Introduction to new concepts in diagnosis of allergy diseases –
Basis of allergy diagnosis
Diagnosis of allergy – requirements

Allergen-specific IgE – not just another immunoassay

Concentration of IgE antibodies in blood is extremely low in comparison to most other substances assayed, even in highly sensitized individuals. Each main allergen (pollen, food etc.) contains large numbers of different allergenic components (proteins). A test must be sensitive enough to find ALL components. The assay must be independent of influence from other immunoglobulin classes. Most allergen sources are complex mixtures of biological material. To achieve a precise and reproducible test system, control of the source material is mandatory, both in content and in allergenic activity, thus reassuring lot to lot reproducibility.

Prerequisites for a quantitative specific IgE ImmunoCAP test:

- Excess of allergen (Allergon)
- Precision - Reproducibility
- Linearity
- Calibration traceable to WHO
Diversity of IgE specificities

Allergogram, Peanut

H. Perborn, 1997
Unparalleled Precision

ImmunoCAP Consistency over 25 years

Pharmacia CAP System 1987, kU/A/L

70 sera 11 allergens

0.1 kU_A/l. LoD = LoQ

0.1

Sample range: 0.08 – 3.8 kU_A/l

100 kU_A/l

<table>
<thead>
<tr>
<th>Platform</th>
<th>Within</th>
<th>Between</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phadia 100</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Phadia 250</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Phadia 1000</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Ref: Internal studies
ImmunoCAP – results you can trust

- Correct measurement of low levels of allergen specific IgE antibodies
- Low non-specific binding

Dilution of 27 samples of 9 allergens in ImmunoCAP Specific IgE

Pool of healthy blood donors tested with 584 single allergens (all allergen lots between 2007-2010 tested)
Prediction – how long to tolerance?

• Median time from first reaction after having eaten egg until tolerance was:
  • Children with low IgE-levels (<1.98 KU/L) - 27 mon
  • Children with high IgE-levels (>1.98 KU/L) - 59 mon
  • Boyano-Martinez et al JACI 2002:110:304-9

"No association has been found between the size of the cutaneous skin prick test reaction or s-tot IgE level and tolerance of the food"
• 1 kU/L specific IgE is equal to 1 kU/L total IgE (=2.42 ng/ml) when measured with ImmunoCAP and are both traceable to WHO IgE reference 75/502.

• Published quantitative relationships with clinical outcomes and decision levels in kU/L for specific IgE established with ImmunoCAP cannot be used for interpretation of results obtained with other systems.
Phadia Systems
Unmatched allergy portfolio

• Specific IgE, Phadiatop, Phadiatop Infant
• total IgE, specific IgG, specific IgG4, specific IgA
• Tryptase,
• ECP
• >600 complete allergens
• > 100 allergen components
• ca 800 allergens for research use
## Broad autoimmune disease panel

- Autoimmunity tests for > 20 clinical indications
- Covering all relevant markers for connective tissue diseases, gastro intestinal diseases, vasculitis, rheumatoid arthritis and antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Condition</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rheumatoid Arthritis</strong></td>
<td>EliA CCP, EliA RF IgM, EliA RF IgA, EliA RF IgG Research</td>
</tr>
<tr>
<td></td>
<td>EliA Cardiolipin IgG, EliA Cardiolipin IgM, EliA Cardiolipin IgA, EliA β2 Glycoprotein I IgG, EliA β2 Glycoprotein I IgM, EliA β2 Glycoprotein I IgA</td>
</tr>
<tr>
<td><strong>Antiphospholipid syndrome</strong></td>
<td>EliA CTD Screen, EliA Symphony, EliA dsDNA, EliA Sm, EliA Rib P, EliA Ro, EliA Ro60, EliA Ro52, EliA La, EliA U1RNP, EliA RNP70, EliA Scl-70, EliA CENP, EliA Fibrillarin, EliA PM-Scl, EliA Jo-1, EliA Mi-2, EliA PM-Scl</td>
</tr>
<tr>
<td><strong>Connective tissue diseases (CTD)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>ANCA-associated diseases and GBM disease</strong></td>
<td>EliA PR3 S, EliA MPO S, EliA GBM</td>
</tr>
<tr>
<td><strong>Inflammatory bowel diseases</strong></td>
<td>EliA Calprotectin</td>
</tr>
<tr>
<td><strong>Thyroid diseases</strong></td>
<td>ImmunoCAP Thyroid Peroxidase, ImmunoCAP Thyroglobulin</td>
</tr>
<tr>
<td><strong>IgA Deficiency</strong></td>
<td>EliA Anti-IgA</td>
</tr>
</tbody>
</table>
ImmunoCAP help identify the allergen(s) that add up to symptoms

