

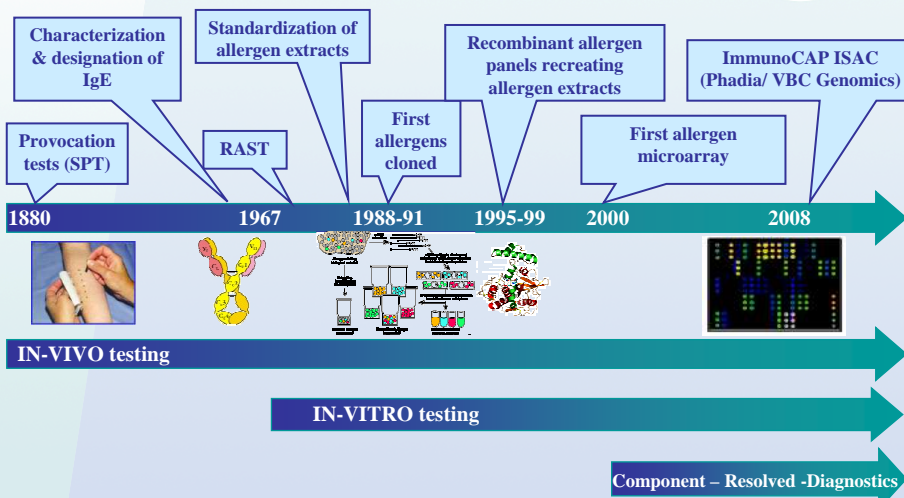


Molecular Allergology
Manchester

Pharm. Biol. Romy GADISSEUR, University Hospital of Liège, Belgium

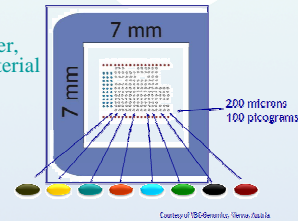
June 28th 2011

Timeline...allergy diagnosis



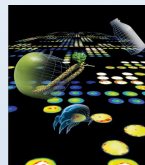
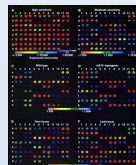
Allergen microarrays Concept and development

- **Microarray concept**
 - Small aqueous volumes of biomolecules, highly dense manner, miniaturised device, hundreds or thousands of biological material probes, immobilised in a matrix component.
- **Nanotechnology advances**
 - Deposit, immobilise large number of protein aliquots on small surfaces (spot sizes: μm)
- **Difficulties :**
 - **Antigens : intrinsic complexity**
(size, charge, solubility, surface activity, three-dimensional structure)
 - Must be carefully selected.
 - **Antigen spotting conditions :** secure efficient immobilisation, prevent denaturalisation of the surface, maintain adequate accessibility to the allergen epitopes.
 - **Optimised test conditions and sensitive detection system.**
(sensitivity, specificity and reproducibility must be validated to ensure the accuracy of the results)



Evolution of in-vitro specific IgE diagnosis

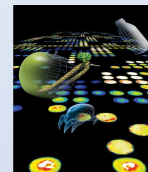
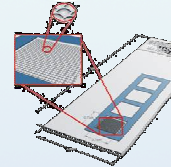
- In the late 1980s, first miniaturised multianalyte immunoassays (microspots arranged in the form of microarrays in which miniaturised ligands were deposited)
 - Quantitative information and great sensitivity in application to a large number of analytes in a single determination.
 - Thanks to developments in image analytical techniques and data analysis.



- **Use of microarrays**
 - Protein microarrays used to develop diagnostic panels and biomarkers
 - Clinical use in different areas such as autoimmunity, oncology, infectious diseases or **allergy**.

➤ **ImmunoCAP ISAC**

- Indirect solid-phase immunoassays.
 - Specific IgE semiquantitative indirect enzymeimmunoassay (EIA).
- Each glass slide contains four identical microarrays.
- The conventional tests use activated cellulose capsules as solid phase for fixing the allergens (UniCAP®, Phadia) while allergen microarrays generally use chemically modified glass as substrate.
- Each microarray is surrounded by a fine Teflon® layer to prevent overflow of the sample during the test.
- Allergenic proteins deposited in triplicate on the matrix to ensure the reproducibility or reliability of the test.
- Arbitrary units, ISAC Standardized Units (ISU),
 - four classes (negative, low, medium and high),
 - * corresponding to the classes 0, 1-2, 3-4 and 5-6, respectively.



ISAC Standardized Units (ISU)	Test
< 0.3	Indetectable
0.3 - 0.9	Faible
1 - 14.9	Moyennement
≥ 15	Très élevé

What is the difference to ImmunoCAP® ?

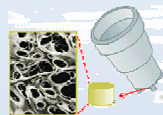
Size and matter

Standard ImmunoCAP®

Microscopic dimensions

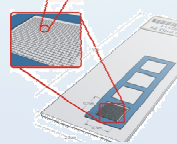
Microgram amount of allergen / CAP

10³ - 10⁴ times more allergen per test required



ImmunoCAP ISAC®

~ 200 µm spot size



Microscopic dimensions

~ 100 picogram / allergen spot
> 100 allergens / cm²



What is the difference to ImmunoCAP® ?

ImmunoCAP

- CV < 15%
- Fully quantitative results
- Automated method
- Single result using 40µL sample
+ 150µL dead instrument volume !
- Extremely high total binding capacity,
- Low non-specific binding
- High sensitivity and very low concentrations of IgE can be detected.

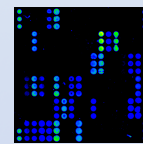
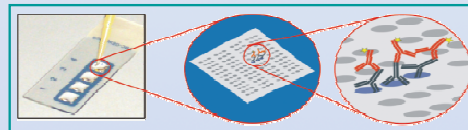


