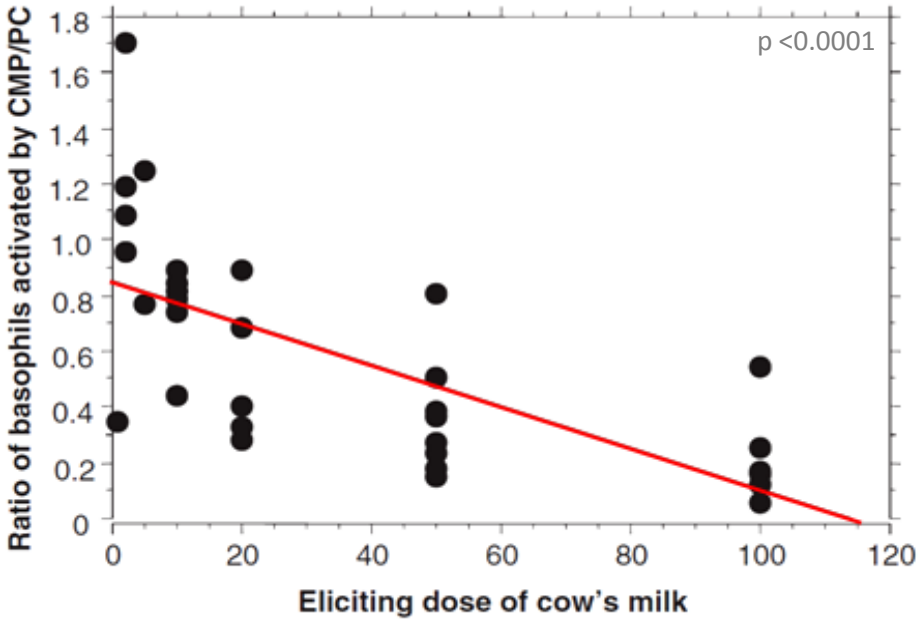
Song Y et al, Ann Allergy Asthma Immunol 2015

