

Update on food allergy: new biomarkers

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Declaration

- 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

New food allergy biomarkers

- Peptide-sIgE methods
- Functional assays
 - Basophil activation test
 - Mast cell activation test



Peptide-sIgE methods

- Spot membranes
 - Microarrays
 - Luminex-based assays (LBA)
 - Bead-based epitope assay (BBEA)
-
- More commonly used for peanut, cow's milk although some have been used for shellfish and legumes

Microarray tests



Advantages

- Ability to test against many allergen components using little serum
 - Helpful in paediatrics if sample volume is a concern
- Helps define which components are causing sensitization
- Useful for complex allergy cases if multiple potential allergens are involved or concerns around cross-reactivities

Disadvantages

- Sensitization doesn't always equate to allergy – risk of false positives
- Cost and access to the tests

Luminex-based assays

Advantages

- Multiplexing – test many different allergens at once
- Small sample volumes needed
- Flexibility as can use panels with different bead sets for different allergens/allergenic components

Disadvantages

- Needs careful standardization
- Cross reactivity binding can occur
- Cost/availability

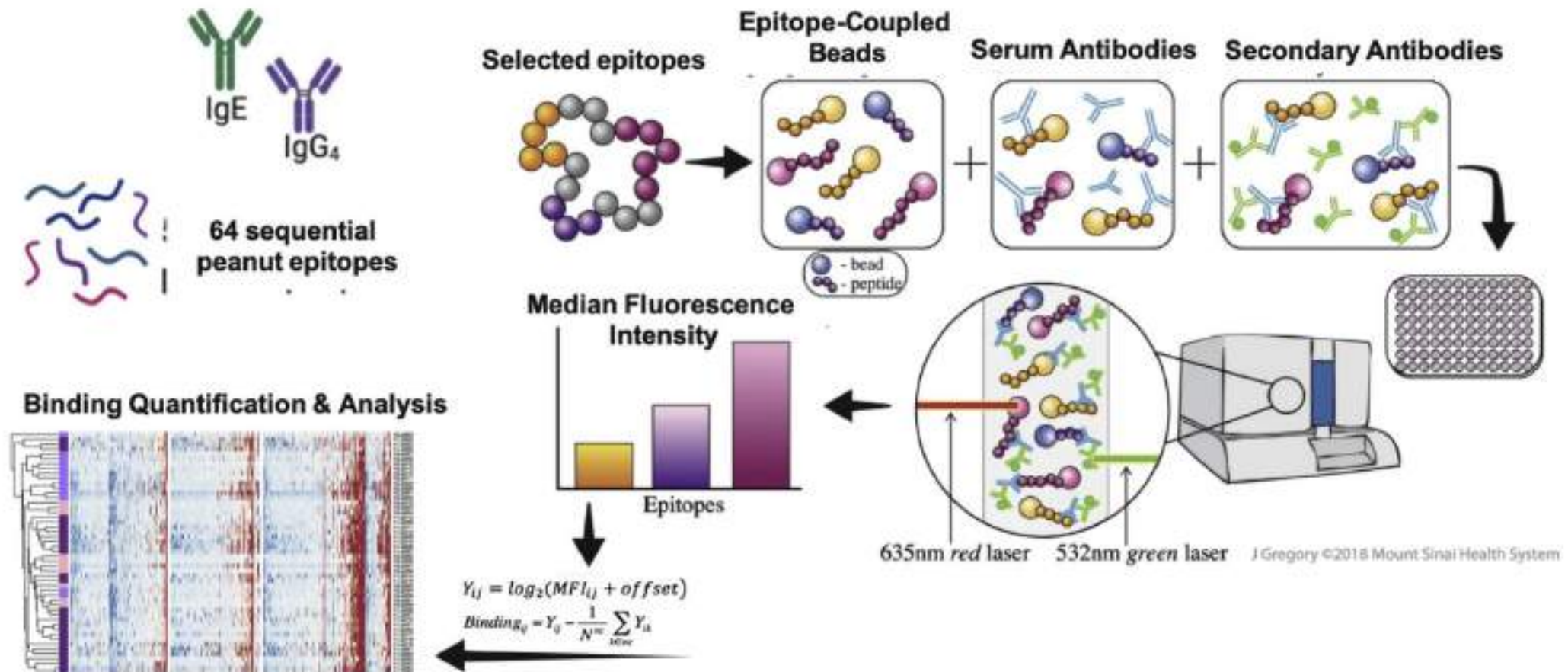


FIGURE 1. Bead-based epitope assay measuring epitope-specific IgE and IgG4.

