Innovative technologies in allergen diagnosis

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COI

I have been paid for lectures in GSK, AZ, MSD, and Novatis sponsored symposiums

Also served as Scientific or Medical consultant through National Cheng Kung University Tainan, Taiwan in OrientMed pharma Co., Promed Biotechn Co, Agnitio Biotech, Co. and Advagene Biopharma Co. Taiwan
The word comes from the Greek word 'alol', meaning, 'change in the original state.' defined the term **allergy** as "altered reactivity" in 1906.
Atopic serum, intradermal

Skin, cross-section (sensitization) → 12 hr

Allergen, intradermal

Sensitized skin (challenge) → 5 min

Increased blood flow, redness → 5 min

Edema, swelling (pale center, surrounded by redness)

Prausnitz-Küstner Reaction
1956 Kimishige Ishizaka and Teruko Ishizaka

Hans Bennich and S. G. O. Johansson

Binding of IgE to FcεRI

- IgE binds to α chain
- β and γ chains are involved in signal transduction

F (ab')₂

Allergen binding site

Cε1

VH

VL

Cε2

Cε3

Cε4

Fc

FcεRI

α₂

α₁

Out

Cell membrane

β

γ

Holgate ST. QJM
IgE and Anti-IgE

Invention
Tanox
1987

Research, initial clinical trials
Novartis joined
1990

Genentech

1990

1996

Prof. Kimishige and Teruko Ishizaka

2003
FDA Approved

Dr. Tze-Wen Chang, Taiwanese scientist worked in Ciba-Geigy. Co.
IgE Milestones - description, discovery, characterization, measurement,

- **1972 RAST**
- **1972: First RAST**
- **1967: Radioimmunoassay published by Wide et al.**
- **1968: P. Ishizaka**
- **1906: Terminus “Allergy” Clemens von Pirquet**
- **1972 RAST**
- **1989 ImmunoCAP**
- **1999 CRD**
- **2009 ISAC**
- **2009: Thermo Scientific takes over ISAC from VBC**
- **2013: A WAO - ARIA - GA2LEN consensus document on molecular-based allergy diagnostics**
  
  *World Allergy Organization Journal* 2013, 6:17
doi:10.1186/1939-4551-6-17WAO
  Consensus paper
FDA approved laboratory based in vitro IgE diagnostic methods—“essentially unchanged” since 1972.

- The ImmunoCAP® System
- Siemens IMMULITE Systems: “3gAllergy”.
- HYCOR Biomedical HYTEC-288.
- Hitachi MAST® systems.

All using heterogeneous allergen extracts for IgE antibody binding and enzyme labelled anti-IgE for detection
Immulite 2000 (Siemens)

- Patient's serum IgE
- Anti-IgE
- Alkaline phosphatase
- Phosphate ester of adamantyl dioxetane
- Light

ImmunoCAP (ThermoFisher)

- Patient's serum IgE
- Anti-IgE
- β-galactosidase
- Methylumbelliferyl-β-D-galactoside
- Fluorescence

Hytec 288 Plus (Hycor)

- Patient's serum IgE
- Anti-IgE
- Alkaline phosphatase
- P-Nitrophenyl phosphate
- Color
1. Performance characteristics with coefficients of variation < 15%, however, results from the three assays are not interchangeable.

2. Separate tests are needed for each allergen

3. Use large sample of serum

4. All laboratory based and require substantial infrastructure
Reasons for measuring specific IgE levels

- Sensitization and common allergic disorders may **develop into severe conditions later** in life.
- Patients with IgE related allergy are in most cases **sensitized to several allergens**.
- Identifying the offending allergen is essential for: **avoidance**, assessing clinical risk for reaction, explaining cross-reactivity, identifying right patients for **SIT**.
- Quantification is one tool to pick out the most **probable offending allergen**.
- Quantification is a tool to **follow up patients** (monitor, manage treatment, compliance).
Is skin testing always sufficient?

Positivity due to cross-reactivity (CCD, Profilin, LTP...)?

