Advance Diagnostic Testing In Food Allergy: What Really Works

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Matthew Greenhawt, MD, MBA, MSc
Assistant Professor of Pediatrics
Section of Allergy/Immunology
Co-director, Food Challenge and Research Unit
Children’s Hospital Colorado
University of Colorado School of Medicine
Disclosures

- Member, Joint Task Force on Allergy Practice Parameters
- Member of Nutricia specialty advisory board and have received honorarium from Nutricia for lectures
- Member of the medical advisory team for Kids With Food Allergies Foundation and the International Association for Food Protein Enterocolitis (non-financial)
- Former member Thermo Fishery Advisory Board regarding Allergen Component Testing
- Have received honorarium from Gerber/Nestle and Adamis Pharmaceuticals
- Consultant to Canadian Transport Agency, Aimmune, Intromune
- Receiving support from 1-K08-HS024599-01 (AHRQ, start date 5/1/16)
- Received support from NIH grants #2KL2TR000434 & UL1RR024986, private foundation (while at U of M)
- Member of AAAAI EGID, Anaphylaxis, Adverse Reaction to Food, Health Technologies and Joint Task Force on Quality Improvement Measures Committees
- Member ACAAI Conferences On-Line Allergy, Abstract, Practice Improvement, and Adverse Reaction to Food committees
- AAAAI/ACAAI advisor to CDC-ACIP on Egg Allergy/Influenza Vaccine Safety
- ACAAI representative to consensus statement on interim consensus on early peanut introduction guidelines
- Member, NIAID Expert Panel on early introduction of peanut to prevent peanut allergy
- Associate Editor, Annals of Allergy, Asthma, and Immunology
- Editorial board: Allergy and Rhinology; Medscape Pediatrics; Infectious Diseases in Children
- Former officer and legislative advocacy liaison Michigan Allergy and Asthma Society (2010-2015)
- Have testified to Michigan State Legislature on behalf of Michigan State Medical Society and Michigan Allergy and Asthma Society
- Member, Scientific Advisory Council, National Peanut Board
- Former Medical Advisory Chair/member, Food Allergy and Anaphylaxis Connection Team
Goals

- Understand the technology available to detect components
- Discuss potential applications where component testing may aid in clinical diagnosis and their limitations
- Discuss basophil activation testing
Current Testing Constraints

- Tests can’t distinguish sensitization vs. allergy
- All tests are “imperfect”
  -- Have high sensitivity, poor specificity, high NPV, low PPV
- Crude extract tests cannot isolate major allergen
  -- Allergens contain multiple major allergens, not all “parts” equal
  -- Regional sensitization differences may be based on these “parts”
- Tests have limited interpretation
  -- Can’t determine significance of cross-reactivity seen
  -- Can’t determine “severity” of reactivity
  -- Can’t determine if there is actual reactivity inferred
- Can can we build a better mousetrap?

Basophil Activation Testing

- Growing importance of the role of the basophil in mediating allergic responses in mice
- Growing body of evidence for utility of measuring basophil activation as a marker in humans as well
- Basophils release histamine from preformed granules similar to mast cells
- Basophils have a half-life of only one week
Basophil Histamine Release

• Basophils release varying degrees of histamine but is a highly individualized response
  --Spontaneous release has been used in food/eczema studies
  --Release can be triggered by FcER1 binding
  --However, other molecules can trigger histamine release, such as C5a, so the process can be non-specific

• Measurement is very technical
  --Involves lymphocyte enrichment, requires careful platelet removal
  --Measurement through RIA or ELISA
  --1ml blood (20,000 basophils) generally sufficient
  --Release from antigen/FcER1 cross-linking is susceptible to dose-response effects, surface receptor density and receptor reductions
Surface Markers of Activation

- Advances in flow cytometry has made it possible to measure surface markers as opposed to histamine/mediator release.
- Method more accessible than auto-analyzers for mediator measurement.
- Can use heparanized whole blood.
- Common markers include CD63, CD203c, and CD69.
Basophil Activation Test

• **CD63 (BAT) is most commonly used**
  --Most closely associated with anaphylactic vs. piecemeal degranulation
  --Because degranulation processes are distinct, CD63 only appears with anaphylaxis and can distinguish histamine release mechanisms
  --Commercial assay available
  --Assay sensitivity enhanced by IL-3