BBEA

Advantages

- Microliter quantities of serum/plasma
- Ease of performance
- Reproducibility
- Greater degree of accuracy – diagnostic discrimination to IgE to whole allergen
- Potential to predict outcomes

Disadvantages

- Most allergenic epitope profiling has focused on milk and peanut allergy
- Requires knowledge of amino acid sequences of component protein allergenic epitopes comprising each individual food
- Need large cohorts to validate the assays
- Tested in isolation (without testing to whole allergen)
 - Risk of false-negative results

Cow's milk allergy (CMA)

- Caubet et al Allergy 2017:
 - Peptide microarray showed greater intensity and broader diversity of IgE binding in children with persistent CMA which decreased as children became tolerant
 - Tolerance associated with matched peptides recognized by IgE and IgG4
- Suarez-Farinas et al JACI 2018–
 - Milk OIT + omalizumab reduced IgE and increased IgG4 binding to milk peptides
 - Lower IgE levels and lower diversity to milk peptides at baseline were associated with sustained unresponsiveness after OIT to cow's milk
 - Models based on sIgE to key epitopes allowed prediction of response to treatment (OIT + omalizumab) with 87% accuracy



Peanut allergy



- Santos et al Allergy 2020
 - 7 key IgE epitope containing peptides from Ara h 1, Ara h 2 and Ara h 3 identified as discriminating peanut allergic from peanut sensitized but tolerant children
 - Peanut profilin Ara h 5 was bound more by IgE of tolerant children
 - 4 peptides from Ara h 2 diagnostically useful when combined with Ara h 2-sIgE (enhancing diagnostic accuracy)
- Suarez-Farinas et al 2025
 - Children who developed early PA that persists or those who develop later onset PA had increasing epitope-sIgE antibodies over time
 - Baseline epitope-sIgE in children sensitized to peanut in first year of life can predict likely persistent PA