BAT reflects the severity and the threshold of allergic reactions

Severity



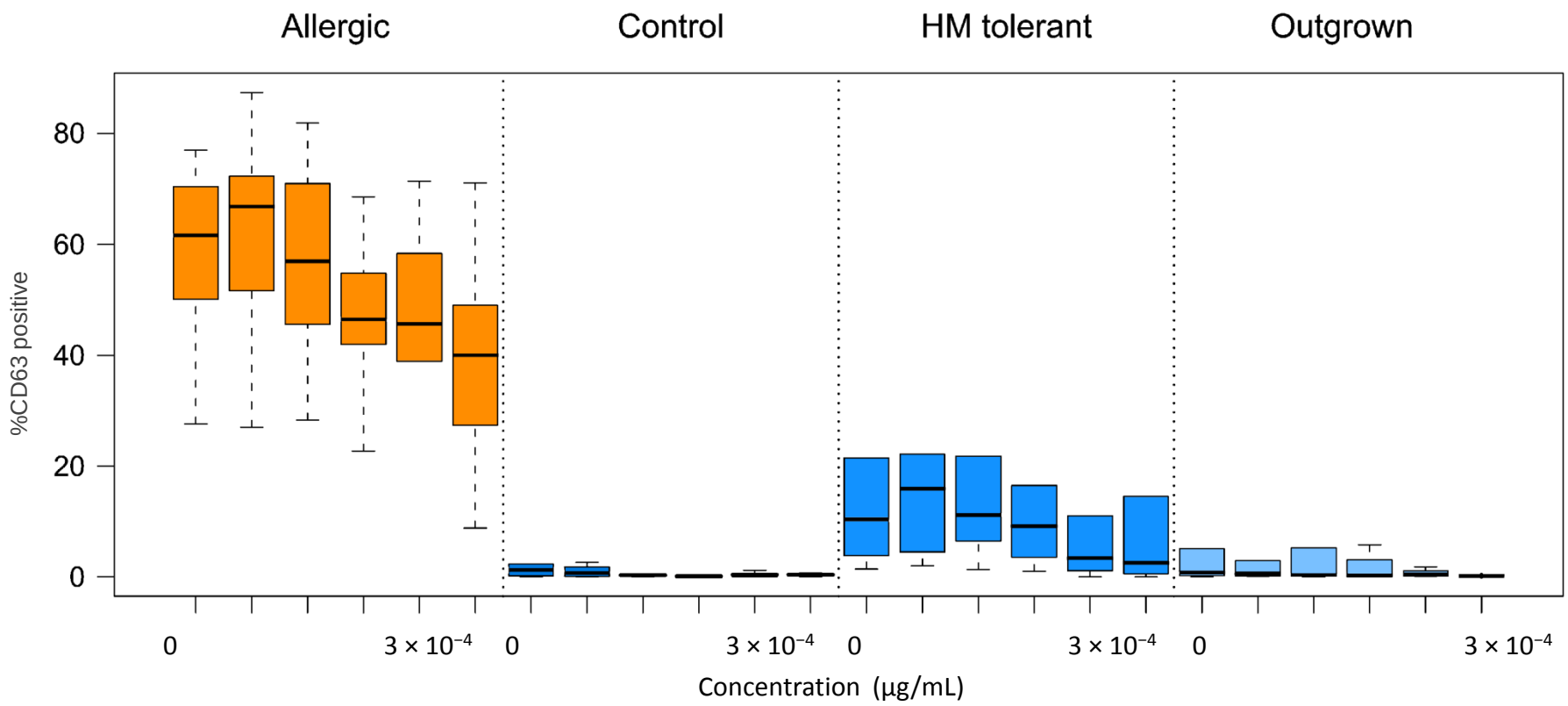
Threshold



N=112

Rubio A et al, Allergy 2011

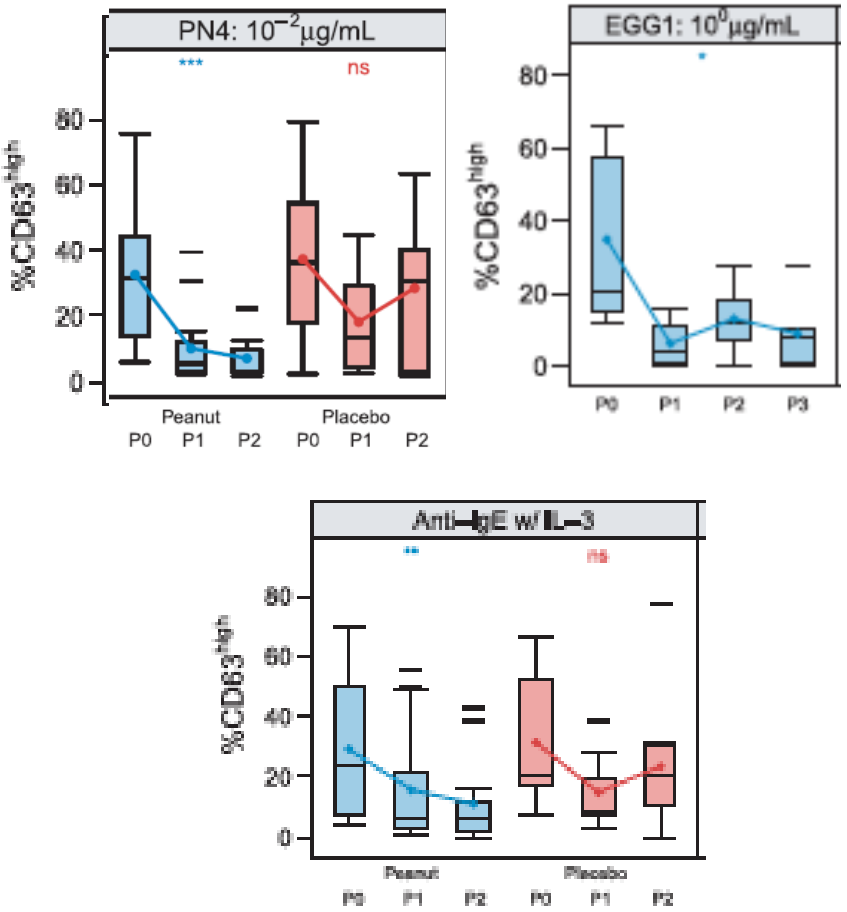
Monitoring natural resolution of food allergy