W. Aberer, 2013
### Indoor Allergens

- **Fel d 1**
- **Der p 1**
- **Fel d 4**
- **Der p 2**

### Outdoor Allergens

- **Alt a 1**
- **Que a 1**
- **Alt a 2**

#### Sources

- **Cat** *(Felis Domesticus)*
- **Dust Mite** *(Dermatophagoides pteronyssinus)*
- **Oak Tree** *(Quercus alba)*
- **Alternaria Mold** *(Alternaria alternate)*
## Food allergy: clinical manifestations

<table>
<thead>
<tr>
<th>IgE</th>
<th>IgE/Non-IgE</th>
<th>Non-IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urticaria/angioedema</td>
<td>Atopic dermatitis</td>
<td>Protein-induced proctocolitis/enterocolitis</td>
</tr>
<tr>
<td>Rhinitis /Asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral allergic syndrome</td>
<td>Eosinophilic gastro-intestinal disorders</td>
<td>Celiac disease</td>
</tr>
<tr>
<td>Gastrointestinal symptoms (GIT)</td>
<td></td>
<td>Contact dermatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Herpetiform dermatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heiner’s syndrome</td>
</tr>
</tbody>
</table>

Adapted from J Allergy Clin Immunol. 1999;103:717-728
Three sites that might be relevant to food sensitization:

- Keratinized Skin Epithelium
- Non-keratinized Esophageal Epithelium
- Gut Epithelium

Eczema  Protein-loss enteropathy  Heiner’s syndrome
Figure 1. The diagnostic subgroup of patients ($n = 2256$). AD = atopic dermatitis; AR = allergic rhinitis; AS = asthma.
Serum specific IgE levels and positive sensitization rates to egg white, ovalbumin, and ovomucoid in different age groups (n = 2256). (A) Serum s-IgE levels for trend (0-2 year age groups are reference); *p < 0.05, **p < 0.01, ***p < 0.001, by ANOVA post hoc Tukey test;

(B) positive sensitization rates for trend (2-4 age groups are reference); *p < 0.05, **p < 0.01, ***p < 0.001, by Mann-Whitney test.
IgE levels of peanut allergen components in all peanut-sensitized children

Yang-Te Lin. Patterns of sensitization to peanut allergen components in Taiwanese Preschool children. JMII 2012; 45; 90-95
Changes of IgE levels children at baseline and at an average of 22 months later
Peanut allergies in Taiwanese preschool children

In conclusion, Ara h 1, Ara h 2, and Ara h 3 were found to be major components of peanut sensitization in children in Taiwan. Ara h 2 was probably the most important component that contributed to clinical symptoms and remained at steady levels in children who were allergic to peanuts.
<table>
<thead>
<tr>
<th>Source</th>
<th>Particles</th>
<th>Allergen</th>
<th>MW (kDa)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust mite</td>
<td></td>
<td>Der p 1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Der p 2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Der p 10</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Der p 11</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Cockroach</td>
<td>Frass</td>
<td>Bla g 1</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bla g 2</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bla g 4</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bla g 5</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td>Fel d 1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fel d 2</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fel d 4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cat IgA</td>
<td>200†</td>
<td></td>
</tr>
<tr>
<td>Alternaria</td>
<td></td>
<td>Alt a 1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alt a 2</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alt a 3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Grass</td>
<td></td>
<td>Lol p 1</td>
<td>17+</td>
<td></td>
</tr>
</tbody>
</table>
Circumstances of potential increase of allergy diagnosis accuracy by Components Resolved Diagnosis (CRD)

- **Indication of allergen immunotherapy**
  - Inhalant oligo/monosensitization
  - Pollen polysensitization
  - Hymenoptera venom allergy

- **Anaphylaxis**
  - Cofactor-enhanced food-dependent anaphylaxis
  - Delayed red meat anaphylaxis
  - Idiopathic anaphylaxis

- **Latex allergy**

- **Polysensitization**
  - Pollen and plant food

- **Food allergy**
  - Risk assessment
  - Identification of unanticipated allergen triggers
Pollen species-specific allergens

