MEDI-LAB Ltd. 2014 Meeting in Buda Mercure Hotel

November 18th 2014 Budapest, Hungary

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

ImmunoCAP Molecular Allergology

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Diagnosis of allergy – requirements

Allergen-specific IgE – not just another immunoassay

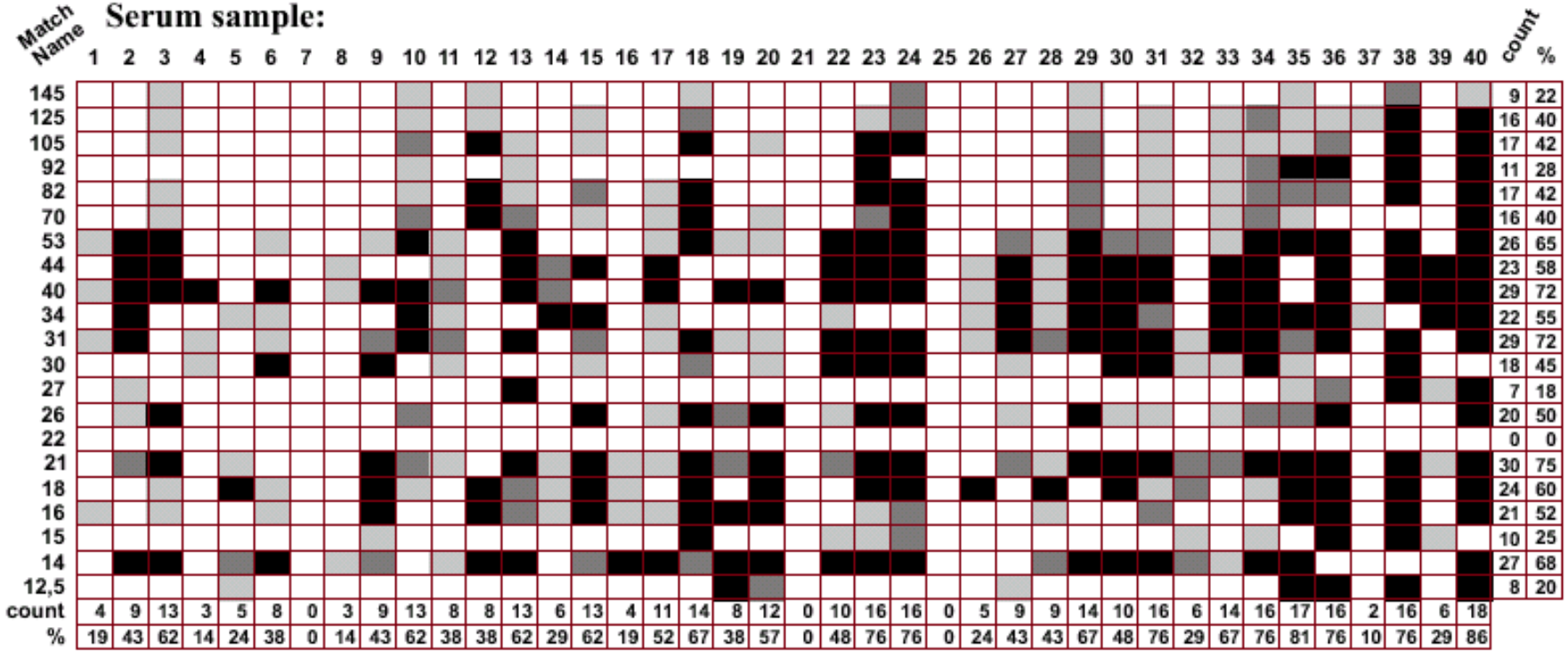
Concentration of IgE antibodies in blood is extremely low in comparison to most other substances assayed, even in highly sensitized individuals
Each main allergen (pollen, food etc.) contains large numbers of different allergenic components (proteins).
A test must be sensitive enough to find ALL components
The assay must 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

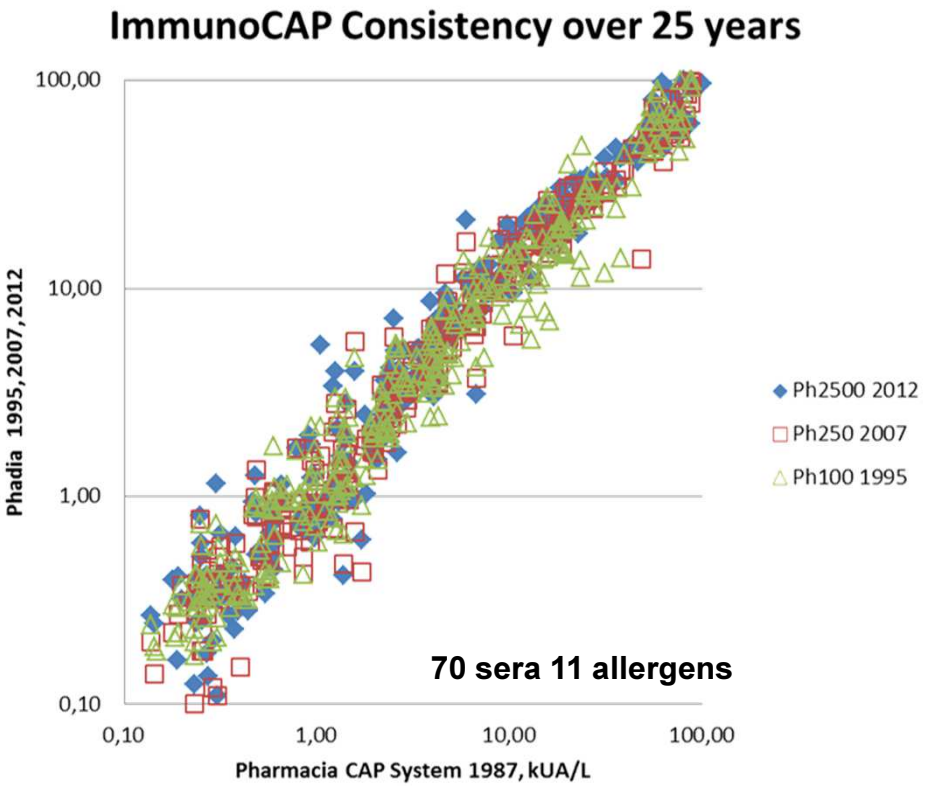
Diversity of IgE specificities

Allergogram, Peanut



H. Perborn, 1997

Unparalleled Precision



0.1 kU_A/l. LoD = LoQ

0.1 — 100 kU_A/l

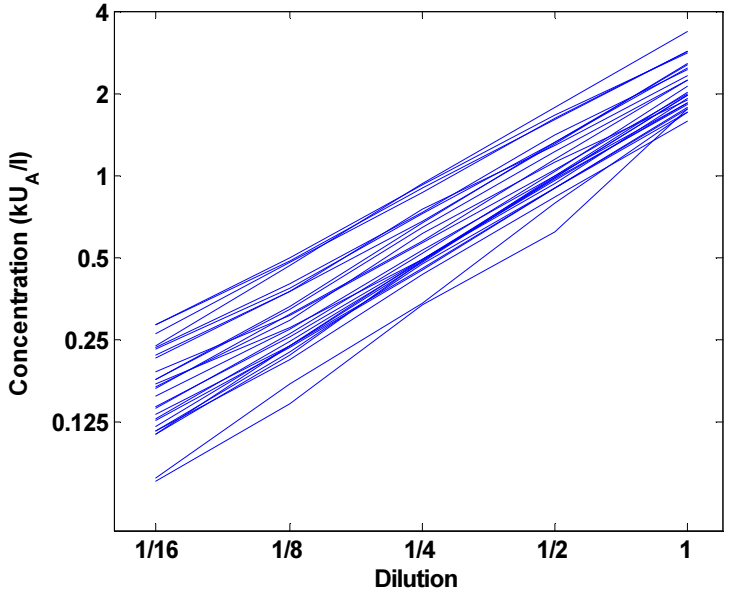
Sample range: 0.08 – 3.8 kU_A/l

	Pooled CV		
Platform	Within	Between	Total
Phadia 100	4	5	6
Phadia 250	4	4	6
Phadia 1000	4	6	6

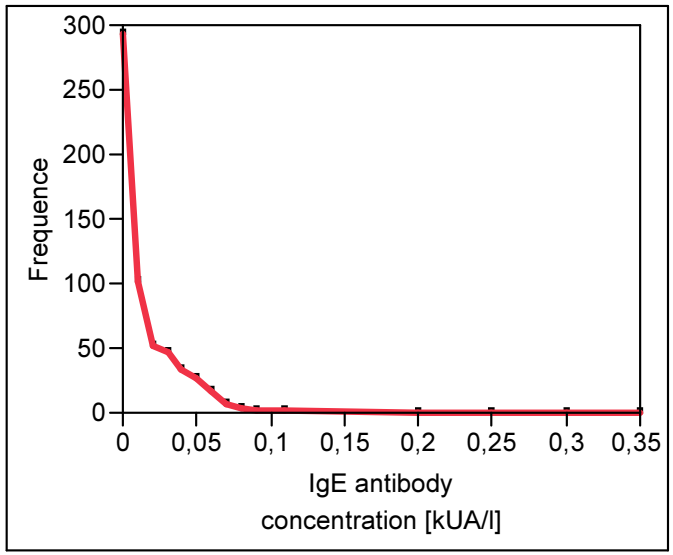
Ref: Internal studies

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



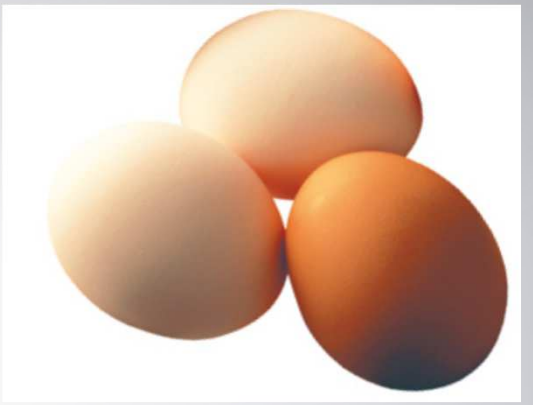
Pool of healthy blood donors tested with 584 single allergens (all allergen lots between 2007-2010 tested)