Suspicion of allergy:

Confirm / identify relevant allergens:

Test interpretation:

Patient management:

### Allergy-like symptoms + Case history

**ImmuNoCAP Complete Allergen testing with relevant tree, grass and weed pollens** or Phadiatop

<table>
<thead>
<tr>
<th>0.1</th>
<th>&gt;100 kU/l</th>
</tr>
</thead>
</table>

Risk of symptomatic allergy increases with increase in IgE antibody level.  

**Negative (<0.1 kU/l)**: Symptoms are probably not caused by IgE mediated allergy.

**Positive (≥0.1 kU/l)**: Symptoms are probably caused by IgE mediated allergy.

### Risk of symptomatic allergy increases with increase in IgE antibody level.**1,2**


**Specific Immunotherapy (SIT)?**

- ImmunoCAP Allergen Components help you identify patients and allergens for improved SIT outcome.

** Regular follow-up testing to evaluate changes in allergy profile**

- Continue examination: Look for other causes.

- Treat the allergies:
  - Provide an allergen avoidance plan to keep patient below symptom threshold.
  - Prescription of relevant medications e.g. antihistamines.

**Factors to consider for a final diagnosis:** age, degree of atopy, allergen load, type of sensitizing allergens, previous symptoms, other triggering factors.

* Symptom profile containing relevant allergens. Local adaptation with respect to age and regional differences is recommended.

**Symptoms are probably not caused by IgE mediated allergy.**
Phadiatop Infant is especially designed for children

Reference: Sigurs N et al. Sensitization in childhood atopic disease identified by Phadebas RAST serum IgE and Phadiatop. Pediatric Allergy Immunology 1990; 74-78
• ECP is released from the activated eosinophils during the inflammation process characteristic of asthma. Testing ECP can be useful in asthmatics to:
  • **Monitor the inflammation**
  • **Guide corticosteroid treatment**
  • Values below 15 ug/l are regarded as normal, but the patient should be his own control

**Cellular Markers**

**ECP Eosinophil Cationic Protein**

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**Monitoring the tapering down of steroid therapy utilising serum ECP**

ImmunoCAP Tryptase

- Elevated baseline Tryptase – risk factor for severe reactions

- Risk patients with severe reactions to insect venoms and drugs

- Elevated baseline Tryptase levels indicate an increased mast cell burden and may serve as a risk factor for severe reactions during surgery.

- Baseline Tryptase should be measured before starting Immunotherapy. (EAACI Position paper 2005).

- Up to 25% of patients with severe reactions to insect stings have mastocytosis.

- Tryptase levels correspond to severity in mastocytosis

- Tryptase a marker for relapse in Acute Myeloid Leukemia
Phadia Laboratory Systems from S to XXL
Automation and quality in allergy & autoimmunity parameter testing
Ideal for medium sized laboratories 250 - 350 tests/day
ImmunoCAP and EliA Well technology
Highly automated, e.g
Continuous random access
Main Frame connection
Quality / Surety Positive identification
Throughput: 60 tests/hour
Additional throughput using option of over-night runs
Results in one-minute intervals
Continuous loading of samples
Automatic sample dilution
6 methods
50 patient sample tubes (5 racks),
180 ImmunoCAP carriers, (38 stat positions)
Automatic “wake-up”
Automatic shut down after last sample
Automatic barcode reading of ImmunoCAP carriers, sample racks/tubes
Paediatric sample tubes possible to define
Manual barcode reading of all other reagents for full traceability
Reflex testing
Reagent load list printed on demand
Remote monitoring
Patient database, capacity:
   100 000 patient name
   500 000 samples
   1 000 000 test results
Patient follow-up
Extended stock management
   Total laboratory stock, Print orders on demand
A precise, quantitative allergen-specific IgE antibody test can detect IgE antibody formation at an early stage, even before symptoms have evolved. This enables the physician to prescribe the best strategy for managing the disease and to avoid the development of a severe chronic condition.

**ImmuNoCAP™ gives real new opportunities – for diagnosis, prognosis and follow-up of your patient**

- **Diagnosis**
  - The sensitizing allergen(s) can accurately be confirmed, and the quantitative IgE antibody levels enable the physician to recommend relevant allergen avoidance.