- Chenopodium
- Olive/Ash
- Grass
- Birch
- Cypress
- Ribwort plantane
- Salsola
- Mugwort
- Plane tree
- Amb a 1
- Ole e 1
- Phl p 1/ Phl p 5
- Bet v 1
- Par j 1/ Par j 2
- Cup a 1/ Cry j 1
- Pla l 1
- Sal k 1
- Pla a 1/ Pla a 2

Luengo and Cardona Clinical and Translational Allergy 2014, 4:28
Cross-Reactive Allergens

- Polcalcins
- Ole e 1 related
- Pectate lyase
- Poligalacturonase

- Storage proteins
  - nsLTP
  - TLP

Pollen-Pollen

Pollen-Plant food

Pollen-Plant food-latex

Plant food-Plant food

Cross-reactive carbohydrate determinants

- Profilin
- Bet v1-homologues
- nsLTP
- TLP

- Profilin
- Beta-1,3-glucanase
- Hevein-like domain proteins

Luengo and Cardona Clinical and Translational Allergy 2014, 4:28
Allergens associated to higher versus lower risk of anaphylaxis

- **Higher risk**
  - Profilins: (Cor a 2, Pru p 4, Mal d 4, Cuc m 2, Dau c 4)
  - PR-10: (Ara h 8, Cor a 1, Mal d 1, Cuc m1, Dau c 1)
  - CCD

- **Lower risk**
  - nsLTPs: (Pru p 3, Cor a 8, Jug r 3, Ara h 9, Tri a 14)
  - Storage proteins: (Ara h 1, Ara h 2, Ara h 3, Cor a 9, Cor a 14, Jug r 1, Jug r 2, Gly m 5, Gly m 6)
  - PR10: (Gly m 4)
  - Wheat ω5-gliadin: (Tri a 19)

Luengo and Cardona Clinical and Translational Allergy 2014, 4:28
### Table 1: High- versus low-risk molecules from foods giving rise to anaphylaxis

<table>
<thead>
<tr>
<th>Source</th>
<th>High risk</th>
<th>Low risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>Ara h 1, 2, 3, 9</td>
<td>Ara h 8, profilin, CCD</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>Cor a 8, 9, 14</td>
<td>Profilin, CCD</td>
</tr>
<tr>
<td>Walnut</td>
<td>Jug r 1, 2, 3</td>
<td>Profilin, CCD</td>
</tr>
<tr>
<td>Soy</td>
<td>Gly m 5, 6, (4)</td>
<td>Profilin, CCD</td>
</tr>
<tr>
<td>Rosacea fruits</td>
<td>Pru p 3, Mal d 3</td>
<td>Pru p 1, Mal d 1, profilin, CCD</td>
</tr>
<tr>
<td>Wheat</td>
<td>Tri a 14, Tri a 19</td>
<td>Profilin, CCD</td>
</tr>
</tbody>
</table>

**KEY:** *CCD = Cross-reactive Carbohydrate Determinant.*
Allergen sources

- Trees
- Grasses
- Food
- Mites
- Cat
- Dog
- Bee

Recombinant allergen molecules

Profiling

Oligosensitized patients

- Prophylactic vaccination?
- Immunotherapy with engineered hypoallergenic derivatives