• **CD203c and CD69 expression can also be quantified**
  --Evidence that CD203c expression is not related to histamine degranulation and can be triggered by multiple stimuli, including IL-3
  --CD69 is expressed by basophil cytokine expression, and occurs slowly
  --Expression of both is highly distinct from CD63

MacGlashan J Allergy Clin Immunol 2013;132: 777-87
BAT Clinical Applications

• CD63 may have diagnostic properties
  --Expression not altered by anti-histamines
  --Can run on blood within 1 year of exposure

• Sensitivity for food allergy diagnosis is 77-98%, and sensitivity 75-100%
  --Has shown enhanced accuracy vs. SPT and sIgE

• Has shown potential ability to determine
  --if peanut OFC is needed and how severe a reaction in an OFC was
  --if baked milk/egg tolerance and if have outgrown milk allergy
  --if OIT subject achieved clinical desensitization
  --if Omalizumab treatment had effect on food tolerance
BAT Limitations

- Still mainly a research tool
- Accessibility
- Price (cost-effectiveness)
- Limited data showing effectiveness

- Is a very promising and potentially useful tool that should play a larger role going forward
What is an Epitope

• 3-D allergen binding site
  --No universally common structure

• Can bind IgE and IgG
  --Can elicit cross-reactivity
  --T cell epitopes exist as well

• Conformational: tertiary
  --Heat labile, subject to hydrolysis

• Linear: sequential
  --Heat stable, not alterable

Sampson HA J Allergy Clin Immunol 2004; 113: 805-819
## Homology Across Families

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>EPITOPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific lipid transfer proteins</td>
<td>Ara h 9 Cor a 8 Pru p 3 Par j 2 Art v 3</td>
</tr>
<tr>
<td>Seed storage proteins</td>
<td>2S albumins Ara h 2, 6 and 7 Ber e 1</td>
</tr>
<tr>
<td></td>
<td>7S albumins Ara h 1 Gly m 5 11S albumins Ara h 3 Gly m 6</td>
</tr>
<tr>
<td></td>
<td>Cor a 9 Gliadins Tri a 19</td>
</tr>
<tr>
<td>Pathogenesis-related protein family 10 proteins (PR-10)</td>
<td>Bet v 1 Ara h 8 Gly m 4 Cor a 1 Pru p 1 Api g 1.01 Mal d 1</td>
</tr>
<tr>
<td></td>
<td>Act d 8 Dau c 1</td>
</tr>
<tr>
<td>Profilins species</td>
<td>Bet v 2 Pru p 4 Hev b 8 Phl p 12</td>
</tr>
<tr>
<td>Calcium-binding proteins</td>
<td>Bet v 4 Phl p 7</td>
</tr>
<tr>
<td>Serum albumins</td>
<td>Fel d 2 Can f 3 Bos d 6 Sus PSA Equ c 3</td>
</tr>
<tr>
<td>Parvalbumins</td>
<td>Cyp c 1 Gad c 1</td>
</tr>
<tr>
<td>Tropomyosins</td>
<td>Pen a 1 Der p 10 Ani s 3</td>
</tr>
<tr>
<td>Lipocalins</td>
<td>Fel d 1 Fel d 4 Can f 1 Can f 2 Equ c 1 Mus m 1</td>
</tr>
</tbody>
</table>
Peanut: A Component Model

- Peanut contains:
  - Major allergens
  - Cross-reactive allergens
  - Other compounds
- Can recognize multiple epitopes
- Positive test is a sum of all recognized parts

Ideal Uses For Components

- Clarifying cross-reactivity vs. primary sensitization with clinical relevance
- Risk stratification
- Longitudinal outcomes
- Predicting response to therapy/challenge
- Predicting ideal therapy candidates
Peanut Component Model of Theoretical Risk

- rAra h 1
- rAra h 2
- rAra h 3
- rAra h 8
- rAra h 9
- CCD

Risk:
- CCD
- Profilin
- PR-10
- LTP
- Storage Proteins
  - Ara h 1
  - Ara h 2/6
  - Ara h 3
  - Ara h 5
  - Pollen cross-reactive
  - Ara h 8
  - Ara h 9

Slide courtesy Anna-Nowak-Wegryzn, Jaffe Food Allergy Institute, Mount Sinai School of Medicine, with permission.
Assessing the Clinical Applicability of Peanut Components
Key Questions to Implementing Use of Components in Practice

- Is the use of Ara h 2 (or any component) superior to peanut ImmunoCAP or PST?
- Is there an optimal diagnostic decision point for any component?
- Does Ara h 2 (or any component) help assess severity of reactivity?
- What degree of bias do the component studies have that could limit their usability?
Is Ara h 2 the Superior Test for Peanut Allergy?