- **Prognosis**
  - Early sensitization can be predictive of future allergies. The higher the IgE antibody level, the higher the risk. IgE antibodies to inhalant allergens prior to symptoms also predict disease progression.

- **Follow-up**
  - Allergen-specific IgE test results help monitoring the effects of avoidance and immunotherapy treatment, and the changes in the patient's sensitization status over time.

**ImmuNoCAP™ quantitative IgE antibody results help explain the progression of allergic disease**
Causing agents

- Faeces
- Dander, Urine, Saliva
- Pollen
An allergen source...
…of which only a few are allergenic

Allergenic molecule = component
From allergen source to component
Specific components
- indicate genuine sensitization

Gal d 1 = *Gallus domesticus*, allergen # 1

Honey bee
Api m 1

Egg
Gal d 1

Timothy
Phl p 1

Birch
Bet v 1

Peanut
Ara h 2
Components are proteins with four important aspects

- Specific
- Cross-reactive
- Different stabilities
- Different amounts

Extract and components - for an improved profile
Understanding the nature of cross-reactivity

- Anti-A antibodies will react in an identical fashion with allergens 1 and 2.
- Anti-B and Anti-D antibodies recognize unrelated epitopes on the two allergens and will show no cross-reactivity.
- Anti-C antibodies react strongly with the homologous epitope C and cross-react weakly with heterologous epitope E.

Similar components may be present also in distantly related species.
### Molecular basis of cross reactivity

<table>
<thead>
<tr>
<th></th>
<th>birch</th>
<th>apple</th>
<th>soy</th>
<th>celery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet v 1</td>
<td>Mal d 1</td>
<td>Gly m 4</td>
<td>Api g 1</td>
<td></td>
</tr>
<tr>
<td>Sequence identity</td>
<td>66 %</td>
<td>49 %</td>
<td>40 %</td>
<td></td>
</tr>
<tr>
<td>Structural identity</td>
<td>71 %</td>
<td>60 %</td>
<td>47 %</td>
<td></td>
</tr>
</tbody>
</table>
Allergy diagnostics before saw only ...
Allergy diagnostics now tell much more ...
Protein stability

Labile protein ➔ Local reaction

Stable protein ➔ Systemic reaction
PR-10-Proteins

- Heat labile
- Cooked and processed foods are often tolerated
- Associated with local symptoms, such as OAS
- High sensitization rate in northern Europe

Bet v 1
Betula verrucosa

Kleine-Tebbe et al. Allergo J 2010
Lipid Transfer Proteins

- Proteins stable to heat and digestion, primarily localized in the peel
- Reactions also to cooked and processed foods
- Often associated with severe systemic reactions additional to OAS
Is it three genuine sensitizations?

Timothy
Mugwort
Birch
### Common clinical practice

**Patient**

**Rudolf, 13 years**

**Previous**

**Anamnesis**
- Milk allergy and eczema as an infant
- Both parents atopic

**At 13 years**

**Clinical History**
- Rhinoconjunctivitis during March-Oct
- Asthma after heavy exercise

**SPT & sIgE to birch**
- +3
- 7.9 kU/l

**SPT & sIgE to timothy**
- +4
- 23

**SPT & sIgE to mugwort**
- +3
- 6.5

**Diagnosis**

- Birch, timothy and mugwort allergy

**Recommendation:**
- SIT with birch and grass pollen extracts

70% of 48 clinicians suggested SIT with grass and birch extracts +/- mugwort
Using components in clinical practice

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rudolf, 13 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous</td>
<td></td>
</tr>
<tr>
<td>Anamnesis</td>
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<td>SPT &amp; sIgE to birch</td>
<td>+3 7.9kU/l</td>
</tr>
<tr>
<td>SPT &amp; sIgE to timothy</td>
<td>+4 23</td>
</tr>
<tr>
<td>SPT &amp; sIgE to mugwort</td>
<td>+3 6.5</td>
</tr>
<tr>
<td>Components</td>
<td></td>
</tr>
<tr>
<td>Phl p 1</td>
<td><strong>6.5</strong></td>
</tr>
<tr>
<td>Phl p 5</td>
<td><strong>11.2</strong></td>
</tr>
<tr>
<td>Phl p 7</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Phl p 12</td>
<td><strong>4.9</strong></td>
</tr>
<tr>
<td>Bet v 1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Art v 1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Diagnosis:</td>
<td>Timothy allergy</td>
</tr>
<tr>
<td>Recommendation:</td>
<td>SIT with timothy pollen extract</td>
</tr>
</tbody>
</table>