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_A/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

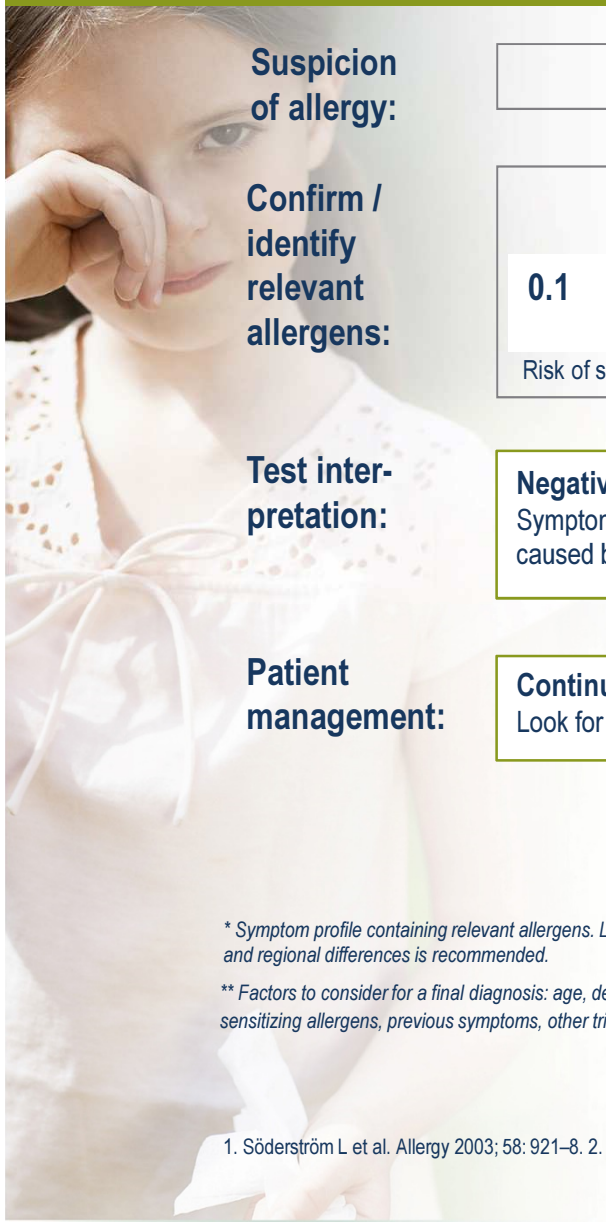
- Specific IgE, Phadiatop, Phadiatop Infant
- total IgE, specific IgG, specific IgG4, specific IgA
- Tryptase,
- ECP
- >600 complete allergens
- > 100 allergen components
- ca 800 allergens for research use

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

Rheumatoid Arthritis	EliA CCP, EliA RF IgM, EliA RF IgA, EliA RF IgG Research
Antiphospholipid syndrome	EliA Cardiolipin IgG, EliA Cardiolipin IgM, EliA Cardiolipin IgA, EliA ß2 Glycoprotein I IgG, EliA ß2 Glycoprotein I IgM, EliA ß2 Glycoprotein I IgA
Connective tissue diseases (CTD)	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
ANCA-associated diseases and GBM disease	EliA PR3^{S}, EliA MPO^{S}, EliA GBM
Celiac disease	EliA Celikey IgA, EliA Celikey IgG, EliA Gliadin IgA, EliA Gliadin IgG, EliA Gliadin^{DP} IgA, EliA Gliadin^{DP}, IgG
Inflammatory bowel diseases	EliA Calprotectin
Thyroid diseases	ImmunoCAP Thyroid Peroxidase, ImmunoCAP Thyroglobulin
IgA Deficiency	EliA Anti-IgA

ImmunoCAP help identify the allergen(s) that add up to symptoms



Suspicion of allergy:

Allergy-like symptoms + Case history

Confirm / identify relevant allergens:

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

0.1 → >100 kU_A/l

Risk of symptomatic allergy increases with increase in IgE anti-body level.[1,2]

Test interpretation:

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

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

Patient management:

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.

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

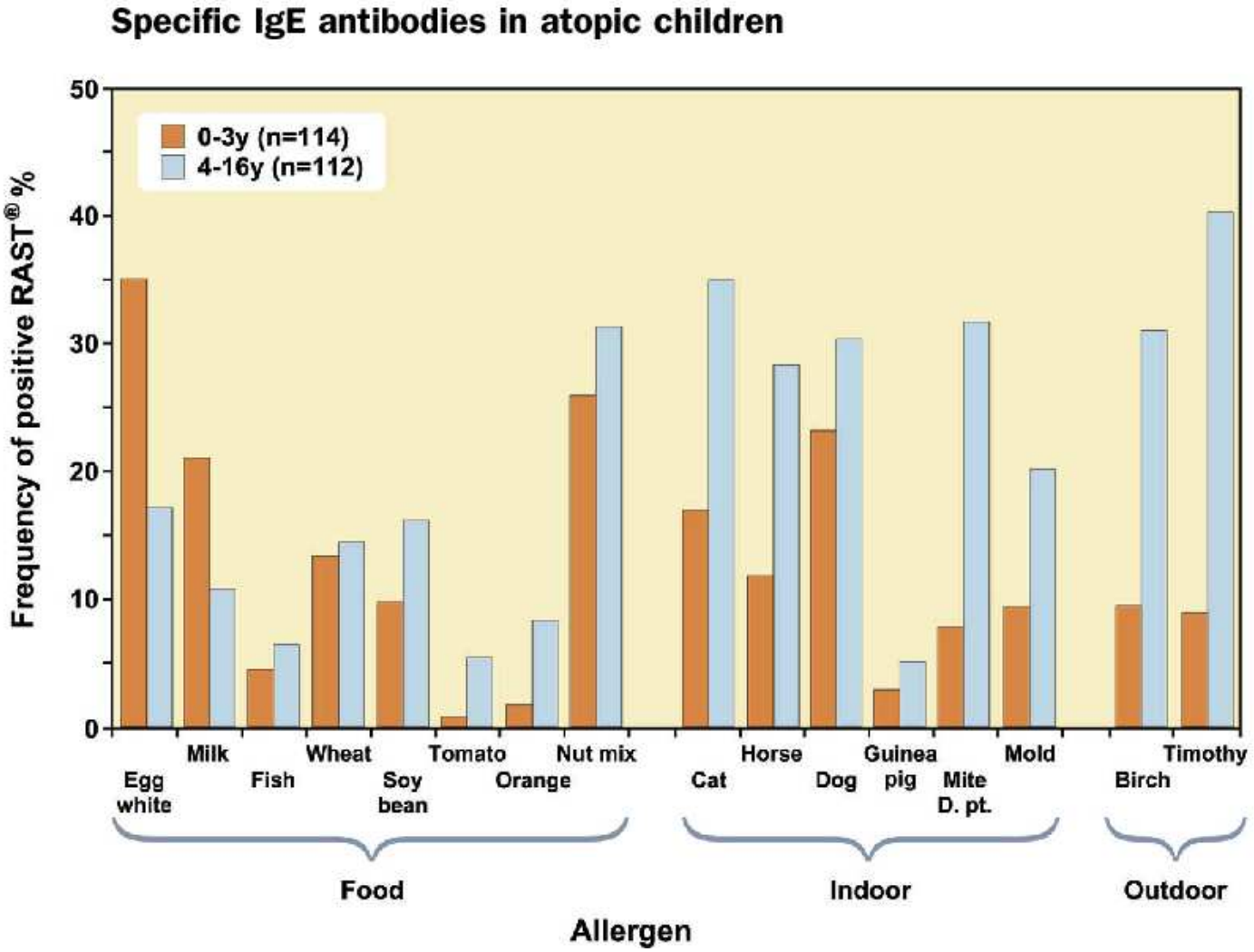
** Symptom profile containing relevant allergens. Local adaptation with respect to age and regional differences is recommended.*

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

1. Söderström L et al. Allergy 2003; 58: 921–8. 2. Sampson HA. J Allergy Clin Immunol 2001; 107: 891–6.

Thermo SCIENTIFIC

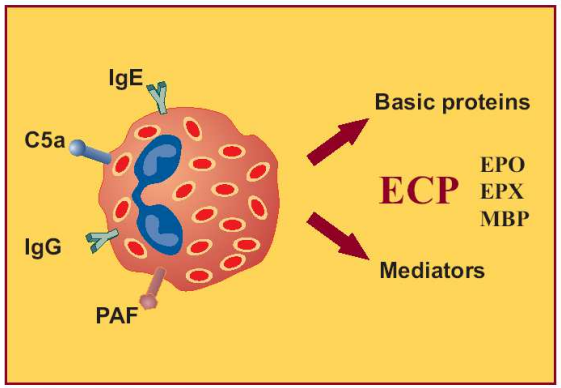
Phadiatop Infant is especially designed for children



Reference: **Sigurs N** et al. Sensitization in childhood atopic disease identified by Phadebas RAST serum IgE and Phadiatop. Pediatric Allergy Immunology 1990; 74-78