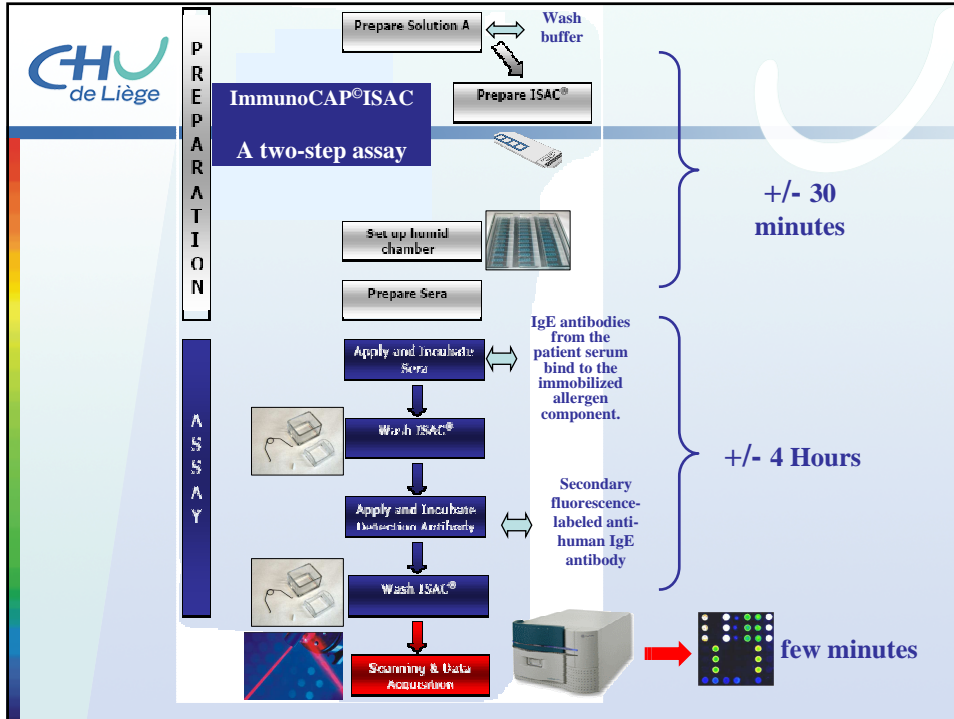
ImmunoCAP ISAC

- CV < 25% above 1 ISU
- Semi-quantitative results
- Manual method
- >103 results using 30µL sample
- Extremely high total binding capacity,
- Low non-specific binding
- Good sensitivity.
- Good specificity.
- Provide a large sensitization profile.



- **VBC Genomics (expert in microarrays) and Phadia (allergy expert, in-vitro diagnostic tests) have combined “innovative biochip technology” with “cutting-edge research in molecular allergology” to develop ImmunoCAP®ISAC.**
 - *Harwanegg C et al, Expert Rev Mol Diagn 2004*
 - **Modern multiplex in vitro diagnostic tool for the allergy specialist, based exclusively on allergen components.**
 - **Miniaturized immunoassay platform for measurement of sIgE antibodies to over 40 common allergen sources in a single step.**
 - **103 purified natural or recombinant allergen components immobilized on a solid support.**
 - **Run CRD assays rapidly and cost-effectively.**





CHU de Liège

ImmunoCAP ISAC

2. Composants allergéniques classés par groupes protéiques

2.1 Origine végétale

Composants principalement spécifiques d'espèce

Kivi	nAct d 1	Cystéine protease	<0.3 ISU
	nAct d 2	Thaumatin-like protein	<0.3 ISU
	nAct d 5	Kiwelin	<0.3 ISU
Noix du Brésil	rBer e 1	Storage protein, 2S albumin	<0.3 ISU
Muscadine	rAra e 3	Leucosialin protein	<0.3 ISU
		sin, 11S globulin	NA ISU
		sin, 2S albumin	<0.3 ISU
		sin, 7S globulin	<0.3 ISU
		sin, Conglutin	<0.3 ISU
		sin, 11S globulin	<0.3 ISU
		sin, Beta-conglycinin	<0.3 ISU
		sin, Glycinin	<0.3 ISU
		lectin 1	<0.3 ISU
		sin	<0.3 ISU
		he/1 Trypsin inhibitor	<0.3 ISU
		1	<0.7 ISU

1. Résumé des résultats des dosages d'IgE spécifiques

Composants spécifiques d'espèce

Châtaignier digitale	rOyn d 1	Grass group 1	
Phléole	rPhl p 1	Grass group 1	

Composants avec une réactivité croisée limitée

Protéines PR10	Protéines PR10	Protéines PR10	Protéines PR10
Bouleau	rBet v 1	PR-10 protein	32 ISU
Auline	rAin g 1	PR-10 protein	10 ISU
Pollen de noisetier	rCor a 1.0101	PR-10 protein	5.2 ISU
Noisetier	rCor a 1.0401	PR-10 protein	2.5 ISU
Pomme	rMal d 1	PR-10 protein	9.7 ISU
Pêche	rPru p 1	PR-10 protein	19 ISU
Saja	rGly m 4	PR-10 protein	1.9 ISU
Arachide	rAra h 8	PR-10 protein	9.9 ISU
Kivi	nAct d 8	PR-10 protein	0.7 ISU
Calicot	rApi g 1	PR-10 protein	2.1 ISU
Carotte	rDau c 1	PR-10 protein	<0.3 ISU

Composants avec une réactivité croisée large

Protéines	Protéines	Protéines	Protéines
Bouleau	rBet v 2	Protin	<0.3 ISU
Oliver	nOle e 2	Protin	<0.3 ISU
Laitue	rLau s 8	Protin	<0.3 ISU
Mercuriale	rMer a 1	Protin	<0.3 ISU
Présole	rPhl p 12	Protin	<0.3 ISU

Protéines liant le calcium

Bouleau	rBet v 4	Calcium binding 2-EF-hand prot.	<0.3 ISU
Présole	rPhl p 7	Calcium binding 2-EF-hand prot.	<0.3 ISU

Marqueur de réactivité croisée entre patients, qui n'est pas présent dans les aliments d'origine végétale.

Marqueur de CCD

Dronimine	nAra c 2	CCD marker	<0.3 ISU
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ISAC Standardized Units (ISU)

<0.3	Taux indétectable
0.3-0.9	Faible
1 - 14.9	Moderé/élevé
≥ 15	Très élevé

Information Echantillon: ID ECHANTILLON: 0L27924_3, Date prélèvement: 19.10.2010, Test QC: Passé, Date impression: 19.10.2010

Information Patient: ID PATIENT: Vanderpoelen, Nom: Vanderpoelen, Prénom: Vanderpoelen, Date de naissance: 19.10.1974, Sexe: M, Age: 36 ans, Adresse: 19, rue de la Chapelle, 1350 Wavre

Information Médecin: Médecin prescripteur: Dr SEGHAÏE M-C, Adresse: 19, rue de la Chapelle, 1350 Wavre

2. Composants allergéniques classés par groupes protéiques

2.1 Origine végétale

Composants principalement spécifiques d'espèce

Composants avec une réactivité croisée limitée

Composants avec une réactivité croisée large

Protéines liant le calcium

Marqueur de réactivité croisée entre patients, qui n'est pas présent dans les aliments d'origine végétale.

Marqueur de CCD

ISAC Standardized Units (ISU)

Information Echantillon: ID ECHANTILLON: 0L27924_3, ID PATIENT: VANDERPOELEN

Dans le nord et le centre de l'Europe, les pollens de bouleau ou d'arbres apparentés sont considérés comme les principaux agents sensibilisants et provoquent souvent des symptômes respiratoires. La présence de protéines PR10 dans de nombreux aliments d'origine végétale est souvent responsable de symptômes par réactivité croisée. La plupart de ces protéines sont thermosensibles et les aliments cuits sont souvent tolérés. Cette sensibilisation est souvent associée à des symptômes locaux tels que le syndrome oral et est également souvent associée à des réactions à des fruits et légumes dans le nord de l'Europe.

systémiques et plus sévères en complément d'un syndrome oral. Souvent associée à des réactions allergiques à des fruits et légumes dans le sud de l'Europe.