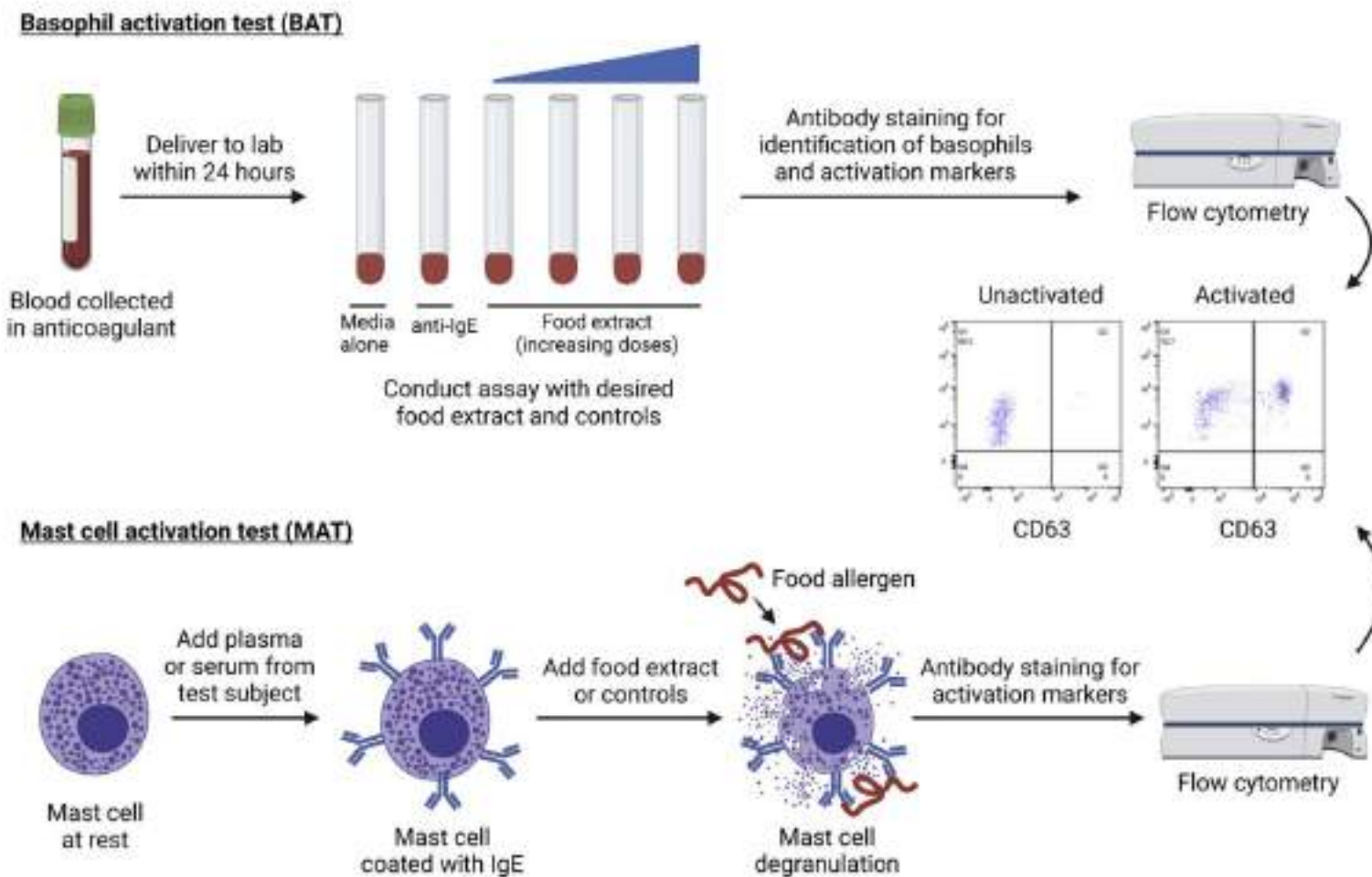


FIGURE 2. Overview of basophil activation test and mast cell activation test.

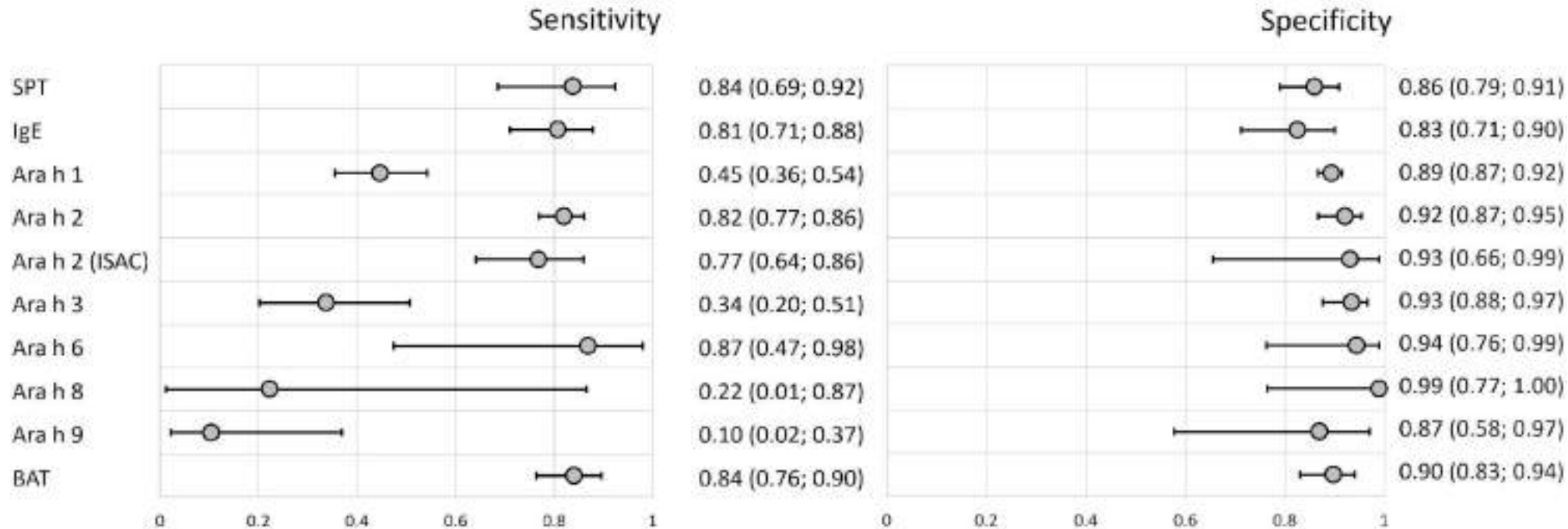
Basophil activation test (BAT)

- Functional assay that uses whole blood of which basophils are stimulated with allergen and analyzed by flow cytometry to detect the expression of activation markers
- Usually needs to be run within 24 hours of blood collection □ timing, transport requires standardisation
- BAT has high accuracy but 10-15% individuals have non-reactive basophils to non-IgE-mediated stimulants
- Measure – CD63 activation or CD203c