Now 80 % of the clinicians suggested SIT with timothy extract only
Fig. 4. Clinically relevant sensitization to cross-reactive calcium-binding allergens. The patient lacks IgE antibodies and skin reactivity to Bet v 1 and Bet v 2 and birch pollen is therefore unlikely as a primary sensitizer. IgE reactivity to Phl p 1, Phl p 2 and Phl p 5 demonstrates sensitization to grass pollen and in addition there are IgE antibodies to the calcium-binding allergen Phl p 7. The immediate type skin reaction to birch pollen extract is apparently caused by IgE cross-reactivity between Phl p 7 and the homologous protein in birch pollen, Bet v 4.
If only cross-reactive markers are identified, further investigation is needed.
Modified from Heiss S …. Valenta R.
J Invest Dermatol. 1999
### The algorithm of efficiency prognosis of ASIT by grass pollens allergen extract

**Diagnosis of patient reactivity to the components:**

**Major pollen components:**
- Allergen g213 - rPhl p 1, rPhl p 5b

**Minor cross sensitive components:**
- Allergen g214 - rPhl p 7, rPhl p 12

<table>
<thead>
<tr>
<th>ASIT efficiency</th>
<th>rPhl p 1, 5 «+»</th>
<th>rPhl p 1, 5 «+»</th>
<th>rPhl p 1, 5 «-»</th>
</tr>
</thead>
<tbody>
<tr>
<td>rPhl p 7, 12 «-»</td>
<td>rPhl p 7, 12 «+»</td>
<td>rPhl p 7, 12 «+/-»</td>
<td></td>
</tr>
<tr>
<td><strong>High</strong></td>
<td><strong>Medium</strong></td>
<td><strong>Low</strong></td>
<td></td>
</tr>
</tbody>
</table>

### The algorithm of efficiency prognosis of ASIT by tree pollens allergen extract

**Diagnosis of patient reactivity to the components:**

**Major pollen components:**
- Allergen t215 - rBet v 1

**Minor cross sensitive components:**
- Allergen t221 - rBet v 2, rBet v 4

<table>
<thead>
<tr>
<th>ASIT efficiency</th>
<th>rBet v 1 «+»</th>
<th>rBet v 1 «+»</th>
<th>rBet v 1 «-»</th>
</tr>
</thead>
<tbody>
<tr>
<td>rBet v 2, rBet v 4 «-»</td>
<td>rBet v 2, rBet v 4 «+»</td>
<td>rBet v 2, rBet v 4 «+/-»</td>
<td></td>
</tr>
<tr>
<td><strong>High</strong></td>
<td><strong>Medium</strong></td>
<td><strong>Low</strong></td>
<td></td>
</tr>
</tbody>
</table>
Successful SIT relies on genuine sensitization

\[ \geq 50\% \text{ clinical improvement after 2 years of SIT} \]

- Only specific: 87%
- Cross-reactive/Specific: 60%
- Only cross-reactive: 18%

n=746 patients with birch and/or grass pollen SIT

Adapted from P. Schmid-Grendelmeier, 2010
Molecular Allergology helps you to

- Assess the clinical risk for reaction
- Explain symptoms due to cross-reactivity
- Identify the right patients for Specific Immunotherapy
### Characteristics of main egg white components

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Common name</th>
<th>Constitute (%)</th>
<th>Heat-treated</th>
<th>Digestive enzyme-treated</th>
<th>Allergenic Activity</th>
<th>Test code #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal d 1</td>
<td>Ovomucoid</td>
<td>11%</td>
<td>Stable</td>
<td>Stable</td>
<td>+++</td>
<td>f233</td>
</tr>
<tr>
<td>Gal d 2</td>
<td>Ovalbumin</td>
<td>54%</td>
<td>Unstable</td>
<td>Unstable</td>
<td>++</td>
<td>f232</td>
</tr>
<tr>
<td>Gal d 3</td>
<td>Conalbumin</td>
<td>12%</td>
<td>Unstable</td>
<td>Unstable</td>
<td>+</td>
<td>f323</td>
</tr>
<tr>
<td>Gal d 4</td>
<td>Lysozyme</td>
<td>3.4%</td>
<td>Unstable</td>
<td>Unstable</td>
<td>++</td>
<td>k208</td>
</tr>
</tbody>
</table>