Parallèlement présentant une grande homologie et responsable d'une large réactivité croisée entre les plantes, y compris d'espèces éloignées. Rarement associé à des symptômes mais peut avoir une importance clinique et même provoquer des réactions sévères chez une petite minorité de patients allergiques notamment au citron, au melon, à la banane, au fitchi et à la tomate.

Components on ISAC Possible cross-reactivity

FOOD ALLERGENS - VEGETAL ORIGIN			
English name	Allergens	Function	Latin name
Apple	Mal d 1	PR-10	Malus domestica
Peach	Pru p 1	PR-10	Prunus persica
	hPru p 3	nsLTP	
	hAct d 1	Systemic protease	Actinidia deliciosa
	hAct d 2	Thaumatin-like protein	
	hAct d 5	kwelmin	
	hAct d 8	PR-10	
Bromelain	hAna c 2	CCD	Annona muricata
	HBP	CCD	
	MUXF3 CCD	CCD	
Cashew nut	Ana o 2	legumin-like protein	Anacardium occidentale
	Ana o 3	Storage protein, 2S albumin	
Peanut	hAra h 1	Storage protein, 7S globulin	Arachis hypogaea
	hAra h 2	Storage protein, Conglutin	
	hAra h 3	Storage protein, Glycinin	
	hAra h 8	PR-10	
	hAra h 9	nsLTP	
Brazil nut	hBer e 1	Storage protein, 2S albumin	Bertholletia excelsa
Hazelnut	hCor a 1,0401	PR-10	Corylus avellana
	hCor a 9	nsLTP	
Walnut	hJug r 1	Storage protein, 11S globulin	Juglans regia
	Jug r 2	Storage protein, 2S albumin	
	Jug r 3	Storage protein, vicillin	
	Jug r 4	nsLTP	
Sesame seed	hSes s 1	Storage protein, 2S albumin	Sesamum indicum
Celery	hDau c 1	PR-10	Apium graveolens
Carrot	hDau c 1	PR-10	Daucus Carota
Soybean	hGly m 4	Storage protein, b-conglycinin	Glycine max
	hGly m 5	Storage protein, 7S globulin	
	hGly m 6	Storage protein, glycinin	
Wheat	hGli m 2S	Storage protein, 2S albumin	Triticum aestivum
	Tri a 14	nsLTP	
	Tri a 18	Agglutinin isolectin 1	
	Tri a 19 Gliadin	Gliadin	
	Tri a 19,0101	Omega-5 gliadin	
Buckwheat	hFag e 1	Storage protein, 2S albumin	Fagopyrum esculentum
	Fag e 16KD	Storage protein, 2S albumin	

CCD
 • A marker for sensitization to cross-reactive compounds in the alimentaria.
 • Sensitization associated with clinical symptoms but may cause severe reactions in a small minority of patients.

11S globulin
 • A protein stable to heat and digestion causing reactions also to cooked foods.
 • Often associated with symptoms in some severe reactions in southern Europe.
 • Other associated with allergic reactions to fruit and vegetables in southern Europe.

Storage protein
 • Protein found in seeds serving as a source of nutrients during the growth of a new plant.
 • Often associated with clinical symptoms but may cause severe reactions in a small minority of patients.

Components on ISAC Possible cross-reactivity

INHALENT ALLERGENS			
English name	Allergens	Function	Latin name
Bermuda Grass	hCyn d 1	Grass group 1	Cynodon dactylon
Timothy grass	hPti p 1	Grass group 1	Phleum pratense
	hPti p 11	Die e 1-related protein	
	hPti p 12	PFL	
	hPti p 2	Grass group 2	
	hPti p 4	Betaine lyase enzyme	
	hPti p 5	Grass group 5	
	hPti p 6	Grass group 6	
Latex	hPti p 7	Ca Binding protein	
	Hev b 1	Rubber elongation factor	Hevea brasiliensis
	Hev b 3	Small rubber particle protein	
	Hev b 5	Acidic protein	
	Hev b 6	Hevein precursor	
	Hev b 8	PFL	
	Hev b 11	Chitinase	
Birch	hBet v 1	PR-10	Betula pubescens
	hBet v 2	PFL	
	hBet v 4	Ca Binding protein	
Hazel pollen	hCor a 1,0101	PR-10	Corylus avellana
Oak	hQue a 1	PR-10	Quercus
Japanese cedar	hCrj 1	Protease lyase	Cryptomeria japonica
Cypress	hCup a 1	Protease lyase	Cupressus arizonica
Olive	hOle e 1	Common olive group 5	Olea europaea
	hOle e 2	PFL	
	hOle e 7	nsLTP	
Plane tree	hPla a 1	Putative invertase inhibitor	Platanus acerifolia
	hPla a 2	Polygalacturonase	
	hPla a 3	nsLTP	
Alder	hAln a 1	PR-10	Alnus glutinosa
Mugwort	hArt v 1	Defensin	Artemisia vulgaris
Annual mercury	hMer a 1	nsLTP	Mercurialis annua
	Mer a 1	PFL	
Wall pellitory	hPar j 2	nsLTP	Parietaria judaica
Saltwort	hSal k 1	Psitin methyltransferase	Salsola kali
Goosefoot	hChe a 1	Trypsin inhibitor	Chenopodium album
Ragweed	hAmb a 1	Protease lyase	Ambrosia artemisiifolia
Plantain	hPla 1	Protease lyase	Plantago lanceolata

PR-10 protein, Bet v 1 homologue
 • A heat labile protein, common foods are often tolerated.
 • Often associated with local symptoms and/or oral allergy syndrome (OAS).
 • Other associated with clinical symptoms but may cause severe reactions in a small minority of patients.

Components on ISAC Possible cross-reactivity

FOOD ALLERGENS - ANIMAL ORIGIN			
English name	Allergens	Function	Latin name
Ovomucoid	nGal d 1	Ovomucoid	Gallus domesticus
Ovalbumin	nGal d 2	Ovalbumin	
Conalbumin	nGal d 3	Ovotransferrin	
Lysozyme	Gal d 4	Lysozyme	
CSA (livetin)	nGal d 5	Serum albumin	
Carp	nCyp c 1	Parvalbumin	
Cod	nGad c 1	Parvalbumin	
Red meat	Alpha gal	Sugar epitope	
	Alpha gal	Sugar epitope	
Alpha-lactalbumin	nBos d 4	Alpha-lactalbumin	Bos domesticus
Beta-lactoglobulin	nBos d 5	Beta-lactoglobulin	
BSA	nBos d 6	Serum albumin	
Casein	nBos d 8	Casein	
Lactoferrin	nBos d lactoferrin	Transferrin	
Anisakis	nAni s 1 nAni s 3	Tropomyosin	Anisakis simplex
Shrimp	nPen a 1 nPen i 1 nPen m 1 Pen m 2 Pen m 4	Tropomyosin Tropomyosin Tropomyosin Arginine kinase Sarcoplasmic Ca binding protein	Penaeus aztecus Penaeus indicus Penaeus monodon
VENOMS			
English name	Allergens	Function	Latin name
Honey bee venom	nApi m 1 nApi m 4	Phospholipase A2 Mellitin	Apis mellifera Apis mellifera
Paper wasp	Pol d 5	Ag 5	
Wasp	Ves v 5	Ag 5	Vespa vulgaris

Parvalbumin
 • A major allergen in fish • A marker for cross-reactivity among different species of fish and amphibians.
 • A certain ability to react and elicit allergic reactions also to cooked foods.