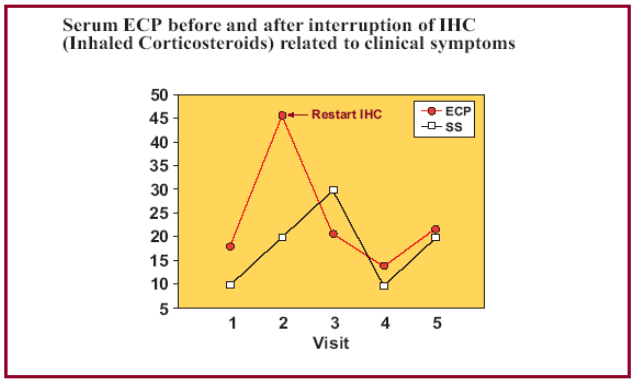
Cellular Markers
ECP Eosinophil Cationic Protein

- ECP is released from the activated eosinophils during the inflammation process charasteristic 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



Reference: Venge P. Allergy 1994; 49: 1-8

Monitoring the tapering down of steroid therapy utilising serum ECP



Reference: De Backer W et al. Am J Respir Crit Care Med 1995; 153(4): A336

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.
- Base line Tryptase should be measured before starting Immunotherapy. (EAACI Position paper 2005).
- Up to 25% of patient 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

Phadia
LAB
COMMUNITY

Phadia® IDM

Phadia®
LABORATORY SYSTEMS

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

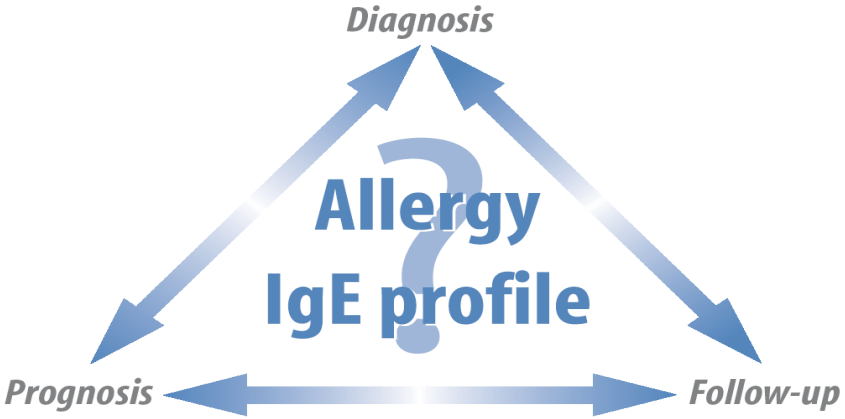
Extended stock management

- Total laboratory stock, Print orders on demand

ImmunoCAP™ gives real new opportunities

– for diagnosis, prognosis and follow-up of your patient

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.



Diagnosis

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

Prognosis

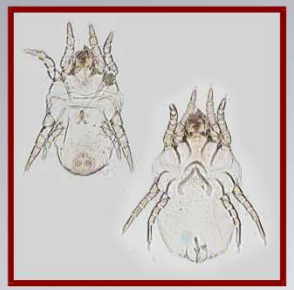
Early sensitization can be predictive of future allergies. The higher the IgE antibody level, the higher the risk. IgE antibodies to inhalant allergens prior to symptoms also predict disease progression.

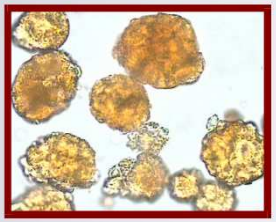
Follow-up

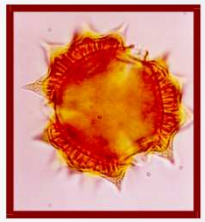
Allergen-specific IgE test results help monitoring the effects of avoidance and immunotherapy treatment, and the changes in the patient's sensitization status over time.

ImmunoCAP™ quantitative IgE antibody results help explain the progression of allergic disease