Peanut studies

- van Erp et al JACI 2017 – key findings:
 - Ara h 2-sIgE and Ara h 6-sIgE were similar in predicting the presence of peanut allergy
 - BAT to Ara h2 and h6 - was able to identify children with peanut allergy who were responsive to Ara h 6 but not Ara h 2
 - BAT takes into account concentration and affinity of sIgE and other blocking antibodies
 - BAT to components may have higher diagnostic accuracy than BAT to peanut

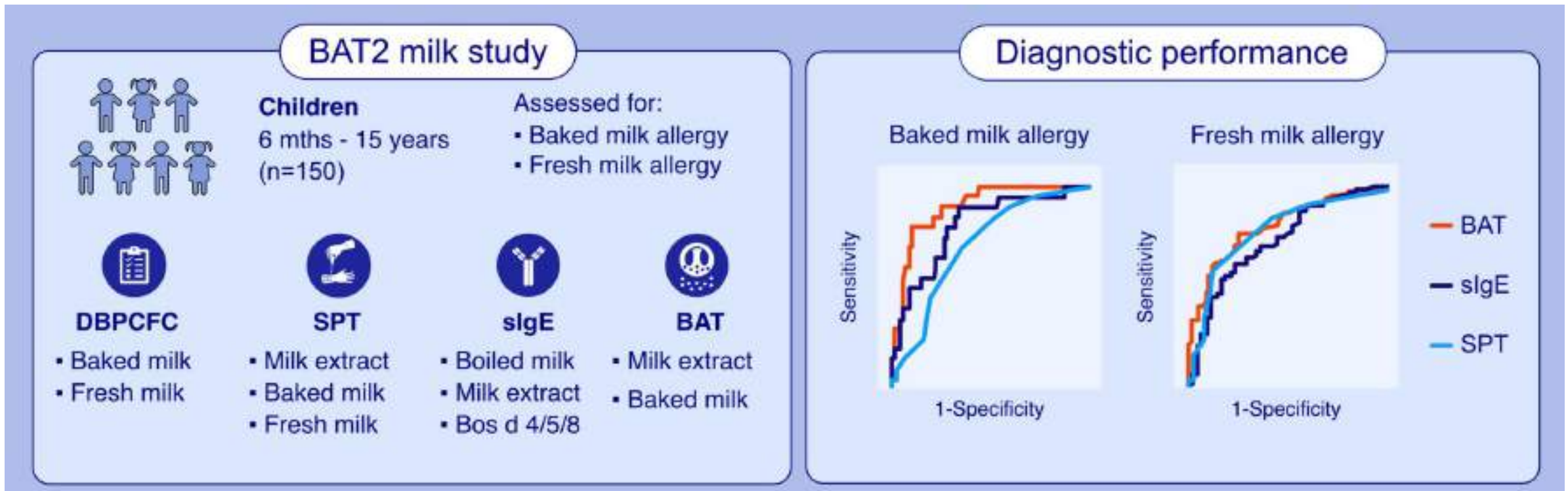
Peanut allergy



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

BAT to cow's milk

Bartha et al Allergy 2025



Diagnostic cutoffs:

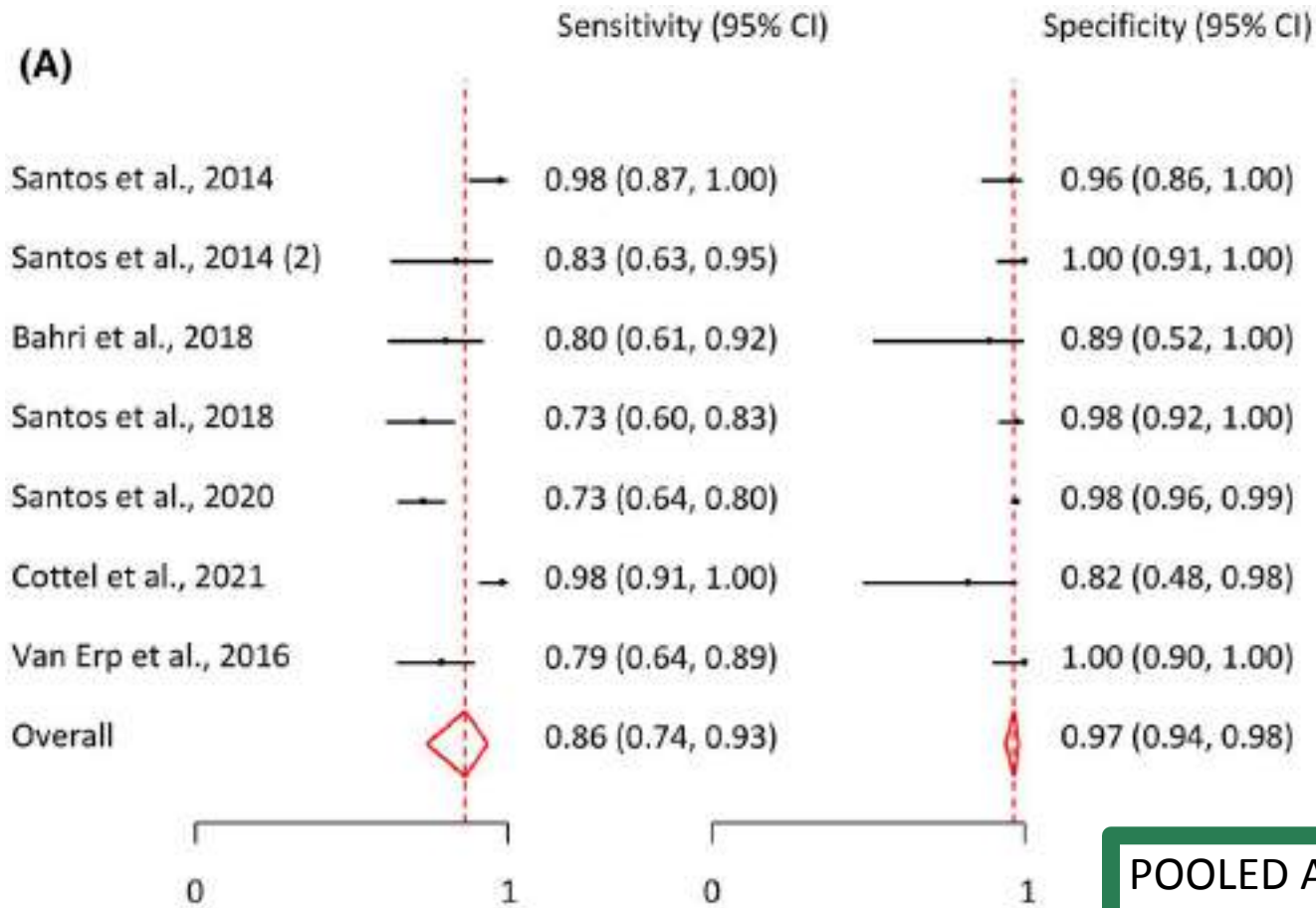
Baked milk – 11.1% CD63+ basophils (sensitivity 91%, specificity 78%)

Fresh milk – 3.57% CD63+ basophils (sensitivity 71%, specificity 76%)