Wanich N et al, J Allergy Clin Immunol 2009

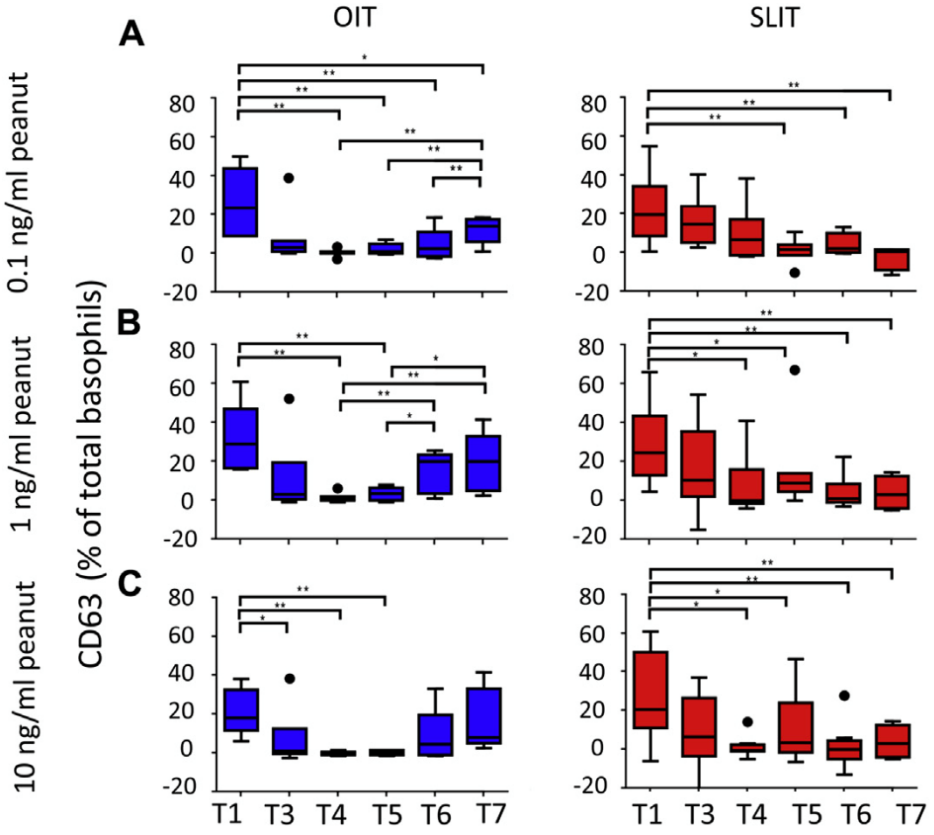
Basophil suppression with allergen immunotherapy