Source: Benhamou AH, state of the art for egg allergy, Allergy 2010, 65:283–289.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Previous Anamnesis</th>
<th>At 2 years Anamnesis</th>
<th>SPT to egg Diagnosis Advice</th>
<th>At 5 years ( \text{slgE to egg} \ Food \text{ challenge} ) Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elin, 5 years</td>
<td><strong>Eczema</strong></td>
<td><strong>Urticaria, asthma</strong></td>
<td>+5 Egg allergy \ Avoid egg</td>
<td>25 kU(_A)/l No symptoms Tolerant to egg</td>
</tr>
<tr>
<td>Nour, 5 years</td>
<td><strong>Eczema</strong></td>
<td><strong>Urticaria, asthma</strong></td>
<td>+4 Egg allergy \ Avoid egg</td>
<td>20 kU(_A)/l Urticaria, cough, rhinitis Egg allergy</td>
</tr>
</tbody>
</table>

Using components in clinical practice

<table>
<thead>
<tr>
<th>Patient</th>
<th>Elin, 5 years</th>
<th>Nour, 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>At 7 months</strong></td>
<td>Eczema</td>
<td>Eczema</td>
</tr>
<tr>
<td>Anamnesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>At 2 years</strong></td>
<td>Urticaria, asthma</td>
<td>Urticaria, asthma</td>
</tr>
<tr>
<td>Anamnesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SPT to egg</strong></td>
<td>+5</td>
<td>+4</td>
</tr>
<tr>
<td><strong>sIgE to egg</strong></td>
<td>25 kU/ml</td>
<td>20 kU/ml</td>
</tr>
<tr>
<td><strong>sIgE to Ovomucoid</strong></td>
<td><strong>0.4 kU/ml</strong></td>
<td><strong>12 kU/ml</strong></td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>Tolerance likely Low risk for reactions</td>
<td>Egg allergy</td>
</tr>
<tr>
<td><strong>Advice</strong></td>
<td>Try cooked egg</td>
<td>Avoid egg</td>
</tr>
</tbody>
</table>

Improved diagnoses and altered advice
Recommended test profile

- **ImmunoCAP® COMPLETE EXTRACTS**
  - Egg white (f1)

- **ImmunoCAP® COMPONENTS**
  - Egg yolk (f75)
  - Gal d 1 + Gal d 2 + Gal d 3 + Gal d 4
    - (f233) (f232) (f323) (k208)
  - Further "investigation": chicken meat f83 (Suggestive of bird-egg syndrome)

- **Gal d 1, Ovomucoid**
  - Heat stable and highly allergenic
  - Risk for reaction to all forms of egg
  - High levels indicate persistent allergy

- **Gal d 2, Ovalbumin**
  - Heat labile
  - Most abundant egg white protein
  - Risk for clinical reaction to raw or slightly heated egg and certain vaccines

- **Gal d 3, Conalbumin**
  - Heat labile
  - Adds information on the complete egg sensitization profile
  - Risk for clinical reaction to raw or slightly heated egg

- **Gal d 4, Lysozyme**
  - Risk for clinical reaction to raw or slightly heated egg
  - Lysozyme is used as an additive in certain pharmaceutical products and foods
Better characterization of egg allergic patients

- Component testing helps in identifying children that are likely to outgrow their egg allergy; by following Gal d 1 IgE levels over time tolerance development may be detected.

- Low levels of specific IgE antibodies to Gal d 1 in early infancy suggest a good prognosis for outgrowing the egg allergy.

- In cases of low levels to Gal d 1, sensitization to egg components Gal d 2, Gal d 3 and/or Gal d 4 can cause clinical reactions to raw and slightly heated egg.

- Egg allergic patients sensitized to Gal d 2 may experience allergic reactions upon influenza and Yellow Fever vaccinations.

- Egg allergic patients with specific IgE antibodies to Gal d 4 may react when unexpectedly exposed to egg lysozyme in hidden forms in pharmaceutical products and foods.
Molecular Allergology helps you to

• Assess the clinical **risk** for reaction

• Explain symptoms due to **cross-reactivity**

• Identify the right patients for **Specific Immunotherapy**
Allergy test strategy

Dr's diagnosis:
- Symptoms
- Case history
- Physical examination

To confirm Diagnosis:
- ImmunoCAP™
- Symptom profiles

Positive

For Prognosis:
- ImmunoCAP™ Specific IgE

For Follow-up:
- ImmunoCAP™ Specific IgE
http://allergyeducation-ma.com/