Causing agents



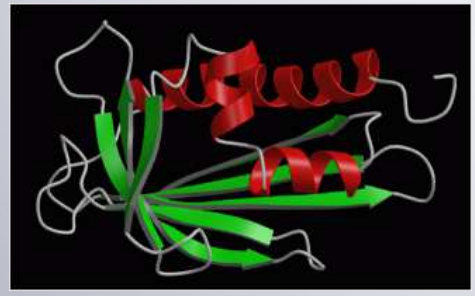


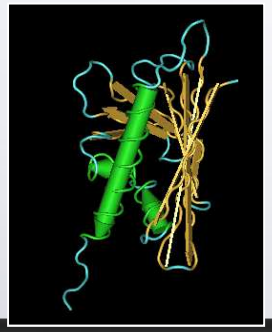


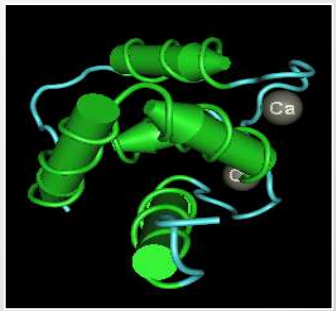
Faeces

Dander
Urine,Saliva

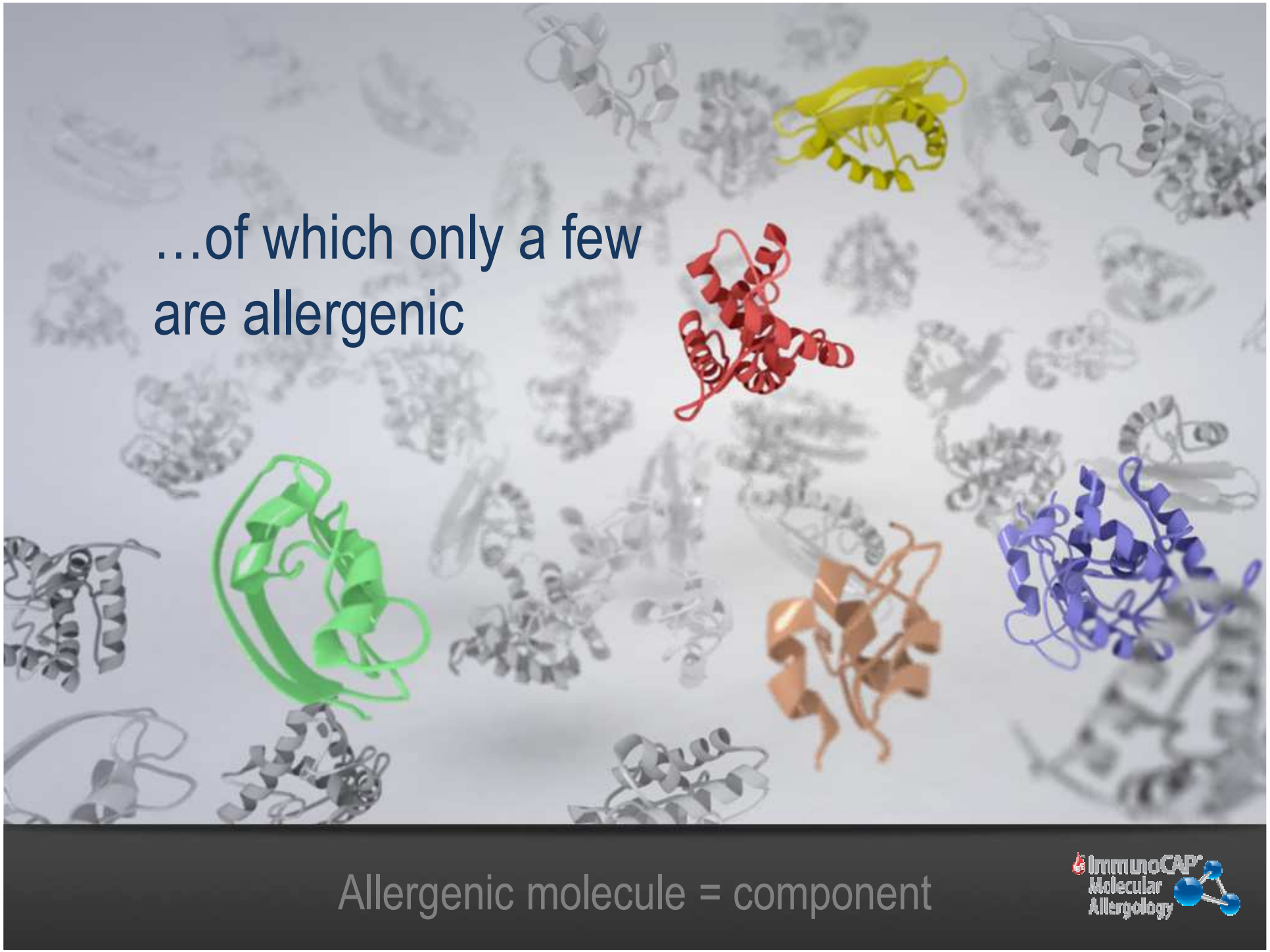
Pollen



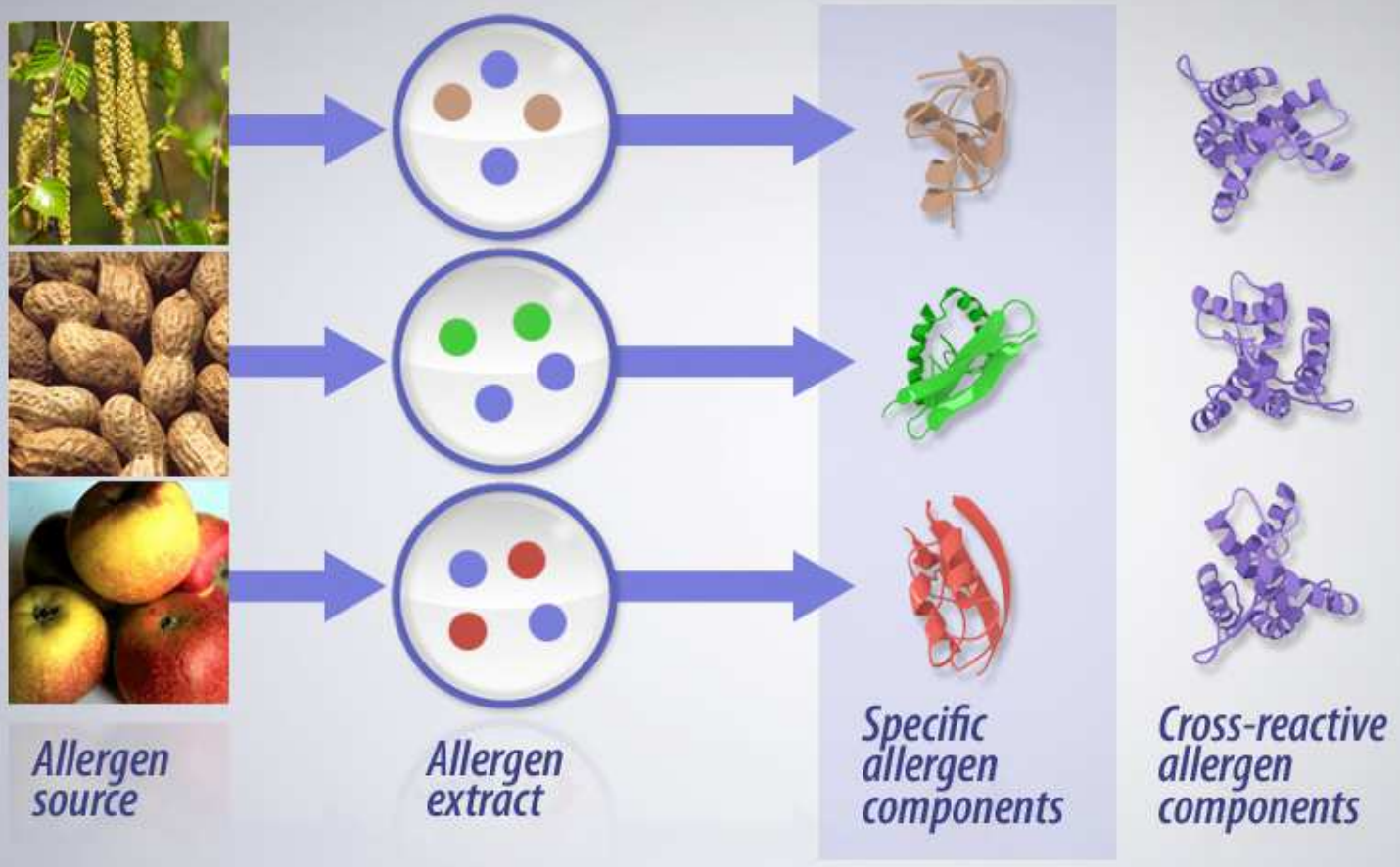




An allergen source…


…of which only a few
are allergenic
Allergenic molecule = component
ImmunoCAP
Molecular
Allergology

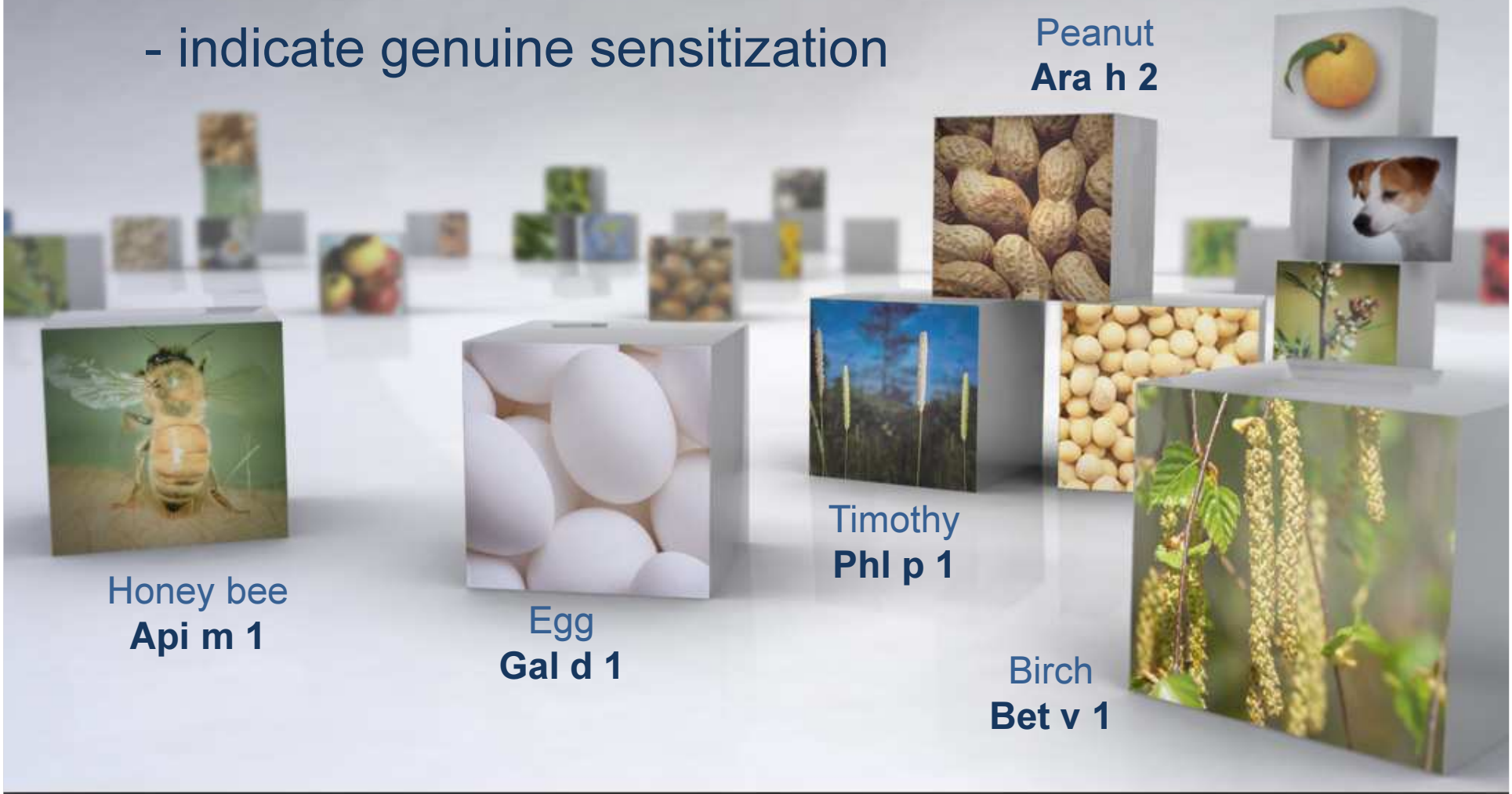
From allergen source to component



ImmunoCAP Molecular Allergology

Specific components

- indicate genuine sensitization



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

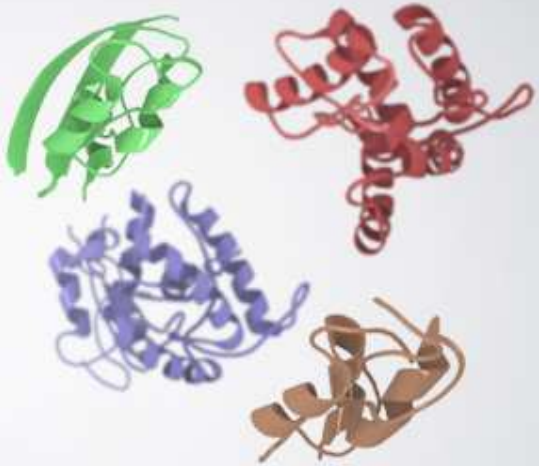
International Union of Immunological Societies Allergen Nomenclature Sub-Committee www.allergome.com