- 22 studies in past 10 years performed to investigate components in diagnosing peanut allergy
- GRADE analysis of studies of components vs. f13 vs. PST as most accurate diagnostic tool
  -- Inclusion: patients w/suspected allergy, components were compared to another test, at least 25% of the population was challenged
  -- Case-control studies excluded unless controls were suspected to have allergy
  -- Assessed selection, index test, reference standard, study flow/timing, risk of bias
- Most of studies performed in Europe, 12/22 studies at high risk for bias (selection), 21 studies pediatric
- Heterogeneity not assessed—deemed too heterogeneous to attempt pooling data

Klemans et al Clin Exp Allergy 2015; 45: 720-30
## Is Ara h 2 the Superior Test for Peanut Allergy?

<table>
<thead>
<tr>
<th>Test</th>
<th>Cut off</th>
<th>Studies</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+ LR</th>
<th>-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PST</td>
<td>3mm</td>
<td>8</td>
<td>66-100%</td>
<td>0-95%</td>
<td>1.391</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>8mm</td>
<td>1</td>
<td>54</td>
<td>98</td>
<td>22.2</td>
<td>0.47</td>
</tr>
<tr>
<td>f13 CAP</td>
<td>0.35 KU/L</td>
<td>12</td>
<td>80-100%</td>
<td>0-63%</td>
<td>0.95-2.15</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>0.23 KU/L</td>
<td>1</td>
<td>95</td>
<td>49</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>24.1 KU/L</td>
<td>1</td>
<td>48</td>
<td>98</td>
<td>23</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>34 KU/L</td>
<td>1</td>
<td>14</td>
<td>99</td>
<td>11.2</td>
<td>0.87</td>
</tr>
<tr>
<td>Ara h 1</td>
<td>0.35 KU/L</td>
<td>6</td>
<td>26-92%</td>
<td>41-95%</td>
<td>1.44-10.7</td>
<td>0.09-0.79</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>0.35 KU/L</td>
<td>10</td>
<td>60-100%</td>
<td>60-96%</td>
<td>2.22-26.3</td>
<td>0-0.47</td>
</tr>
<tr>
<td>Ara h 3</td>
<td>0.35 KU/L</td>
<td>1</td>
<td>21-84%</td>
<td>41-91%</td>
<td>1.4-4.8</td>
<td>0.23-0.87</td>
</tr>
<tr>
<td>Ara h 5</td>
<td>0.35 KU/L</td>
<td>2</td>
<td>16-35%</td>
<td>77-95%</td>
<td>1.53-3.29</td>
<td>0.84-0.88</td>
</tr>
<tr>
<td>Ara h 8</td>
<td>0.35 KU/L</td>
<td>5</td>
<td>16-42%</td>
<td>31-81%</td>
<td>0.52-2.18</td>
<td>0.72-2.08</td>
</tr>
<tr>
<td>Ara h 9</td>
<td>0.35 KU/L</td>
<td>4</td>
<td>8.6-26%</td>
<td>83-95%</td>
<td>0.51-5.48</td>
<td>0.77-1.1</td>
</tr>
<tr>
<td>ISAC (ara h 2)</td>
<td>0.3 ISU/L</td>
<td>2</td>
<td>81-94%</td>
<td>74-77%</td>
<td>3.53-3.62</td>
<td>0.08-0.25</td>
</tr>
</tbody>
</table>

- **Ara h 2 has best overall diagnostic accuracy based on highest +LR**
- Studies of poor quality: retrospective, biased to available serum, selection not at random
- Ara h 2 superiority was independent of geography, but no Spanish studies included
Is there an Optimal Cut-off Point