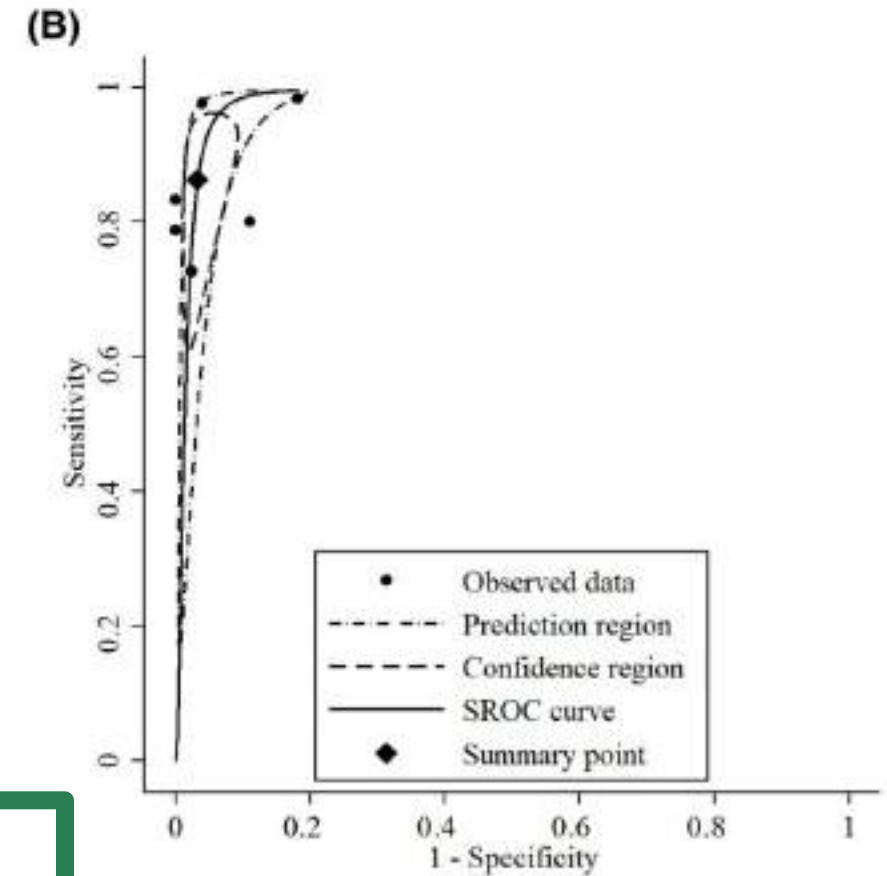
BAT -- Severity and threshold

Boyd et al Allergy 2025 (accepted, unpublished)

- BAT was the only biomarker able to distinguish severity and threshold groups for baked milk and fresh milk
 - Optimal cut-off for severity had 71% sensitivity and 100% specificity to identify severe reactions to baked milk (50.7% CD63+basophils)
 - Optimal cut-off for threshold identified children who were reacting to ≤ 0.143 g of fresh milk (1.12% CD63+ basophils) had 96% sensitivity, 41% specificity



POOLED ANALYSIS
 Sensitivity 0.86
 Specificity 0.97



BAT

ADVANTAGES

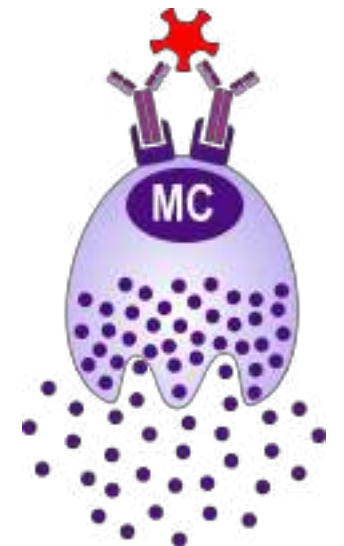
- High specificity and sensitivity
- High PPV and NPV
- In vitro test and thus no risk of allergic reactions/anaphylaxis
- Does not require stopping antihistamines or clear skin

DISADVANTAGES

- Must be performed within 24h of blood collection
- Requires flow cytometry
- 10-15% non-responders
- More expensive than sIgE or SPT

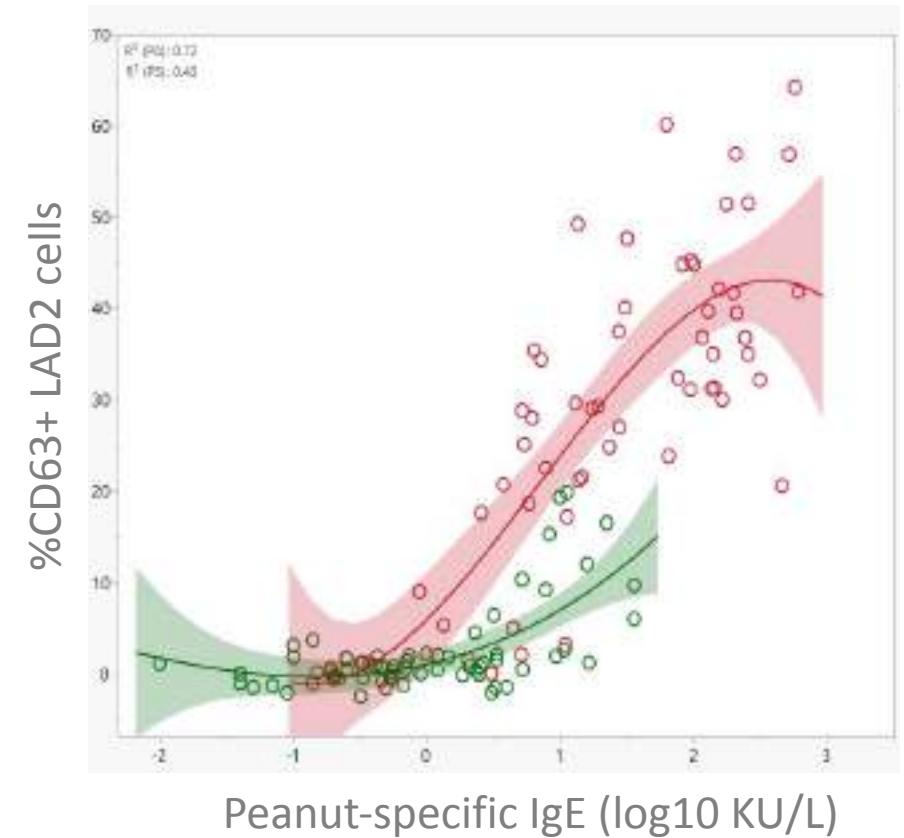
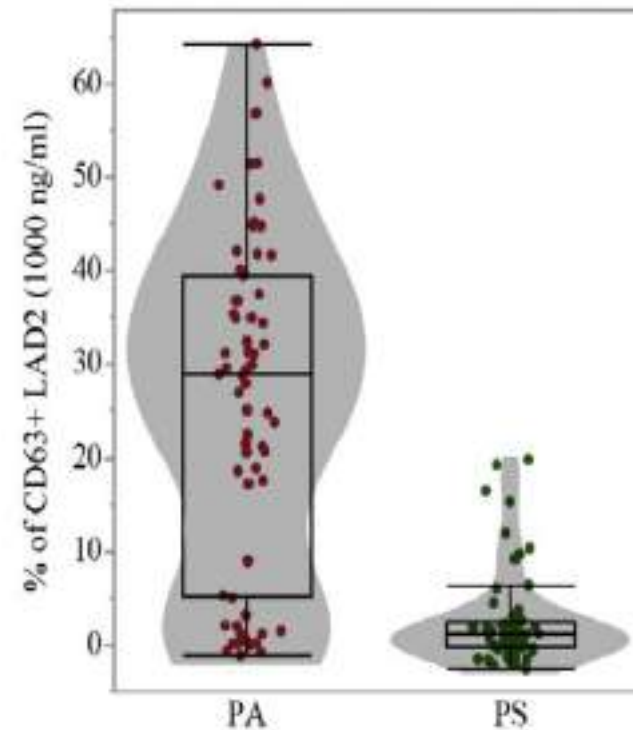
Mast cell activation test (MAT)