ImmunoCAP Molecular Allergology

Components are proteins with four important aspects

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

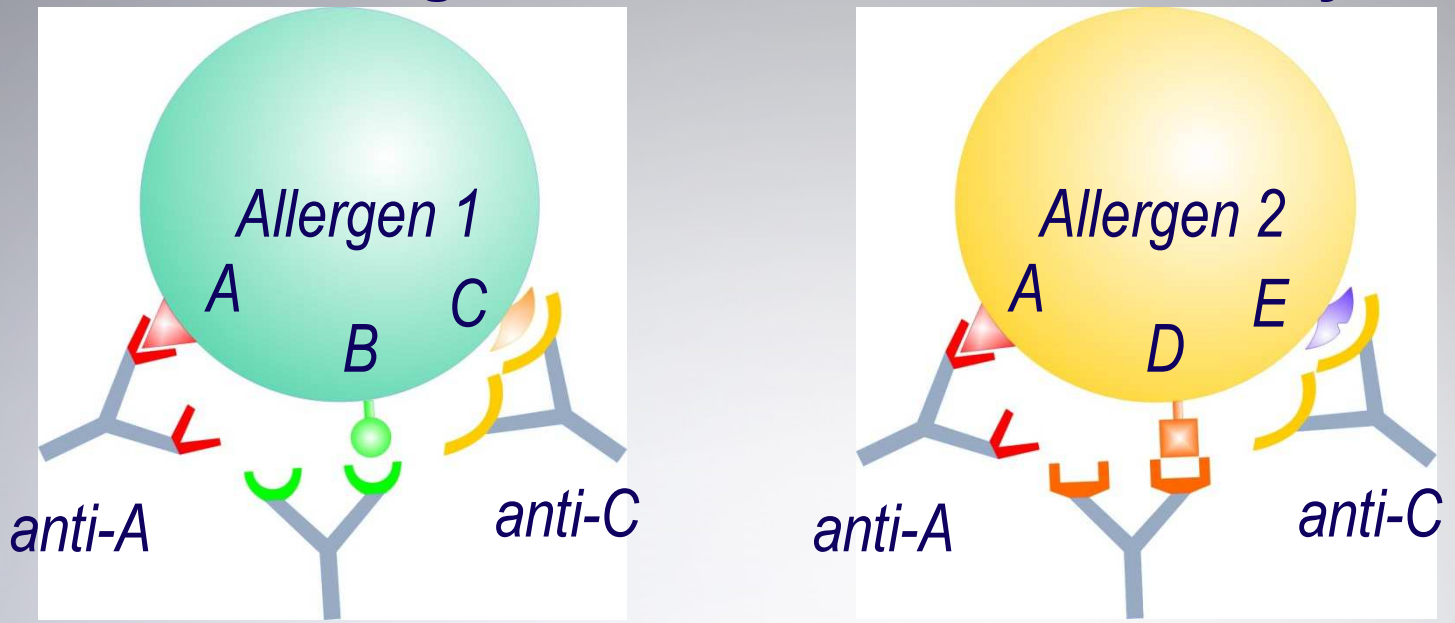
+



Extract and components - for an improved profile

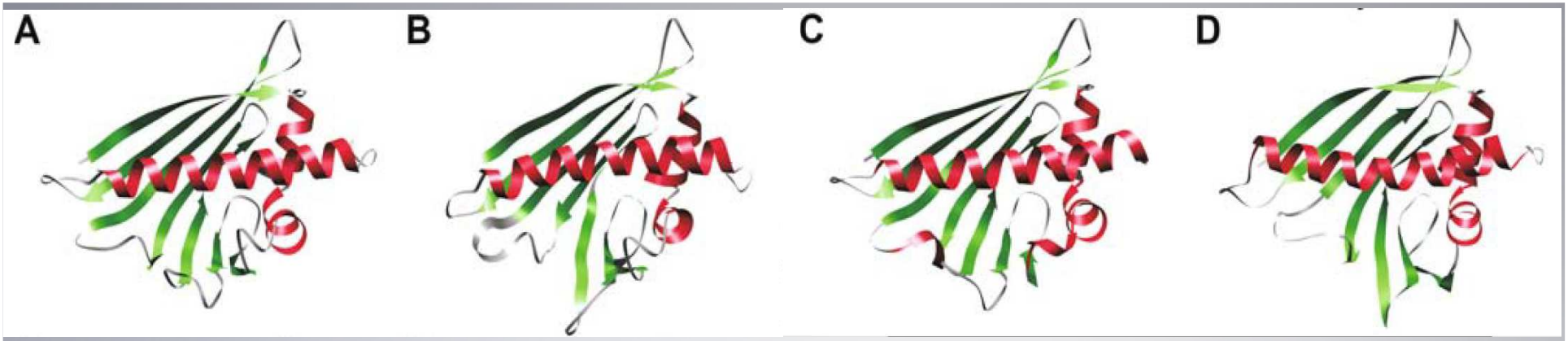
ImmunoCAP Molecular Allergology

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-ractivity**
- **Anti-C antibodies react strongly with the homologous epitope C and cross-react weakly with heterologous epiotpe E**

Similar components may be present also in distantly related species

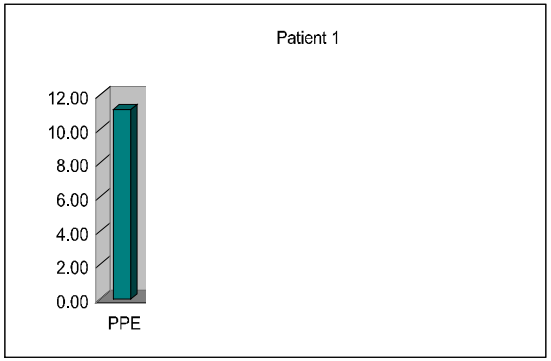


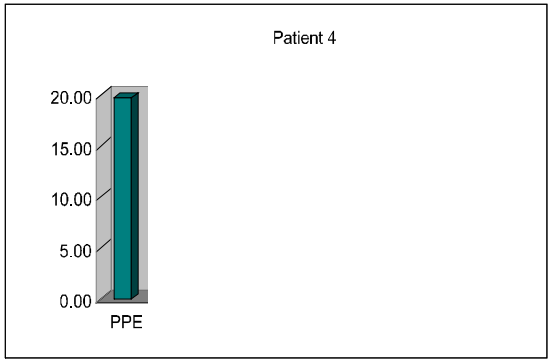
birch	apple	soy	celery
Bet v 1	Mal d 1	Gly m 4	Api g 1
Sequence identity	66 %	49 %	40 %
Structural identity	71 %	60 %	47 %

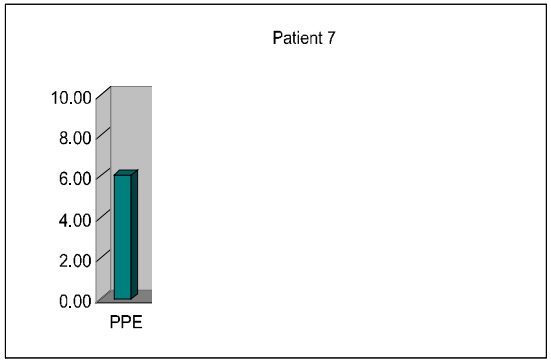
Molecular basis of cross reactivity

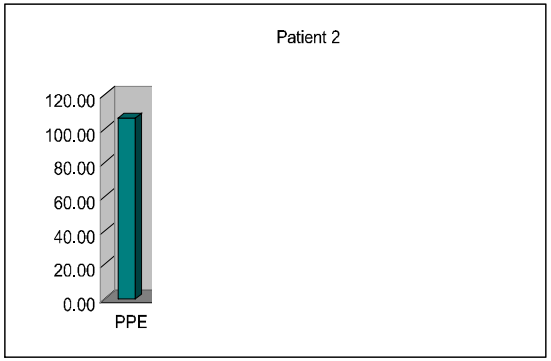
ImmunoCAP Molecular Allergology

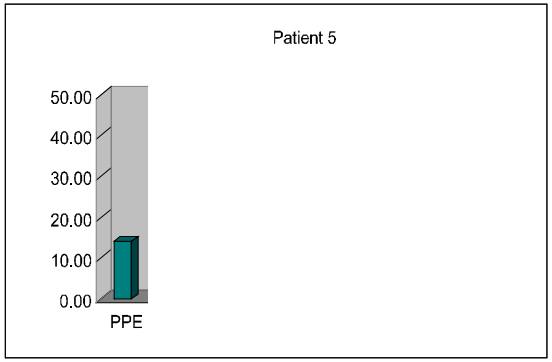
Allergy diagnostics before saw only ...