- Cut off points represent a probability a patient encounters particular outcome
  -- PPV (probability pt w/ + test has disease), NPV (pt w/- test doesn’t)
  -- LR (probability of test finding in pt w/disease vs. in pt w/o disease)
  -- Predictive value depends on prevalence of disease, LR does not
  -- Predictive values should be assumed applicable to only the group studied

- Must consider pre-test probability person has disease
- Must understand the sample being studied

Understanding the Tests

TABLE VI. Predictive value of food allergen-specific IgE levels

<table>
<thead>
<tr>
<th>Allergen</th>
<th>[kU/L]</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>7</td>
<td>98</td>
</tr>
<tr>
<td>- Infants ≤ 2 yrs+</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>Milk</td>
<td>15</td>
<td>95</td>
</tr>
<tr>
<td>- Infants ≤ 2 yrs++</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Peanut</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Fish</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Tree nuts+++</td>
<td>~15</td>
<td>~95</td>
</tr>
<tr>
<td>Soybean</td>
<td>30</td>
<td>73</td>
</tr>
<tr>
<td>Wheat</td>
<td>26</td>
<td>74</td>
</tr>
</tbody>
</table>

PPV = Positive predictive value

Increasing probability of clinical reactivity with increasing level of food-antigen specific IgE value; note: values <0.35 do not exclude allergic reactivity

References:

Sampson, HA. J Allergy Clin Immunol 2004;113:805-1
Is There an Optimal Ara h 2 Cut Off Point?

- Multiple studies suggest Ara h 2 as best discriminator
- But are wide variations in cut off points based on methods or geography
- There probably is no absolute “predictive” level for general use
- We would need to know the accurate prevalence of peanut allergy first!

<table>
<thead>
<tr>
<th>Study</th>
<th>95% PPV Cutoff (ku/L)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampson 2001</td>
<td>15 (peanut)</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>Nicolaou 2011</td>
<td>0.35 (optimal cut point)</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Codreanu 2011</td>
<td>0.23 (optimal cut point)</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>Eller 2013</td>
<td>1.28 (not a PPV)</td>
<td>76</td>
<td>97</td>
</tr>
<tr>
<td>Dang 2012</td>
<td>1.96</td>
<td></td>
<td>96%</td>
</tr>
<tr>
<td>Keet 2013</td>
<td>2 (75% PPV, 62.3% NPV)</td>
<td>23</td>
<td>94</td>
</tr>
<tr>
<td>Klemans 2013</td>
<td>0.35 (74% PPV)</td>
<td>91</td>
<td>72</td>
</tr>
<tr>
<td>Beyer 2015*</td>
<td>42 [0.35 (50%)14.4 (90%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kukkonen 2015</td>
<td>0.35 (PPV 91%, NPV 84% for severe rxn)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leo 2015</td>
<td>2 (91% PPV, 47% NPV)</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>Ballmer-Weber 2015</td>
<td>2 (97% prob. in sample for severe rxn,)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ara h 2 PPV Performance

80% PPV 4.5 KU/L

95% PPV 42.2 KU/L
Whole Peanut PPV Performance

80% PPV 87.9 KU/L

20% PPV 0.63 KU/L
Does Ara h 2 Help Indicate Severity?

- Do component value correlate to reaction severity?
  - Few studies address this, and do so without significant selection bias
- Some indication Ara h 2 may correlate w/ severity but more data needed—cannot support this claim yet

<table>
<thead>
<tr>
<th>Study</th>
<th>Ara h 2 level</th>
<th>Finding</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klemans 2015</td>
<td>Not specified</td>
<td>Higher levels had increased HR (1.004) of objective rxn</td>
<td>No findings for Ara h 8</td>
</tr>
<tr>
<td>Leo 2015</td>
<td>0.1-8.79</td>
<td>Range for pts w/anaphylaxis during OFC, but Ara h 2 level was not associated with anaphylaxis</td>
<td>Excluded if Ara h 2 &gt;10</td>
</tr>
<tr>
<td>Kukkonen 2015</td>
<td>&gt;0.35</td>
<td>84% PPV, 91% NPV for severe reaction Ara h 6 &gt;0.35 94% PPV, 97% NPV for severe reaction</td>
<td>No effect for ara h 8/9, polyclonality best predictor</td>
</tr>
<tr>
<td>Ballmer-Weber 2015</td>
<td>2</td>
<td>97% probability for severe rxn in a multi-center sample, not significant risk below this level. Region of Europe has significant effect on odds of severe rxn.</td>
<td>Southern/west-central Europe had less % severe rxn</td>
</tr>
<tr>
<td>Eller 2013</td>
<td>Not specified</td>
<td>Significant correlation between OFC symptom score and Ara h 2 sensitization (rho=0.6)</td>
<td>Rho of -0.02 for Ara h 8</td>
</tr>
</tbody>
</table>
## Degree of Bias in Studies Supporting Use of Ara h 2