- MAT uses plasma or serum to sensitise mast cells (cell line or primary cells from peripheral blood/tissue), stimulated with allergen and analyzed by flow cytometry to detect expression of activation markers
- Can be run on older/frozen samples
- Measure – CD63 activation

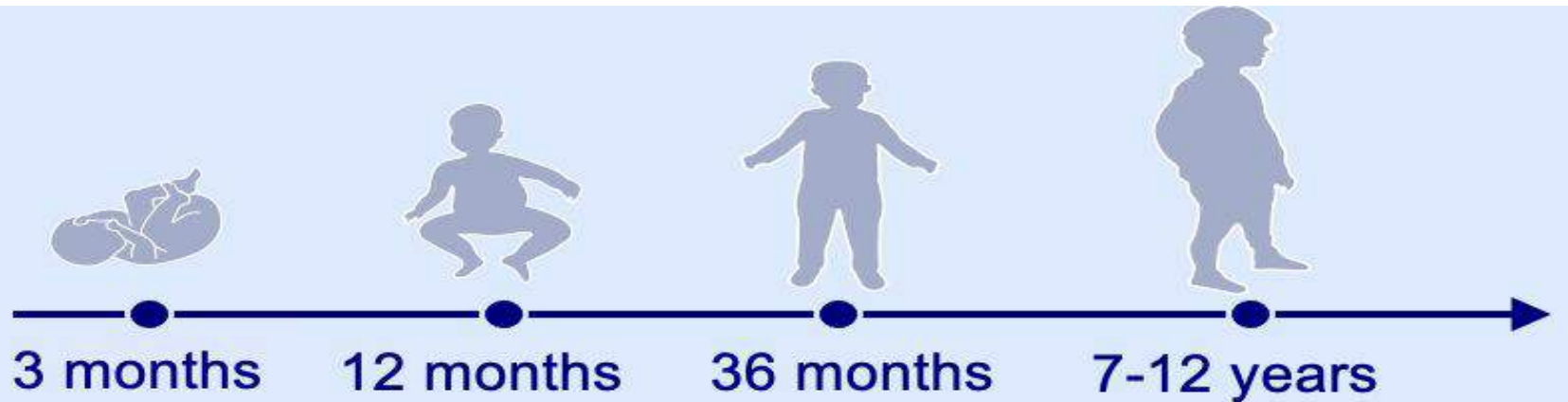


MAT distinguishes allergic from tolerant with similar IgE levels

Optimal cut-off	17.2% CD63+ LAD2 cells
N	N=174
Sensitivity	73%
Specificity	98%
PPV	96%
NPV	83%