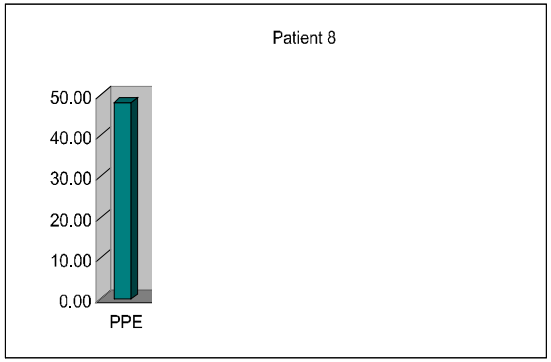


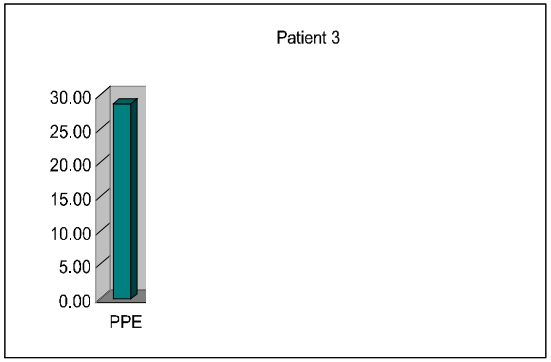


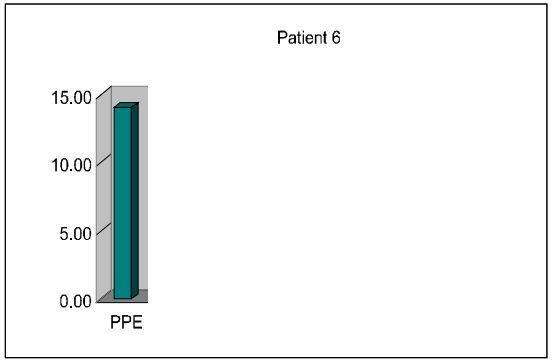


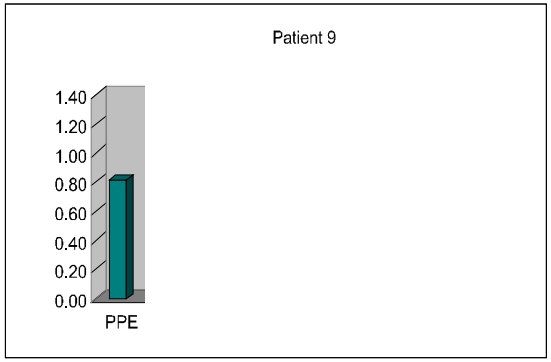




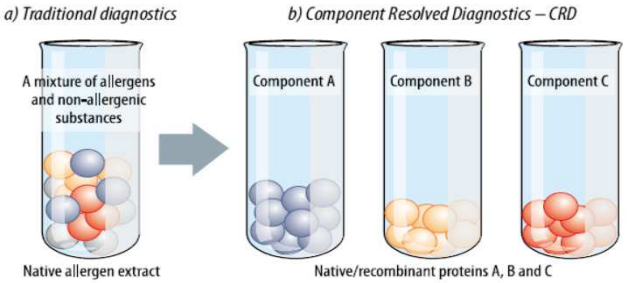


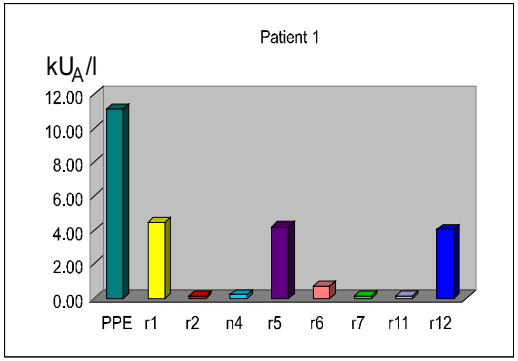


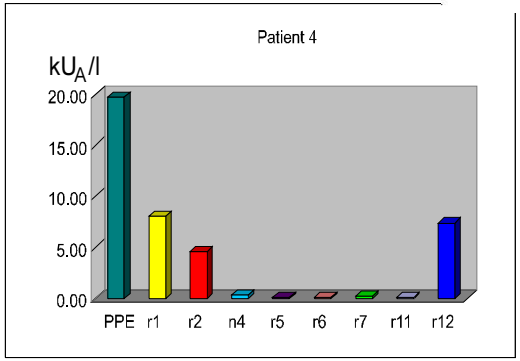


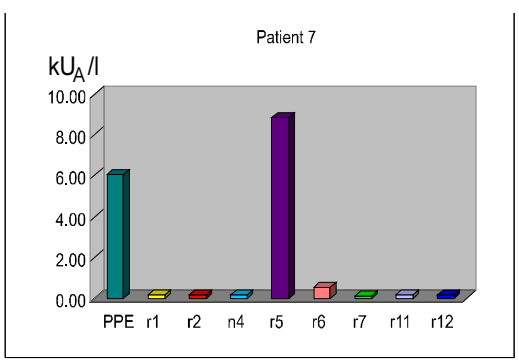


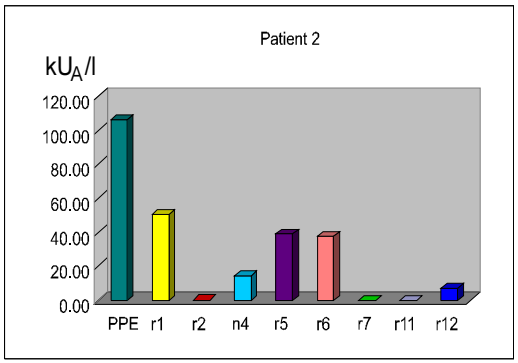
Allergy diagnostics now tell much more ...

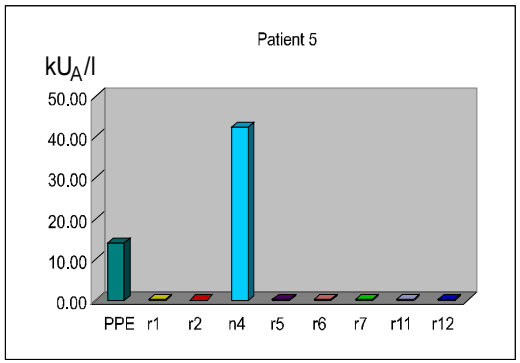


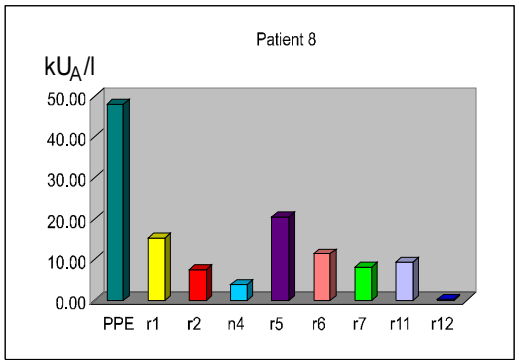


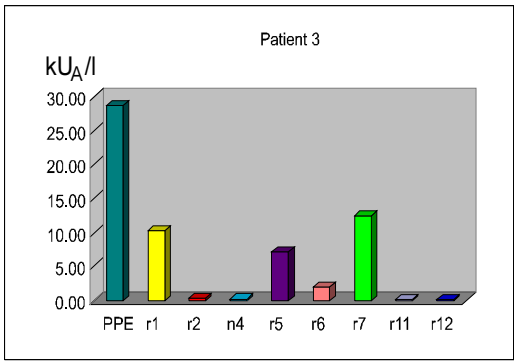


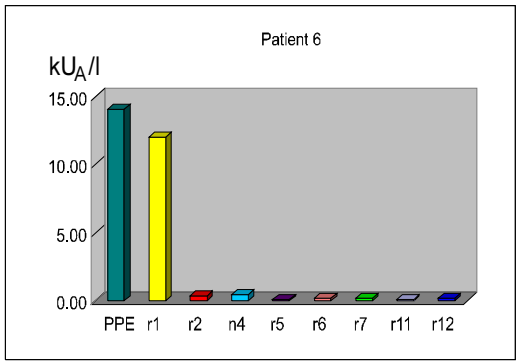


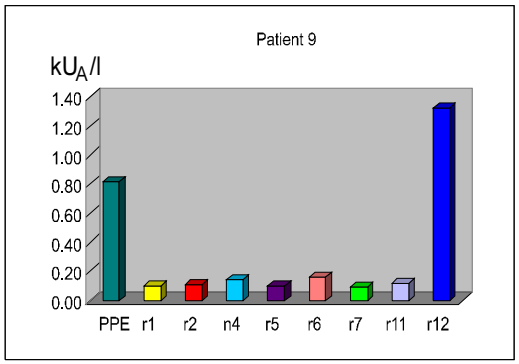








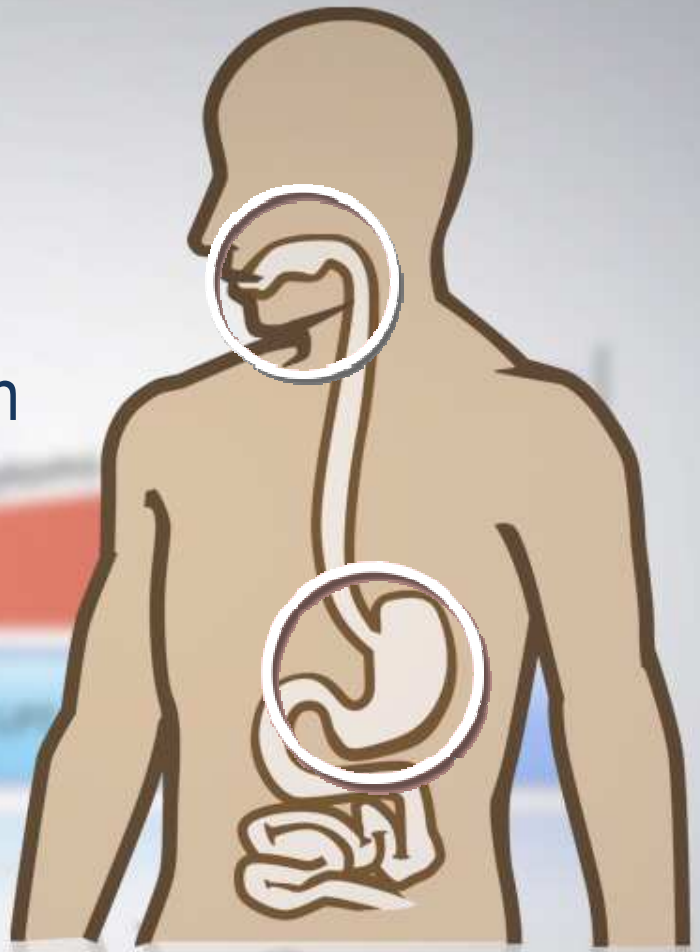




Protein stability

Labile protein ➡ Local reaction

Stable protein ➡ Systemic reaction



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PR-10-Proteins

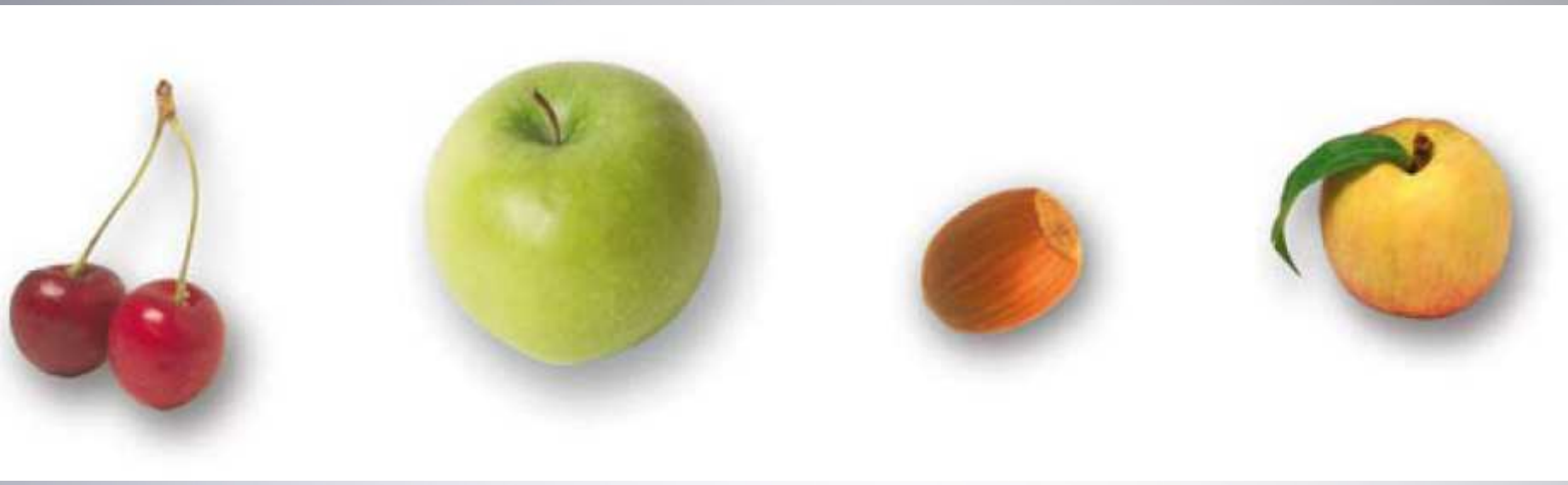
Bet v 1

Betula **v**errucosa

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

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?