Polysensitized patients

- Symptomatic therapy
## Evaluation of ISAC test result

<table>
<thead>
<tr>
<th>Result</th>
<th>Control Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (-)</td>
<td>- Appropriate test requested?</td>
</tr>
<tr>
<td></td>
<td><strong>Note:</strong> In general, if the patient has dermatological symptoms or there is a suspicion of food allergy, microarray testing could be indicated, but the number of negative results may be high.</td>
</tr>
<tr>
<td>Mono Positive (+)</td>
<td>- Appropriate test requested?</td>
</tr>
<tr>
<td></td>
<td><strong>Note:</strong> Mono-sensitization at molecular component level is rare and questionable.</td>
</tr>
<tr>
<td>Multi Positive (+++)</td>
<td>1. Do positive components explain patient signs and symptoms?</td>
</tr>
<tr>
<td></td>
<td>2. Are results consistent with SPT or slgE results? If not, does the clinical history support whole extract or component results?</td>
</tr>
<tr>
<td></td>
<td>3. Do cross-reactive components explain many positive SPT or slgE results?</td>
</tr>
<tr>
<td></td>
<td>4. Are there any unexpected results? If yes, could the collection of patient history be improved?</td>
</tr>
<tr>
<td></td>
<td>5. Can the cross-reactive components explain complex clinical syndromes (e.g., pollen-food, pollen-pollen, mite-shrimp, or cat-pork syndrome)?</td>
</tr>
<tr>
<td></td>
<td>6. Are there any “low risk” (e.g. PR-10, profilin or CCD) or “high risk” markers (e.g. storage proteins, Tri a 19 or LTP)?</td>
</tr>
<tr>
<td></td>
<td>7. Are there single components or component combinations that indicate an increased risk for systemic reactions?</td>
</tr>
<tr>
<td></td>
<td>8. Are all, some, or none of the relevant components of the suspected allergen source represented on the chip? If none or only some: Are there cross-reactive components that may act as representative markers for the suspected allergen (e.g., Ole e 1 (olive) is also a marker for ash pollen)?</td>
</tr>
</tbody>
</table>
Multiplex and quantitative IgE detection

ImmunoCAP ISAC® vs. ImmunoCAP® immunoassay

- Ambient Analyte measurement
- Analyte present in excess (ng)
- Analyte depletion only in proximity of the reaction site
- Mass transport depending
- Influenced by: Surface chem., shape of reaction chamber, spot size, affinity, avidity, etc.

- End point measurement
- Allergen present in excess
- Analyte depletion in sample
- Precise quantitative assay
## Advantages and disadvantages of ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 30 µl of serum or plasma (capillary or venous blood)</td>
<td>• Manual method</td>
</tr>
<tr>
<td>• 112 allergens can be assayed in parallel</td>
<td>• Semi-quantitative assay</td>
</tr>
<tr>
<td>• Natural and recombinants proteins</td>
<td>• Less sensitive</td>
</tr>
<tr>
<td>• Less allergen needed (approximately 100,000-fold, pg vs. µg) per assay</td>
<td>• More variability in the inter-assay analysis for certain allergens</td>
</tr>
<tr>
<td>• No interference from very high total IgE</td>
<td>• Greater coefficient of variation</td>
</tr>
<tr>
<td></td>
<td>• Some allergen sources are not included</td>
</tr>
<tr>
<td></td>
<td>• Less appropriate for monitoring sensitization</td>
</tr>
<tr>
<td></td>
<td>• Potential interference between IgE and other isotypes, principally IgG</td>
</tr>
</tbody>
</table>
Performance Characteristics of an Automated, Microfluidic Protein Microarray System for Allergy Diagnosis

Analytical Biochemistry
Methods in the Biological Sciences

An automated microfluidic-based immunoassay cartridge for allergen screening and other multiplexed assays

Authors:

Affiliations:
*National Taiwan University, Taipei, Taiwan
**Department of Pediatrics, College of Medicine, and Center for Microbiology and Molecular Medicine, National Taiwan University, Taipei, Taiwan

Abstract:
A microfluidic cartridge and system for multiplexed immunoassays is described. The passive microfluidic cartridge was comprised of three layers of transparent-molded plastic sealed together using a thermal sealing technique. Using this platform technology, a specific immunoassay module (EIA) was constructed. Allergen target antigens, positive and negative controls, and IgE capture antibodies were immobilized within the module as a microarray. A disposable central immobilization strip provided the assay results. The disposable module was inserted into the instrument to perform automated, simultaneous antigen detection and quantitation with rapid analysis times of 20-25 min. Allergen screening showed 98% agreement with 3 hour dot blot hybridization and IgE enzyme-linked immunosorbent assay (ELISA) agreement overall of 99%. Average coefficient of variation (CV) was measured as 20% for brevities and 15% for histamine levels. The lower limit of detection of 3 ng/mL was assessed at 0.1 ng/mL, and overall limits of 1-2 ng/mL were estimated at an 1 RIA (0.4 μg/ml). Such a system has potential applications in decentralized allergen screening as well as in other multiplexed diagnostic immunoassays where multiplex analysis, ease of use, and short analysis times are critical.