Peanut allergy



%CD63+ LAD2 cell activation



Peanut allergic n=20	0.01%	0.7%	11.5%	12.2%
Peanut sensitized n=225	0.01%	0.01%	0.01%	0.01%

MAT

ADVANTAGES

- High specificity and PPV
- Uses plasma so can be performed from stored/frozen samples
- Provides result for individuals with non-responder basophils
- In vitro test and thus no risk of allergic reactions/anaphylaxis
- Does not require stopping antihistamines or clear skin

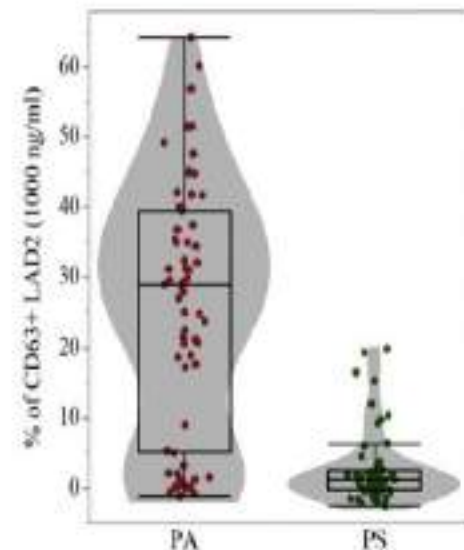
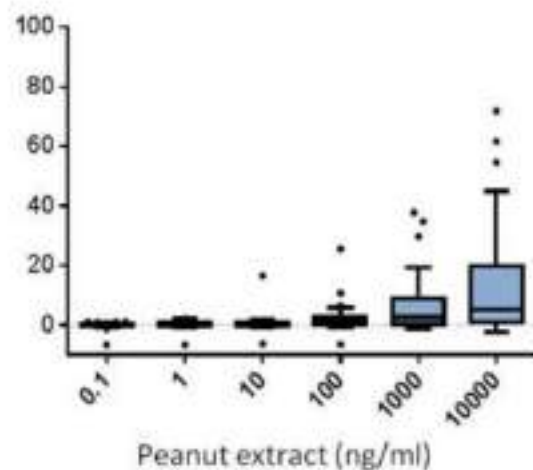
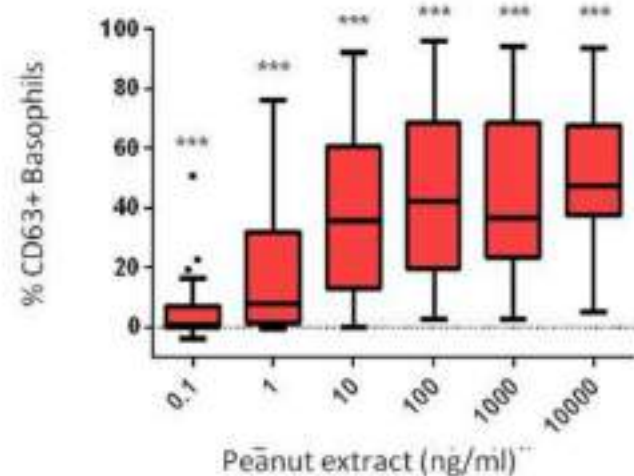
DISADVANTAGES

- Lower sensitivity and NPV than BAT
- More expensive than BAT, sIgE or SPT

TABLE I. Diagnostic performance of next-generation food allergy diagnostic tests

Diagnostic test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Epitope testing				
Bead-based epitope assay peanut ¹¹	92%	94%	91%	95%
Basophil activation test				
To peanut ^{12,13}	83% to 98%	96% to 100%	95% to 100%	83% to 98%
To cow's milk ¹⁴	91%	90%	81%	96%
To egg ¹⁵	90%	100%	100%	92%
Mast cell activation test to peanut ¹⁶	75%	99%	95%	92%

BAT and MAT accurately reflect the allergic status



Optimal cut-off	4.78% CD63+ Basophils	
N	N=104	N=65
Sensitivity	98%	83%
Specificity	96%	100%
PPV	95%	100%
NPV	98%	90%

Santos AF et al. J Allergy Clin Immunol 2014

Optimal cut-off	17.2% CD63+ LAD2 cells	
N	N=174	
Sensitivity	73%	
Specificity	98%	
PPV	96%	
NPV	83%	

Santos AF et al. J Allergy Clin Immunol 2018

Summary

- Increased utilization and research around Epitope testing, BAT and MAT for food allergies;
- Limitations – cost, time, availability, appropriateness
- Move towards making some of these tests more translational to clinical practice (i.e. BAT)
- Paving the way for better allergy diagnostics



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