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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_A/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

ImmunoCAP Molecular Allergology

Using components in 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	7.9kU$_A$/l
SPT & sIgE to timothy	+4	23
SPT & sIgE to mugwort	+3	6.5
Components	**Phl p 1**	**6.5**
	Phl p 5	**11.2**
	Phl p 7	<0.1
	Phl p 12	**4.9**
	Bet v 1	<0.1
	Art v 1	<0.1
Diagnosis:	**Timothy allergy**	
Recommendation:	**SIT with timothy pollen extract**	

Now 80 % of the clinicians suggested SIT with timothy extract only

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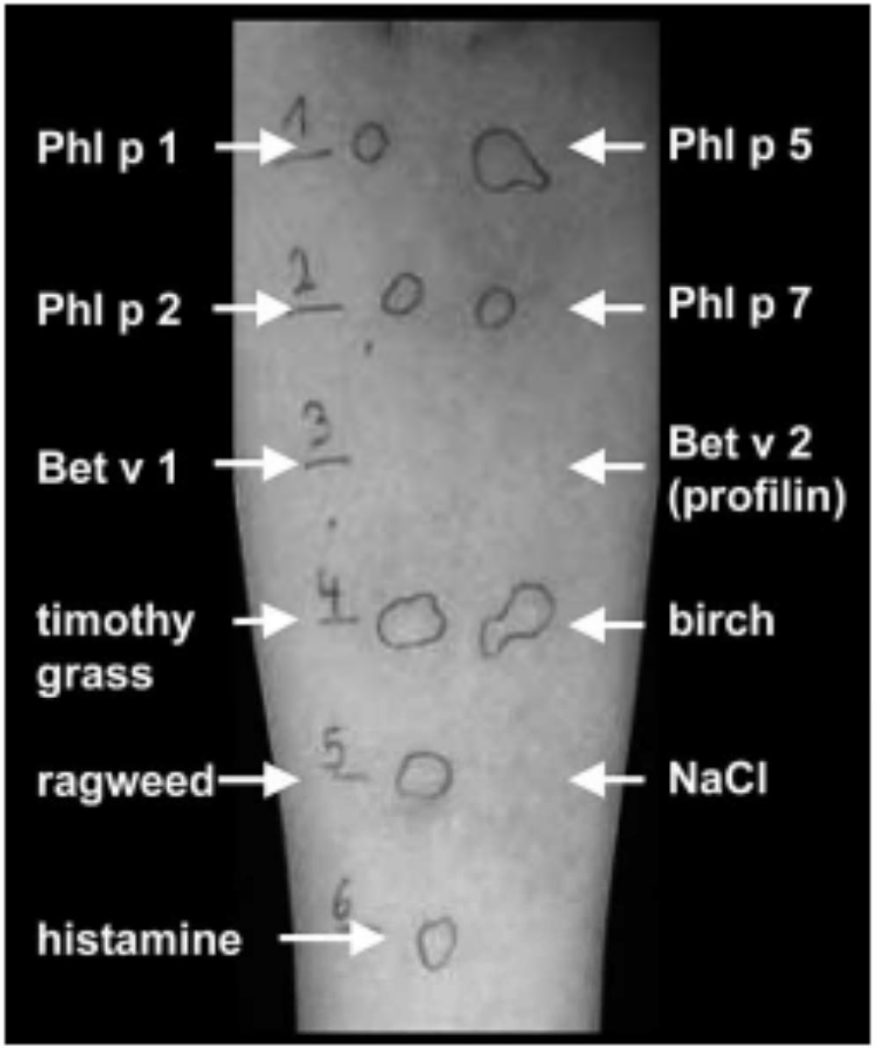

Fig. 4. Clinically relevant sensitization to cross-reactive calcium-binding allergens. The patient lacks IgE antibodies and skin reactivity to Bet v 1 and Bet v 2 and birch pollen is therefore unlikely as a primary sensitizer. IgE reactivity to Phl p 1, Phl p 2 and Phl p 5 demonstrates sensitization 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.

L. Kazemi-Shirazi,V.Niederberger B. Linhart,J Lidholm, D. Kraft, R. Valenta
Int. Arch Allergy Immunol 2002; 127:259-268

Indication for SIT with corresponding extract	Specific allergen components		Cross-reactive allergen components	
			Profilin	*Polcalcin*
	Phl p 1 *Phl p 5*		Phl p 12	Phl p 7
	Art v 1		Art v 4	Art v 5
	Bet v 1		Bet v 2	Bet v 4

If only cross-reactive markers are identified further investigation is needed

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Birch pollen extract

⇩

IgE-mediated birch pollen allergy

⇩

Grass pollen extract

⇩

IgE-mediated grass pollen extract allergy

⇩

Component resolved diagnosis

Major allergen : Bet v 1

Cross-reactive minor allergens: Bet v 2, Bet v 4

Major allergen : Phl p 1, Phl p 5

Cross-reactive minor allergens: Phl p 7, Phl p 12

⇩

Suitability of patients for the specific immunotherapy (SIT)

⇩

Birch-SIT

Bet v 1: +	Bet v 1: +	Bet v 1: -
Bet v 2, 4: -	Bet v 2, 4: +	Bet v 2, 4: +/-

⇩

Grass-SIT

Phl p 1, 5: +	Phl p 1, 5: +	Phl p 1, 5: -
Phl p 7, 12: -	Phl p 7, 12: +	Phl p 7, 12: +/-

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: Minor cross sensitive components:

Allergen g213- rPhl p 1, rPhl p 5b Allergen g214 - rPhl p 7, rPhl p 12

ASIT efficiency	rPhl p 1, 5 **«+»**	rPhl p 1, 5 **«+»**	rPhl p 1, 5 **«-»**
	rPhl p 7, 12 **«-»**	rPhl p 7, 12 **«+»**	rPhl p 7, 12 **«+/-»**
	High	**Medium**	**Low**

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

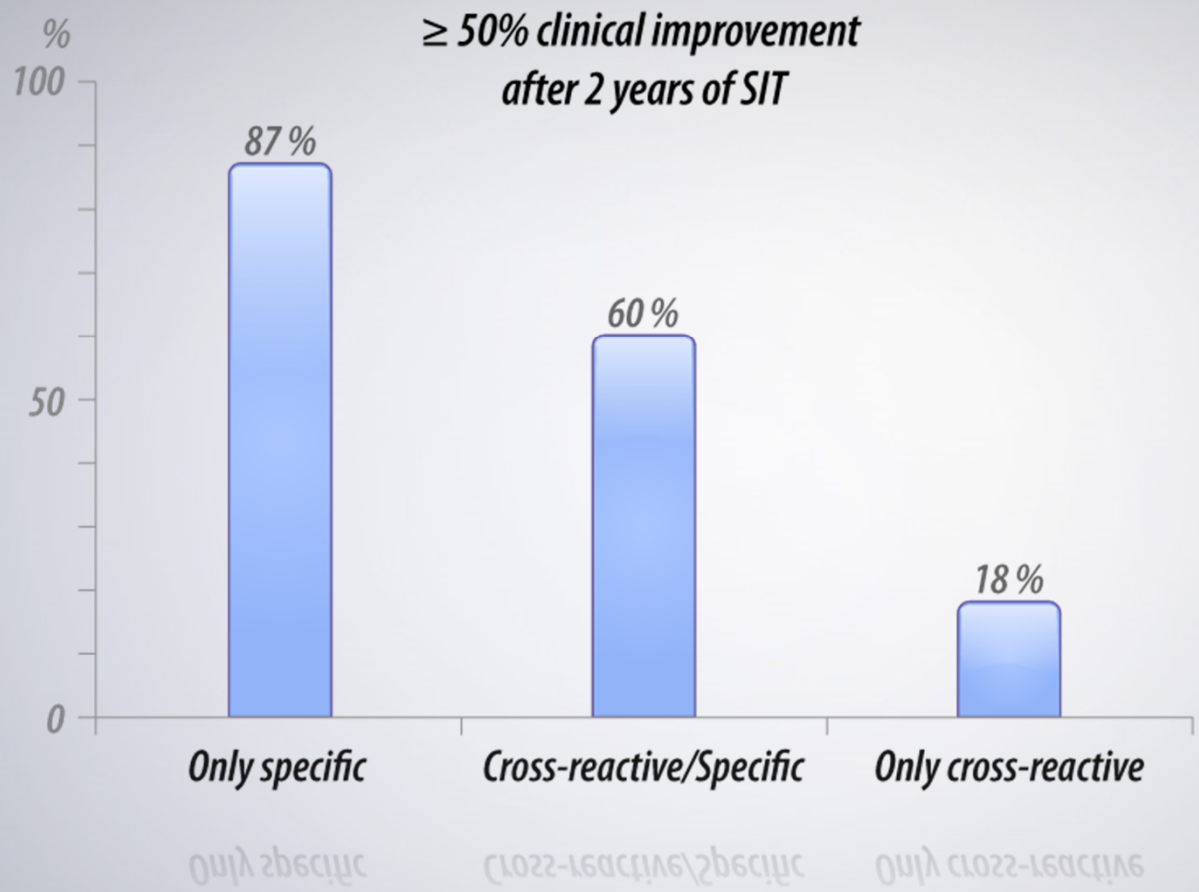
Diagnosis of patient reactivity to the components:

Major pollen components: Minor cross sensitive components:

Allergen t215 - rBet v 1 Allergen t221 - rBet v 2, rBet v 4

ASIT efficiency	rBet v 1 **«+»**	rBet v 1 **«+»**	rBet v 1 **«-»**
	rBet v 2, rBet v 4 **«-»**	rBet v 2, rBet v 4 **«+»**	rBet v 2, rBet v 4 **«+/-»**
	High	**Medium**	**Low**

Successful SIT relies on genuine sensitization



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

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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

ImmunoCAP Molecular Allergology

Characteristics of main egg white components

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

Source: Benhamou AH, state of the art for egg allergy, Allergy 2010, 65:283–289.