Analytical Biochemistry
Volume 391, Issue 2, 15 August 2009
ISSN 0003-2697

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**BIoIC® Technology Overview:**

Lab-on-Chip/microfluid

- Nitrocellulose Coatings for Protein Arrays
- Microfluidics leads Automated Protein Arrays
- Process Automation in both IVD & Life Science
BioIC Cartridge

Reaction zone

Loading position

After pumping
BiolC™ Technology
Fig. 2

(A) BioIC (AU) vs. UniCAP (kU/ml)

\[ y = 0.5911x + 1.4313 \]
\[ R^2 = 0.9958 \]

(B) BioIC (AU) vs. (dilution fold)

\[ y = -0.8891x + 103.54 \]
\[ R^2 = 0.9445 \]

(C) Specificity vs. Sensitivity

\[ y = -0.6472x + 100.24 \]
\[ R^2 = 0.9845 \]
Determination of multiple allergen-specific IgE by microfluidic immunoassay cartridge in clinical settings


Determination of multiple allergen-specific IgE by microfluidic immunoassay cartridge in clinical settings.

Shyh-Dar Shyur¹, Ren-Long Jan², James R. Webster³, Ping Chang³, Yu-Jung Lu⁴ and Jiu-Yao Wang⁵,⁶
Longitudinal pattern of multiplexed immunoglobulin E sensitization from prenatal stage to the first year of life

Jiu-Yao Wang¹,², Chih-Ann Chen¹,², Yung-I Hou¹,², Wan-Lin Hsiao¹,², Ya-Wen Huang³, Yu-Ting Tsai³ & Hui-Ju Tsai³,⁴,⁵

South Taiwan Allergy Research (STAR) Birth Cohort Consortium
<table>
<thead>
<tr>
<th></th>
<th>Der p</th>
<th>Der f</th>
<th>Blo t</th>
<th>Cat</th>
<th>Dog</th>
<th>Blg.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A.U.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother-Cord blood</td>
<td>0.13</td>
<td>0.23</td>
<td>0.53</td>
<td>0.59</td>
<td>0.11</td>
<td>0.66</td>
</tr>
<tr>
<td>Mother-Infant(1 ys)</td>
<td>0.08</td>
<td>0.10</td>
<td>0.99</td>
<td>0.76</td>
<td>0.13</td>
<td>0.04*</td>
</tr>
<tr>
<td>Cord blood- Infant(1 ys)</td>
<td>0.68</td>
<td>0.60</td>
<td>0.05</td>
<td>0.51</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother-Cord blood</td>
<td>0.04</td>
<td>0.15</td>
<td>0.79</td>
<td>0.42</td>
<td>0.13</td>
<td>0.72</td>
</tr>
<tr>
<td>Mother-Infant(1 ys)</td>
<td>0.24</td>
<td>0.56</td>
<td>0.76</td>
<td>0.76</td>
<td>0.19</td>
<td>0.08</td>
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<tr>
<td>Cord blood- Infant(1 ys)</td>
<td>0.66</td>
<td>0.63</td>
<td>0.12</td>
<td>0.43</td>
<td>0.78</td>
<td>0.55</td>
</tr>
</tbody>
</table>
## Food Allergens Sensitization