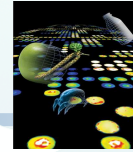
Components on ISAC Possible cross-reactivity

OTHER INHALENT ALLERGENS			
English name	Allergens	Function	Latin name
Cat	nFel d 1 nFel d 2 nFel d 4	Uteroglobulin Serum albumin Lipocalin	
Cockroach	nBla g 1 nBla g 2 nBla g 4 nBla g 5 nBla g 7	Cockroach group 1 Aspartic protease Calycin Glutathione S-transferase Tropomyosin	
Moth	nPilo 1	Arginin kinase	
Dog	nCan f 1 nCan f 2 nCan f 3 nCan f 5	Lipocalin Lipocalin Serum albumin Arginin esterase	
Horse	nEqu c 3 nEqu c 1	Serum albumin Lipocalin	
House dust mite	nDer f 1 nDer f 2 nDer p 1 nDer p 10 nDer p 2	Cysteine protease NCP2 family Cysteine protease Tropomyosin NCP2 family	Dermatophagoides farinae Dermatophagoides farinae
Storage mite	nEur m 2	NCP2 family	
Blomia	Blo t 5	?	
Storage mite	nLsp d 2	Group 2	
Mouse	nMus m 1	Lipocalin	Mus musculus
MOULDS			
English name	Allergens	Function	Latin name
Aspergillus	nAsp f 1 nAsp f 2 nAsp f 3 nAsp f 4 nAsp f 6	Mitogillin family Flavinogen Binding Proteins Peroxisomal protein MnSOD	Aspergillus fumigatus
Cladosporium	nCla h 6	Mannitol dehydrogenase	Cladosporium herbarum
Alternaria	nAlt a 1 nAlt a 6	Enolase	Alternaria alternata

Lipocalin
 • Soluble proteins and important allergens in animals
 • Allergen components showing limited cross-reactivity between species.

Serum albumin
 • A nonimmunizable protein in different animal fluids and soluble in cow's milk and eggs and collagen.
 • Cross-reactivity between albumins from different animal species are well known, for example between cow and sheep and not among.

Arginase
 • An acid-binding enzyme in muscle fibres.
 • A marker for cross-reactivity between vertebrates, fishes and amphibians.



« CRD »

Component-Resolved Diagnosis uses defined recombinant allergens to dissect the individual patient's IgE reactivity profile with the aim of identifying the disease-eliciting molecules.

Valenta R et al, Clin Exp Allergy 1999

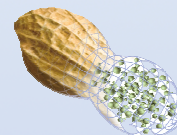
Molecules, genuine markers tell you the truth !

Mari A., VIP ISAC Customers day, Brussels, 17th November 2009.

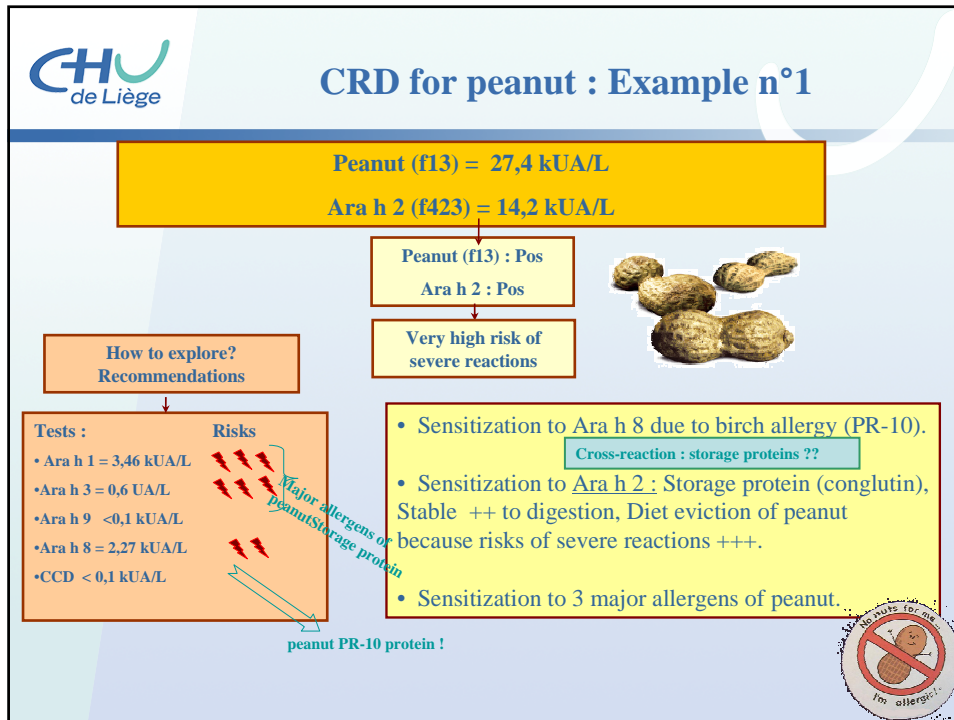
The presence of IgE to cross-reactive allergen components can be determined and used to predict clinically relevant sensitization to allergen sources which contain immunologically related allergens.

1. Julie, F :

- **f13 (peanut) = 27.4 kUA/L.**
 - SPT + : peanut, walnut, hazelnut, sesame seed, birch, weeds, pine nut.
 - SPT - : soybean, egg white et walnut.
- Asthma, gastrointestinal intolerance and erythema after ingestion of nuts.
- What are the risks ? Birch?



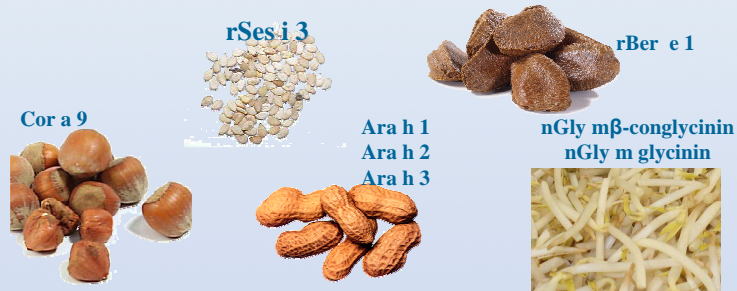
CRD for peanut : Example n°1



CRD for peanut : Example n°1

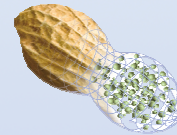
- *Storage protein*

- Protein found in seeds serving as source material during the growth of a new plant
- Often stable and heat-resistant proteins causing reactions also to cooked foods



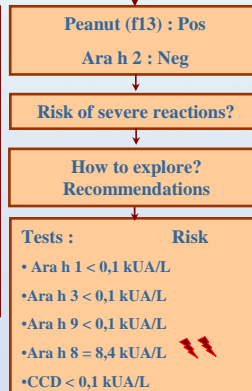
2. Laurent, H :

- **f13 (peanut) = 1.22 kUA/L.**
 - sIgE + : birch, apple, pear, peach, apricot, hazelnut, walnut and recently to celery and to fennel.
- Atopy, polysensitized, oral allergy syndrome(OAS) and a recent evolution to breathing difficulties.
- What are the risks?
 - What about the Birch?
 - Recent evolution of the allergy?