…is observed to culprit and bystander allergen and anti-IgE during OIT



Thyagarajan A et al, Clin Exp Allergy 2012

…is more marked with OIT than with SLIT and is often transient

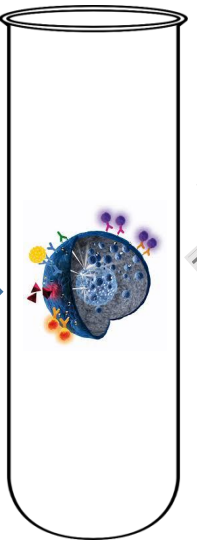


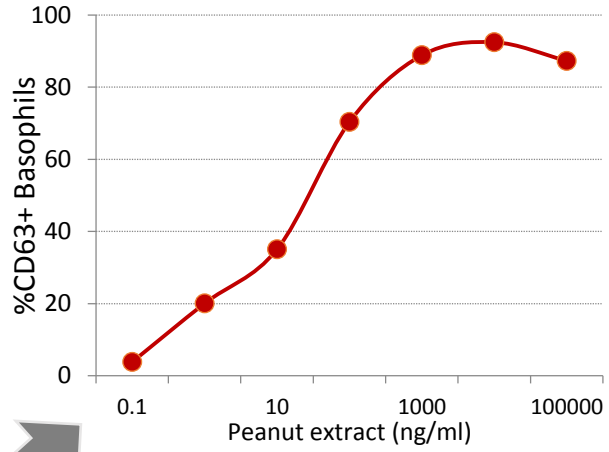
Gorelik M et al, J Allergy Clin Immunol 2015

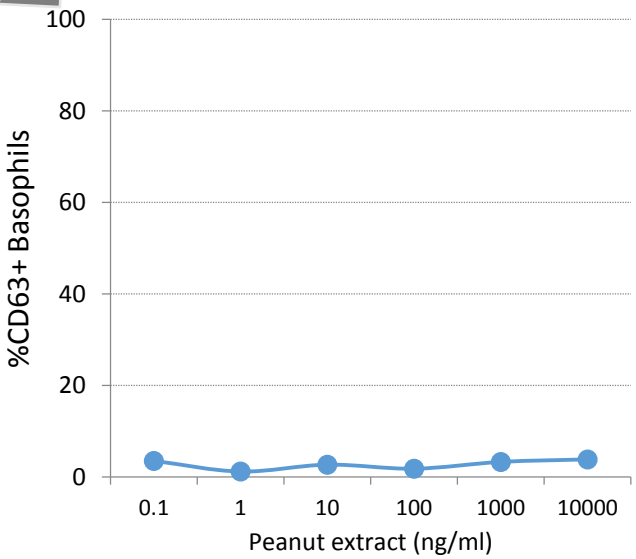
Outline

- Diagnosis of food allergy
- Utility of BAT in food allergy
- Bringing BAT to clinical practice

Equivocal cases after SPT sIgE







Factors influencing the cut-offs and results of BAT

Study population

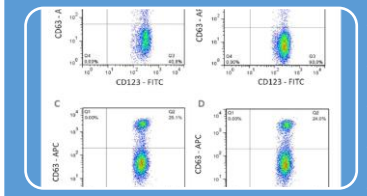
- Prevalence of food allergy
- Origin: general population versus specialised clinic
- Geographical location
- Associated respiratory and food allergies

Study design

- Inclusion criteria (e.g. sensitised/non-sensitised patients)
- Reference standard
- Criteria to refer patients for oral food challenges
- Oral food challenge protocol

BAT Procedure

- Allergen extracts: quality, concentration, stability, standardisation
- Interval between blood collection and performance of BAT
- Pre-incubation with IL-3
- Markers and antibodies used for staining ID and activation markers