ImmunoCAP Molecular Allergology

Common clinical practice

Patient	**Elin, 5 years**	**Nour, 5 years**
Previous		
At 7 months		
Anamnesis	Eczema	Eczema
At 2 years		
Anamnesis	Urticaria, asthma	Urticaria, asthma
SPT to egg	+5	+4
Diagnosis	Egg allergy	Egg allergy
Advice	Avoid egg	Avoid egg
At 5 years		
sIgE to egg	25 kU_A/l	20 kU_A/l
Food challenge	No symptoms	Urticaria, cough, rhinitis
Diagnosis	Tolerant to egg	Egg allergy

ImmunoCAP Molecular Allergology

Using components in clinical practice

Patient	**Elin, 5 years**	**Nour, 5 years**
Previous		
At 7 months		
Anamnesis	Eczema	Eczema
At 2 years		
Anamnesis	Urticaria, asthma	Urticaria, asthma
SPT to egg	+5	+4
sIgE to egg	25 kU_A/l	20 kU_A/l
sIgE to Ovomucoid	**0.4 kU_A/l**	**12 kU_A/l**
Diagnosis	Tolerance likely Low risk for reactions	Egg allergy
Advice	Try cooked egg	Avoid egg

Improved diagnoses and altered advice

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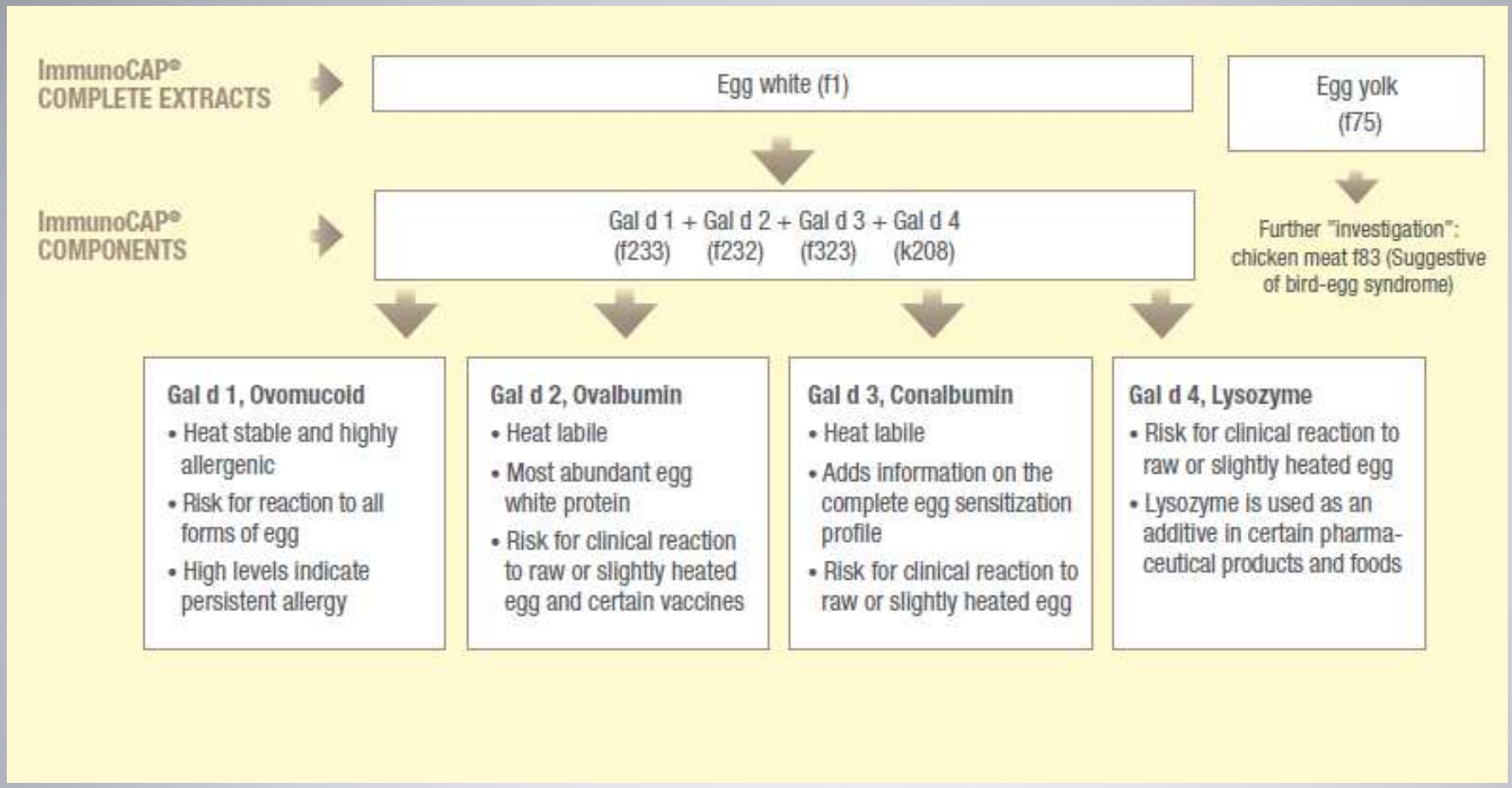
Recommended test profile

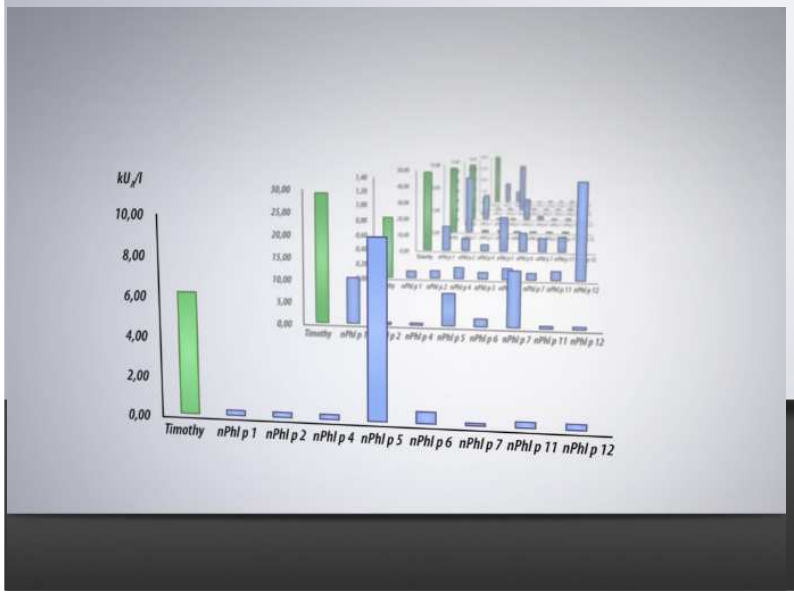


Better characterization of egg allergic patients

- 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



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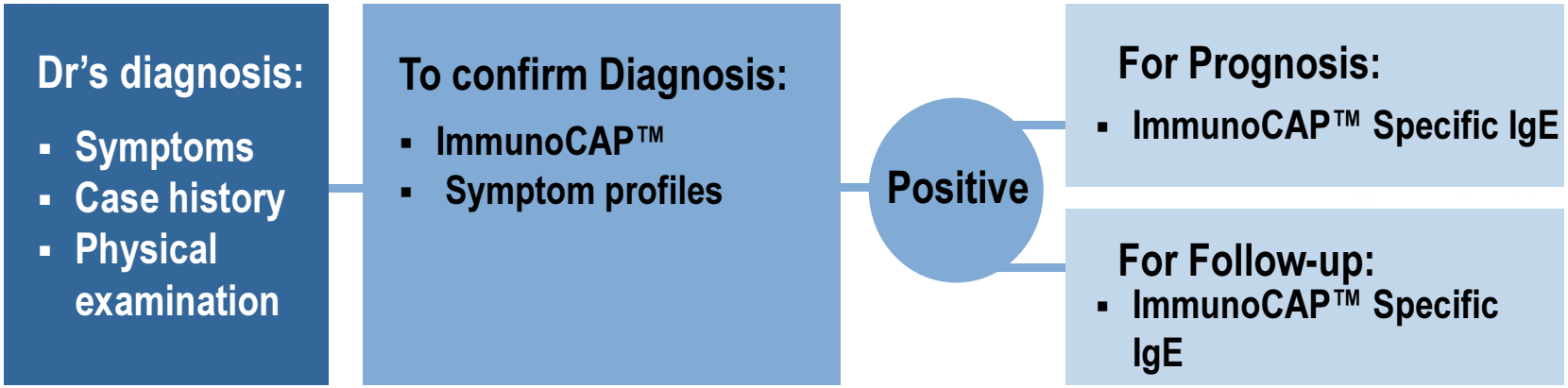
Allergy test strategy



A WAO - ARIA - GA2LEN consensus document on molecular-based allergy diagnostics

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