Peanut (f13) = 1,22 kUA/L
Ara h 2 (f423) < 0,10 kUA/L

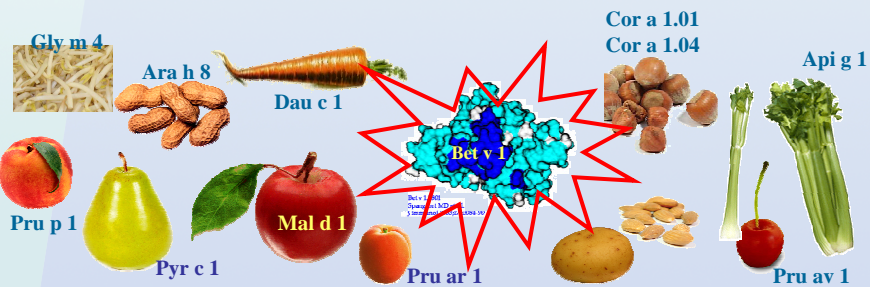
- Sensitization to peanut after birch allergy (PR-10 proteins).
- Sensitization to other PR-10 :
 - Apple (Mal d 1), pear (Pyr c1), peach (Pru p 1), apricot (Pru ar 1), hazelnut (Cor a 1) and recently to celery (Api g 1)
- In this case, evolution of the OAS with respiratory distress explained by recent sensitization to Api g 1.



⇒ PR-10, Bet v 1-homologue

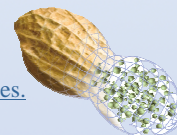
• *PR-10 protein, Bet v 1 homologue*

- A heat labile protein, cooked foods are often tolerated.
» Breiteneder H, *Biotechnol Adv*, 2005
- Often associated with local symptoms such as oral allergy syndrome (OAS).
- Often associated with allergic reactions to fruits, vegetables and spices in northern Europe.



3. Maud, F :

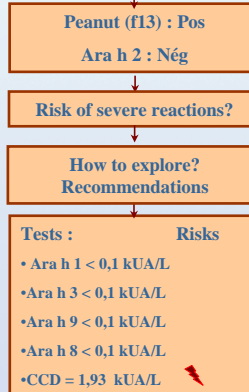
- **f13 (peanut) = 1.23 kUA/L**
 - sIgE + : pollens of weeds, cow milk, carrot, orange, tomato, wheat, potato, garlic, onion, celery, banana, peach and peanut.
- Sensitisation to a wide variety of foods from plants.
- Gastro-intestinal disorders.
- What are the risks ?
 - Multiple sensitizations to fruits and vegetables.



CRD for peanut : Example n°3

Peanut (f13) = 1,23 kUA/L
Ara h 2 (f423) < 0,1 kUA/L

- Sensitization to **CCD**:
 - Marker of sensitization to Carbohydrate Determinants.
 - Multiple cross-reactions.
 - Seldom associated to clinical symptoms.
- Test CCD if sensitization to fruits and vegetables, seeds.

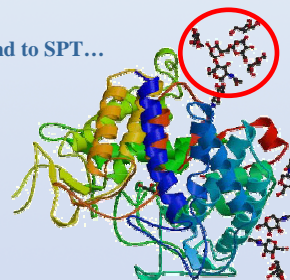


⇒ CCD

CRD for peanut : Example n°3

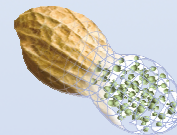
- **CCD (MUXF3)** :
 - Marker of sensitization to Carbohydrate determinants (cross-reacting carbohydrate determinants). Glyco-epitopes.
 - Present in most plants,
 - sIgE directed towards glycans appear to show the widest pattern of cross-reactivity among allergenic extracts and are often responsible for observed in-vitro cross-reactions.
 - « in-vivo » effects ?
 - Seldom associated to clinical symptoms ...
 - » But, publications ?
 - » To compare to the clinical informations and to SPT...

In-vitro cross-reactions



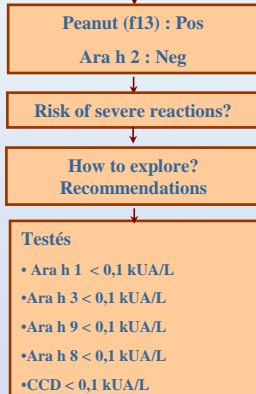
4. Eddy, M :

- f13 (peanut) = 0,44 kUA/L
 - SPT+ : birch, apple, peach, banana, garlic, peanut
- Swollen lips
- What are the risks ?
 - Sensitization to birch...



Peanut (f13) = 0,44 kUA/L
Ara h 2 (f423) < 0,1 kUA/L

- Sensitization to profilins (Bet v 2)
- Seldom associated with clinical symptoms except OAS and rarely severe reactions in a minority of patients.
- Presence of profilins in many fruits and vegetables.
 - Birch (Bet v 2), apple (Mal d 4), peach (Pru p 4), banana (Mus a 1), garlic (All c 4), peanut (Ara h 5).



Bet v 2 = 4,6 kUA/L

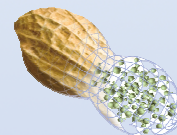
• **Profilin**

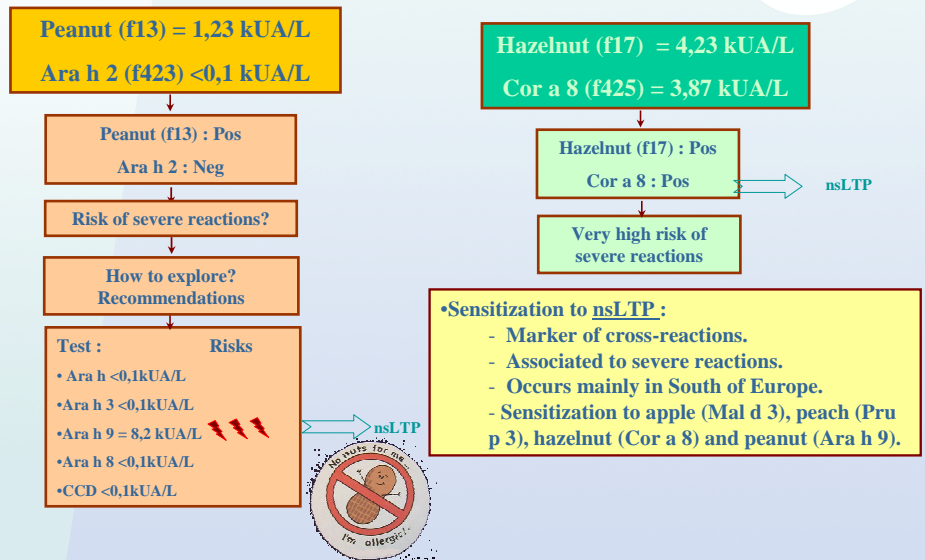
- Seldom associated with clinical symptoms but may cause demonstrable or even severe reactions in a small minority of patients
- Profilins are present in a broad range of pollens and foods including trees, grasses and weeds.
- There is a wide range of homology between profilins from different fruits and vegetables.