<table>
<thead>
<tr>
<th></th>
<th>Egg</th>
<th>Milk</th>
<th>Salm</th>
<th>wheat</th>
<th>Pean</th>
<th>Soy</th>
<th>Alm</th>
<th>Crab</th>
<th>Shrm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A.U.</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Mother-Cord blood</td>
<td>0.94</td>
<td>0.42</td>
<td>0.64</td>
<td>0.30</td>
<td>0.43</td>
<td>0.91</td>
<td>0.55</td>
<td>0.05*</td>
<td>0.17</td>
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<tr>
<td>Mother-Infant(1 ys)</td>
<td>0.04</td>
<td>0.15</td>
<td>0.59</td>
<td>0.04</td>
<td>0.72</td>
<td>0.17</td>
<td>0.59</td>
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<td>0.79</td>
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<td>Cord blood- Infant(1 ys)</td>
<td>0.69</td>
<td>0.28</td>
<td>0.08</td>
<td>0.25</td>
<td>0.65</td>
<td>0.82</td>
<td>0.40</td>
<td>0.88</td>
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<td></td>
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</tr>
<tr>
<td>Mother-Cord blood</td>
<td>0.82</td>
<td>0.49</td>
<td>0.52</td>
<td>0.48</td>
<td>0.32</td>
<td>0.71</td>
<td>0.53</td>
<td>0.06</td>
<td>0.22</td>
</tr>
<tr>
<td>Mother-Infant(1 ys)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.59</td>
<td>0.03</td>
<td>0.72</td>
<td>0.15</td>
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<td>0.98</td>
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</tbody>
</table>
A significant level of agreement was observed for the 20 examined allergen-specific IgE levels in the blood samples of mothers, cord blood and infants at 12 months of age. The findings from this study suggest that the influence of maternal allergen-specific IgE levels on infant immune response might occur at birth and then wane in infants at 12 months of age in an Asian study sample.
Next Generation Innovations for in vitro IgE diagnosis

1. Purified Allergen and recombinant allergenic peptides
2. Microarray and multiplex testing
3. Biosensors and Quantum dots detecting systems
4. Point-of-Care testing
5. Application to test Biomarkers for precision medicine
From source to allergen

Allergen source

Allergen extract

Natural allergens

Purified from source

Recombinant allergens

Biotechnologic manufacturing in bacteria, yeast, plants

- Test solutions
- Lyophilisates for OIT, PT
- SCIT
Allergen-Diagnosis Protein Chip
Technological Innovations for High-Throughput Approaches to In Vitro Allergy Diagnosis

Martin D. Chapman¹ · Sabina Wuenschmann¹ · Eva King¹ · Anna Pomés¹
SPRi microarray configured to detect IgE binding to Ara h2 peptide, BXG, and anti-IgE using antibody-loaded magnetic particles (MP-Ab2) for capture and signal amplification.
Schematic showing the principle of immobilization on a biosensor.
IN VITRO ALLERGY DIAGNOSIS-CELLULAR ALLERGY TESTING
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<th>Subjects' Sera</th>
<th>Assay Tested Specificities</th>
<th>Color on Graphs</th>
<th>ISAC 89°</th>
<th>ABA*</th>
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Direct ex vivo analysis of allergen-specific CD4⁺ T cells using tetramers
Point-of-Care Testing

Convention Laboratory Testing  VS.  Testing in Your Office
Potential biomarkers in relationship to asthma, beginning with prenatal risk for disease and extending to markers associated with treatment selection. JACI 2016, 137:1317–1324
The concept of longitudinal biomarkers (BM) in the management of Allergy at different time points throughout the natural history of the disease.


**Primary prevention of the disease together with early diagnosis.**

**Predict responses, and/or adverse reactions to treatment and can guide targeted, endotype-driven interventions with safety profile.**

**Prognostic biomarkers relate to disease severity, disease flares, or occurrence of remission.**
In summary

1. The technology of in vitro IgE diagnosis is undergoing a renaissance
2. Innovation technologies developed involving multiplex, microarrays, biosensors and other point-of-care tests
3. Clinicians to take patient’s history, order specific allergens panel of choice to confirm the diagnosis.
4. Including additional biomarkers of asthma or anaphylaxis
5. Point-of-care tests provide immediate feedbacks in clinical setting
6. Personized medicine applied in allergy fields.