<table>
<thead>
<tr>
<th>Author, year of publication</th>
<th>Risk of bias</th>
<th>Applicability concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient selection</td>
<td>Index test</td>
</tr>
<tr>
<td>Bernard [22]</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Kagan [33]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Perry [29]</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Roberts [34]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nolan [9]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wainstein [7]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nieuwaaal [35]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DunnGalvin [36]</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Johansson [6]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nicolaou [11]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dang [21]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ebisawa [14]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glaumann [37]</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Eller [10]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Keet [24]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Klemans/Otte [15]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Klemans/Broekman [8]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lieberman [17]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lopes de Oliveira [23]</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>Ludman [38]</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Peters [39]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Suratannoon [25]</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Klemans et al Clin Exp Allergy 2015; 45: 720-30
Degree of Bias in Studies Supporting Use of Ara h 2

- Most studies had some bias
- Most bias in patient selection due to retrospective design
- Few studies had multiple reference standards or enrolled pts lacking a standard
- Applicability concerns in some had to do with selection sites, severity, use of
Cautions from True Population Level Data

- Dang et al noted Ara h 2 of 1.19 had 95% PPV -
  -Australian population level study, 98% specificity and 60% sensitivity
    --Sampled both tolerant and allergic individuals, all underwent challenge

- Natural history f/u study at age 4 looked at markers of predicted tolerance

- Ara h 2 levels at age 1 or 4 poorly predictive of OFC proven allergy at age 4
  --PST 8mm, f13 of 2.1 KU/L best predictors at age 4
  --PST 13mm, f13 5KU/L at age 1 best predictor for age 4
  --No difference in Ara h 2 levels at age 1 between persistent vs. tolerant kids

- Unclear how to use Ara h 2 beyond diagnosis

Dang et al J Allergy Clin Immunol 2012; 129: 1056-63
Peters et al. J Allergy Clin Immunology 2015; 135: 1257-66
So Should I Use These Tests?
## What Does the Evidence Show

<table>
<thead>
<tr>
<th>Question</th>
<th>Magic 8 Ball Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is Ara h 2 superior to peanut ImmunoCAP or PST for diagnosis?</td>
<td>Most likely</td>
</tr>
<tr>
<td>Does Ara h 2 (or any component) help assess severity of reactivity?</td>
<td>Concentrate and ask again</td>
</tr>
<tr>
<td>Is there an optimal diagnostic decision point for any component?</td>
<td>Outlook not so good</td>
</tr>
<tr>
<td>Does study bias limit usability of results?</td>
<td>Signs point to yes</td>
</tr>
</tbody>
</table>
Final (Serious) Comments

• Ara h 2 likely superior test, but data still limited
  --Need for population level, prospective study for this concept
  --Need for studies to determine how and when to use/apply Ara h 2

• No “true” cut point applicable to everyone
  --Cutoffs are population specific and thus vary widely
  --Studies must adjust for confounding effects, not just Ara h 2 level
  --Regional sensitizations key and are completely unknown across the US

• Cannot correlate Ara h 2 recognition to severity yet

• ? role of polyclonal recognition, Ara h 6, B/T cell epitopes as additional key diagnostic factors

Vereda et al J Allergy Clin Immunol 2011; 127: 603-7
Back to Bobby—
Would Ara h 2 Help?

• Positive Ara h 2 could help confirm history
  --But was the vomiting really unrelated, and the + tests irrelevant?
  --If there is doubt, then yes, this could help

• Would Ara h 2 influence decision to challenge?
  --For me no because there are no predictive levels, and my decision to
    challenge would be based on a questionable history, not on a lab value

• Would obtaining Ara h 2 help future decisions?
  --Quite possibly. LEAP-on data indicates some decline in Ara h 2 among
    persistently tolerant
  --May be strong future value in obtaining Ara h 2 if cost not prohibitive
Think Beyond Peanut!