5. **Nadia, F :**

- f13 (peanut) = 1,73 kUA/L
- f17 (Hazelnut) = 4,23 kUA/L
- sIgE + : apple, peach, hazelnut, peanut
- SPT +++ : peanut, hazelnut, walnut.
- Atopy, polyallergy, rhinoconjunctivitis, OAS, urticaria et angioedema
- What are the risks ? ...angioedema.;





- **LTP (non-specific Lipid Transfer Protein, nsLTP)**
 - A protein stable to heat and digestion causing reactions also to cooked foods
 - Often associated with systemic and more severe reactions in addition to OAS
 - LTPs often cause food allergy to fruit in the absence of pollen allergy.
 - Often associated with allergic reactions to fruit and vegetables in southern Europe.

LTP

- nPru p 3
- rCor a 8
- rPar j 2
- nArt v 3
- Ara h 9
- Mal d 3
- Zea m 4
- Jug r 3

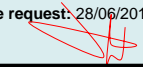
- ♂, 15 years old (Nicolas)
 - Anamnesis :
 - 2 parents + 2 brothers : allergic
 - Symptoms :
 - Atopic Dermatitis,
 - Perennial Rhinitis
 - Conjunctivitis from february to october.
 - Asthma
 - Food allergy :
 - » 2 angioedema after a dinner in a restaurant.
 - » OAS after eating fruits
 - Suspicion of polyallergy
 - Skin prick tests (SPT) :
 - Positive SPT pneumallergens : HDM, birch, grass pollens, henn's egg
 - Positive SPT food allergens : fish
 - » Negative SPT food allergens : seafood.
 - In-vitro tests :
 - Tot IgE : 410 kU/L ++
 - ImmunoCAP ISAC

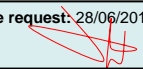


- ♂, 15 years old (Nicolas)
 - Anamnesis :
 - 2 parents + 2 brothers : allergic

Thanks to all the recombinants,
We would be able to explain everything !!

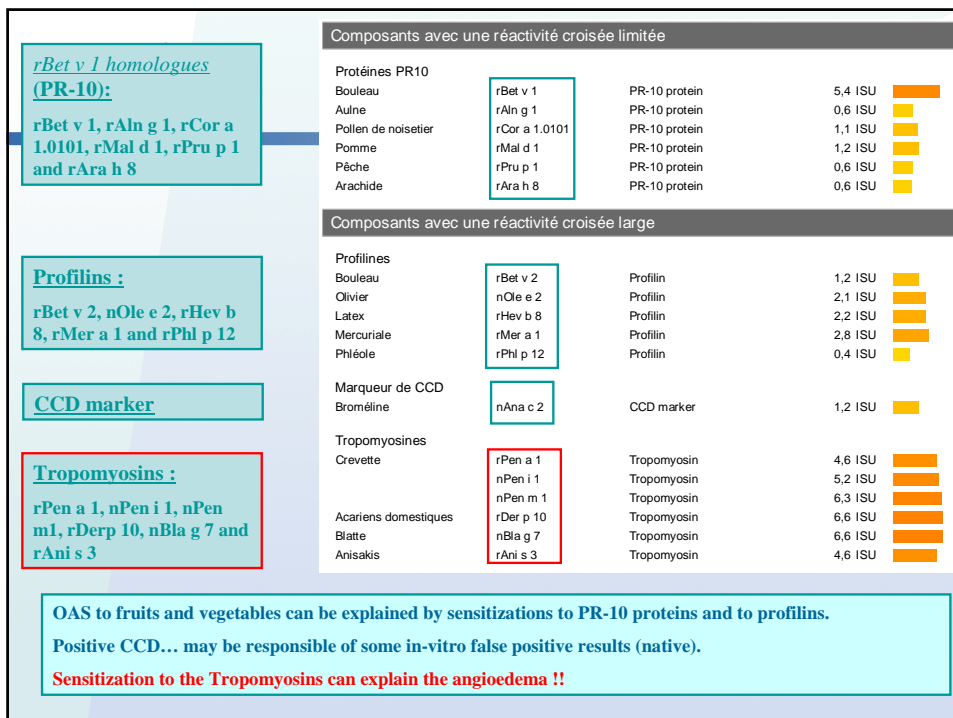
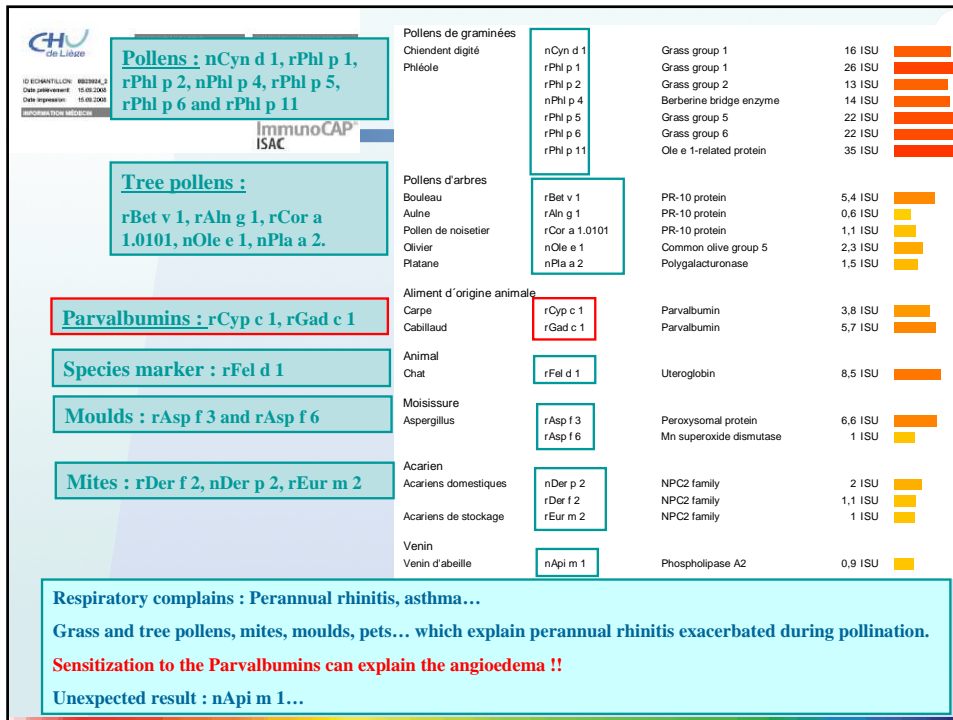
- ImmunoCAP ISAC