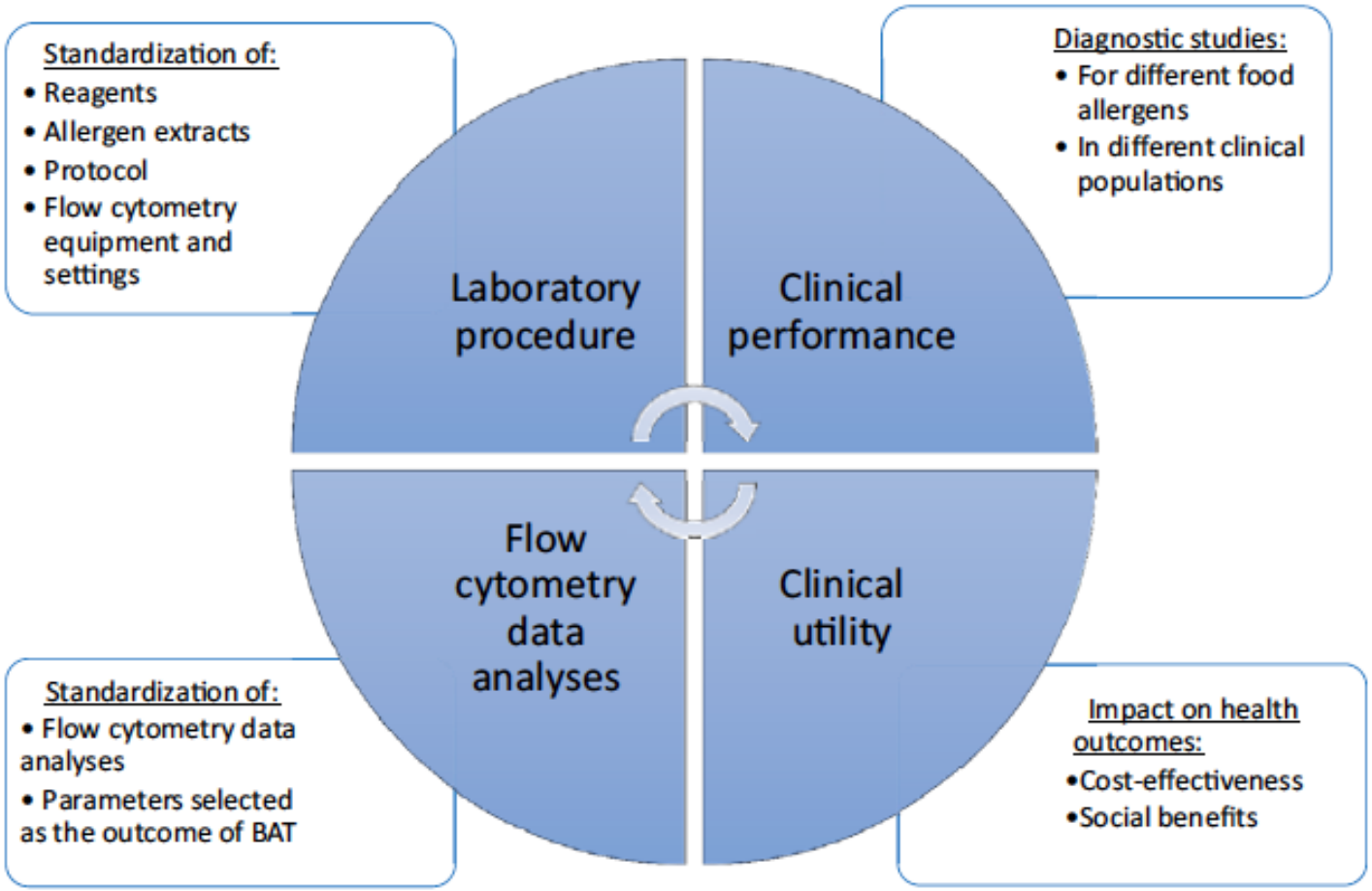


Flow cytometry data analyses

- Cytometer and application settings
- Gating strategy
- Parameters used as outcomes of the test
- Definition of negative gate
- Whether results were corrected for background

Santos AF et al, Clin Transl Allergy 2016

Road map to bring BAT to clinical practice



Santos AF & Shreffler WG. Clin Exp Allergy 2017

Take-home messages:

- BAT has high specificity and sensitivity to diagnose food allergy and can be used to monitor clinical response to treatment and possible resolution of food allergy.
- A positive BAT confirms the diagnosis of food allergy and averts OFC (mainly positive OFC). Patients with a negative BAT or non-responder basophils still require OFC.
- In order to make the transition to clinical practice, standardisation of the laboratory procedure, flow cytometry and data analyses is required and rigorous clinical validation and an assessment of the impact of BAT on health and social outcomes and its cost-effectiveness are warranted.

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Bühlmann Laboratories

Paed Allergy Lab

BRC Flow Core Lab @GSTT