Component Case Studies for Egg and Milk
(Components long commercially available!)
Components for Milk

- Milk proteins are casein (80%) and whey
  - α-lactalbumin (bos d 4), β-lactoglobulin (bos d 6), casein (bos d 8), whey
  - 5 caseins (κ, α_{s1-2}, γ_{1-3}), most with α_{s1} and κ (likely sequential)
  - 5 proteins (α-lactalb., β-lactaglob., BSA, immunoglobulin, lactoferrin)

- Persistent allergy associated w/ higher number of sequential epitopes

- Casein > 0.78 kU/L, α-lact. > 34 kU/L, β-lact. > 9.9 kUL are *proposed* 95% predictive decision points

- Baked milk *proposed* 95% predictive decision point 5 kU/L
Different Epitopes, Different Phenotypes

- Persistent milk, egg, peanut allergy related to increased sequential epitope recognition
- Epitope mapping can elucidate clinical phenotypes
- Shows that α-s1, α-s2, κ-casein associated with persistence of milk allergy and β-lactoglobulin with transient allergy

Components for Egg

- **Egg white has > 20 proteins**
  - Ovomucoid heat/digestion stable, dominant allergen (gal d 1)
  - Ovalbumin, ovotransferrin, lysozyme (gal d 2, 3, 4) all heat/acid labile
  - Egg yolk (gal d 5)—seen in bird-egg syndrome

- **Ovomucoid associated with persistent allergy**
  - 95% PDP’s of 11, 30, 50, and kU/L for baked egg reactivity
  - 1 kU/L reported as “safe” level for baked/cooked egg
  - Persistent egg allergy seen with increased number of sequential epitopes

- **HealthNuts:** 80% of children failing egg white challenge were TOLERANT of baked egg

Lemon Mule J Allergy Clin Immunol 2008; 122:977–983.e1
Osborne et al J Allergy Clin Immunol 2011; 127: 668-76
Components: Cautions

• Validation and quality of present data
  --Retrospective data from clustered, biased, small samples

• Information overload
  --Danger of detecting sensitization of unknown relevance
  --Particularly a problem with multiplex chip detection

• Cultural relevance
  --Differing major allergen patterns in Europe
  --Unclear if this is a US issue

• Marketing
  --This test is directly marketed to PCP’s

An Optimistic Application
## Summary: Key Food Components

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Epitope</th>
<th>Class</th>
<th>Believed risk</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>Ara h 2 &gt; Ara h 1 or 3</td>
<td>Seed storage</td>
<td>Severe sx</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ara h 6 and 9</td>
<td>LTP</td>
<td>Mediterranean-only</td>
<td>Ara h 9 also noted in tolerant pts</td>
</tr>
</tbody>
</table>
| Hazelnut | Cor a 9, Cor a 14 | Seed storage | Challenge verified sx         | -Retrospective analysis  
  -Higher levels in those w/o birch allergy  
  -Cor a 9 pattern seen in other countries  
  -Cor a 9 & 14 seen in younger pts w/sx   |
|          | Cor a 8  | LTP          | Mediterranean-only            | -Reported in 50-80% w/severe sx                                       |
| Apple    | Mal d 3 | LTP          | Severe sx                     |                                                                       |
| Soy      | Gly m 5, Gly m 6 |              | Severe sx                     |                                                                       |
|          | Gly m 4 | PR-10        |                                |                                                                       |
| Milk     | Bos     | Serum protein| Persistent allergy, including |                                                                       |
|          |         |              | baked milk                    |                                                                       |
| Egg      | Gal d   | Serum protein| Persistent allergy, including |                                                                       |
|          |         |              | baked egg                     |                                                                       |

Luengo and Cardona Clin Translational Allergy 2014; 4: 1-9  
Agabriel et al Pediatr Allergy Immunol 2015; 25: 662-7  
Masthoff et al J Allergy Clin Immun 2013; 132: 393-9
Thanks!

The view from the Food Challenge Unit, Children’s Hospital Colorado