Request form ImmunoCAP™ Components Some other components less useful in Belgium could be available. Don't hesitate to ask to your Lab in case of specific clinical history.		Patient: Name:..... Address:..... Birth date:...../...../.....	
April 2010			
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Prot. <i>Phleum pratense</i> g210 <input type="checkbox"/> rPhl p 11 <i>Phleum pratense</i> g211 <input type="checkbox"/> rPhl p 12 <i>Profilin Phleum pratense</i> g212 <input type="checkbox"/> Mix rPhl p 1, rPhl p 5b <i>Phleum pratense</i> g213 <input type="checkbox"/> Mix rPhl p 7, rPhl p 12 <i>Phleum pratense</i> g214 <p>Weed pollens :</p> <ul style="list-style-type: none"> <input type="checkbox"/> w6: <i>Artemisia vulgaris</i> <input type="checkbox"/> nArt v1 <i>Artemisia vulgaris</i> w231 <input type="checkbox"/> nArt v3 LTP <i>Artemisia vulgaris</i> w233 <input type="checkbox"/> w11: <i>Salsola kali</i> <input type="checkbox"/> nSal k1 <i>Salsola kali</i> w232 <p>Tree pollens :</p> <ul style="list-style-type: none"> <input type="checkbox"/> i3: <i>Betula verrucosa</i> <input type="checkbox"/> rBet v 1 PR10 <i>Betula verrucosa</i> t215 <input type="checkbox"/> rBet v 2 <i>Profilin Betula verrucosa</i> t216 <input type="checkbox"/> rBet v 4 Calc. Bind. Prot. <i>Betula verrucosa</i> t220 <input type="checkbox"/> rBet v 6 <i>Betula verrucosa</i> t225 <input type="checkbox"/> Mix rBet v 2, rBet v 4 <i>Betula verrucosa</i> t221 <input type="checkbox"/> i9: <i>Olea europaea</i> <input type="checkbox"/> nOle e 1 <i>Olea europaea</i> t224 <input type="checkbox"/> i222: <i>Cupressus arizonica</i> <input type="checkbox"/> nCup a1 <i>Cupressus arizonica</i> t226 <p>Mites</p> <ul style="list-style-type: none"> <input type="checkbox"/> d1: <i>Dermatophagoides pteronyssinus</i> <input type="checkbox"/> nDer p1 <i>Dermatophagoides pteronyssinus</i> d202 <input type="checkbox"/> nDer p2 <i>Dermatophagoides pteronyssinus</i> d203 <input type="checkbox"/> nDer p10 <i>Tropomyosin Dermatophagoides pteronyssinus</i> d205 <p>Microorganisms</p> <ul style="list-style-type: none"> <input type="checkbox"/> m3 : <i>Aspergillus fumigatus</i> <input type="checkbox"/> rAsp f 1 <i>Aspergillus fumigatus</i> m218 <input type="checkbox"/> rAsp f 2 <i>Aspergillus fumigatus</i> m219 <input type="checkbox"/> rAsp f 3 <i>Aspergillus fumigatus</i> m220 <input type="checkbox"/> rAsp f 4 <i>Aspergillus fumigatus</i> m221 <input type="checkbox"/> rAsp f 6 <i>Aspergillus fumigatus</i> m222 <input type="checkbox"/> m6 : <i>Alternaria alternata</i> <input type="checkbox"/> rAlt a 1 <i>Alternaria alternata</i> m229 	<ul style="list-style-type: none"> <input type="checkbox"/> f13: Peanut <i>Arachis hypogaea</i> <input type="checkbox"/> rAra h 1 <i>Arachis hypogaea</i> t422 <input type="checkbox"/> rAra h 2 <i>Arachis hypogaea</i> t423 <input type="checkbox"/> rAra h 3 <i>Arachis hypogaea</i> t424 <input type="checkbox"/> rAra h 8 PR10 <i>Arachis hypogaea</i> t352 <input type="checkbox"/> rAra h 9 LTP <i>Arachis hypogaea</i> t427 <input type="checkbox"/> i17: Hazelnut <i>Corylus avellana</i> <input type="checkbox"/> rCor a 1 PR10 <i>Corylus avellana</i> t428 <input type="checkbox"/> rCor a 8 LTP <i>Corylus avellana</i> t425 <input type="checkbox"/> i18: Brazil nut <i>Bertholletia excelsa</i> <input type="checkbox"/> rBer e1 <i>Bertholletia excelsa</i> t354 <input type="checkbox"/> i4: Wheat <i>Triticum spp</i> <input type="checkbox"/> rTri a 19: Omega-5 Gliadin <i>Triticum spp.</i> t416 <input type="checkbox"/> i14: Soy <i>Glycine max</i> <input type="checkbox"/> rGly m 4 PR10 <i>Glycine max</i> t353 <input type="checkbox"/> rGly m 5 B-conglutinin <i>Glycine max</i> t431 <input type="checkbox"/> rGly m 6 <i>Glycinin Glycine max</i> t432 <input type="checkbox"/> i95: Peach <i>Prunus persica</i> <input type="checkbox"/> rPru p 1 PR10 <i>Prunus persica</i> t419 <input type="checkbox"/> rPru p 3 LTP <i>Prunus persica</i> t420 <input type="checkbox"/> rPru p 4 <i>Profilin Prunus persica</i> t421 <input type="checkbox"/> i85: Celery <i>Apium graveolens</i> <input type="checkbox"/> rApi g 1.01 PR10 <i>Apium graveolens</i> t417 <input type="checkbox"/> i84: Kiwi <i>Actinidia chinensis</i> <input type="checkbox"/> rAct d 8 PR10 <i>Actinidia chinensis</i> t430 <p>i1 Egg white</p> <ul style="list-style-type: none"> <input type="checkbox"/> nGal d 1 <i>Ovomucoid Gallus spp.</i> t233 <input type="checkbox"/> nGal d 2 <i>Ovalbumin Gallus spp.</i> t232 <input type="checkbox"/> nGal d 3 <i>Conalbumin Gallus spp.</i> t323 <input type="checkbox"/> nGal d 4 <i>Lysozyme Gallus spp.</i> k208 <p>i2: Milk Bos spp</p> <ul style="list-style-type: none"> <input type="checkbox"/> nBos d 4 α-lactalbumin <i>Bos spp.</i> t76 <input type="checkbox"/> nBos d 5 β-lactoglobulin <i>Bos spp.</i> t77 <input type="checkbox"/> nBos d 8 <i>Casein Bos spp.</i> t78 <input type="checkbox"/> nBos d 9 <i>Bovine lactoferrin Bos spp.</i> t334 <p>i24 Shrimp</p> <ul style="list-style-type: none"> <input type="checkbox"/> rPen a 1 <i>Tropomyosin Penaeus aztecus</i> t351 <input type="checkbox"/> rCyp c 1 <i>Parvalbumin Cyprinus carpio</i> t355 <input type="checkbox"/> rGad c1 <i>Parvalbumin Gadus morhua</i> t426 <p>Carbohydrate Determinants (CCD)</p> <ul 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allergens</p> <ul style="list-style-type: none"> <input type="checkbox"/> k82: <i>Hevea brasiliensis</i> <input type="checkbox"/> rHev b 1 <i>Hevea brasiliensis</i> k215 <input type="checkbox"/> rHev b 3 <i>Hevea brasiliensis</i> k217 <input type="checkbox"/> rHev b 5 <i>Hevea brasiliensis</i> k218 <input type="checkbox"/> rHev b 6.01 <i>Hevea brasiliensis</i> k219 <input type="checkbox"/> rHev b 6.02 <i>Hevea brasiliensis</i> k220 <input type="checkbox"/> rHev b 9 Profilin <i>Hevea brasiliensis</i> k221 <input type="checkbox"/> rHev b 9 Enolase <i>Hevea brasiliensis</i> k222 <input type="checkbox"/> rHev b 11 <i>Hevea brasiliensis</i> k224 <p>Enzymes</p> <ul style="list-style-type: none"> <input type="checkbox"/> nCar p 1 Papain <i>Carica papaya</i> k201 <input type="checkbox"/> nAsp o 1 α-amylase From <i>Aspergillus oryzae</i> k87 <input type="checkbox"/> Alkalase From <i>Bacillus sp.</i> k205 <input type="checkbox"/> Maxatase From <i>Bacillus licheniformis</i> k204 <input 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Doctor: Name:...GADISSEUR Romy Address: CHU de Liège N°RIZIV/ INAMI: .	Date of the request: 28/06/2011 Signature: 	Phadia	

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Prot. <i>Betula</i> <input type="checkbox"/> rBet v 6 <i>Betula</i> <input type="checkbox"/> Mix rBet v 2, rBet v 4 <i>Betula</i> <input type="checkbox"/> i9: <i>Olea eu</i> <input type="checkbox"/> nOle e 1 <i>O</i> <input type="checkbox"/> i222: <i>Cup</i> <input type="checkbox"/> nCup a1 <i>C</i> <p>Mites</p> <ul style="list-style-type: none"> <input type="checkbox"/> d1: <i>Dermatophagoides pteronyssinus</i> <input type="checkbox"/> nDer p1 <i>Dermatophagoides pteronyssinus</i> d202 <input type="checkbox"/> nDer p2 <i>Dermatophagoides pteronyssinus</i> d203 <input type="checkbox"/> nDer p10 <i>Tropomyosin Dermatophagoides pteronyssinus</i> d205 <p>Microorganisms</p> <ul style="list-style-type: none"> <input type="checkbox"/> m3 : <i>Aspergillus fumigatus</i> <input type="checkbox"/> rAsp f 1 <i>Aspergillus fumigatus</i> m218 <input type="checkbox"/> rAsp f 2 <i>Aspergillus fumigatus</i> m219 <input type="checkbox"/> rAsp f 3 <i>Aspergillus fumigatus</i> m220 <input type="checkbox"/> rAsp f 4 <i>Aspergillus fumigatus</i> m221 <input type="checkbox"/> rAsp f 6 <i>Aspergillus fumigatus</i> m222 <input type="checkbox"/> m6 : <i>Alternaria alternata</i> <input type="checkbox"/> rAlt a 1 <i>Alternaria alternata</i> m229 	<ul style="list-style-type: none"> <input type="checkbox"/> f13: Peanut <i>Arachis hypogaea</i> <input type="checkbox"/> rAra h 1 <i>Arachis hypogaea</i> t422 <input type="checkbox"/> rAra h 2 <i>Arachis hypogaea</i> t423 <input type="checkbox"/> rAra h 3 <i>Arachis hypogaea</i> t424 <input type="checkbox"/> rAra h 8 PR10 <i>Arachis hypogaea</i> t352 <p>i1 Egg white</p> <ul style="list-style-type: none"> <input type="checkbox"/> nGal d 1 <i>Ovomucoid Gallus spp.</i> t233 <input type="checkbox"/> nGal d 2 <i>Ovalbumin Gallus spp.</i> t232 <input type="checkbox"/> nGal d 3 <i>Conalbumin Gallus spp.</i> t323 <input type="checkbox"/> nGal d 4 <i>Lysozyme Gallus spp.</i> k208 <p>i2: Milk Bos spp</p> <ul style="list-style-type: none"> <input type="checkbox"/> nBos d 4 α-lactalbumin <i>Bos spp.</i> t76 <input type="checkbox"/> nBos d 5 β-lactoglobulin <i>Bos spp.</i> t77 <input type="checkbox"/> nBos d 8 <i>Casein Bos spp.</i> t78 <input type="checkbox"/> nBos d 9 <i>Bovine lactoferrin Bos spp.</i> t334 <p>i24 Shrimp</p> <ul style="list-style-type: none"> <input type="checkbox"/> rPen a 1 <i>Tropomyosin Penaeus aztecus</i> t351 <input type="checkbox"/> rCyp c 1 <i>Parvalbumin Cyprinus carpio</i> t355 <input type="checkbox"/> rGad c1 <i>Parvalbumin Gadus morhua</i> t426 <p>Carbohydrate Determinants (CCD)</p> <ul style="list-style-type: none"> <input type="checkbox"/> i Bromelin k202 <input type="checkbox"/> CCD: MUXF3 (from bromelin) Ro214 	<p>Epidermals & other proteins</p> <ul style="list-style-type: none"> <input type="checkbox"/> e1: Cat <i>Felis domesticus</i> <input type="checkbox"/> rFel d 1 <i>Felis domesticus</i> e94 <input type="checkbox"/> nFel d 2 serum albumin <i>Felis domesticus</i> e220 <input type="checkbox"/> e5: Dog <i>Canis familiaris</i> <p>Occupational allergens</p> <ul style="list-style-type: none"> <input type="checkbox"/> k82: <i>Hevea brasiliensis</i> <input type="checkbox"/> rHev b 1 <i>Hevea brasiliensis</i> k215 <input type="checkbox"/> rHev b 3 <i>Hevea brasiliensis</i> k217 <input type="checkbox"/> rHev b 5 <i>Hevea brasiliensis</i> k218 <input type="checkbox"/> rHev b 6.01 <i>Hevea brasiliensis</i> k219 <input type="checkbox"/> rHev b 6.02 <i>Hevea brasiliensis</i> k220 <input type="checkbox"/> rHev b 9 Profilin <i>Hevea brasiliensis</i> k221 <input type="checkbox"/> rHev b 9 Enolase <i>Hevea brasiliensis</i> k222 <input type="checkbox"/> rHev b 11 <i>Hevea brasiliensis</i> k224 <p>Enzymes</p> <ul style="list-style-type: none"> <input type="checkbox"/> nCar p 1 Papain <i>Carica papaya</i> k201 <input type="checkbox"/> nAsp o 1 α-amylase From <i>Aspergillus oryzae</i> k87 <input type="checkbox"/> Alkalase From <i>Bacillus sp.</i> k205 <input type="checkbox"/> Maxatase From <i>Bacillus licheniformis</i> k204 <input type="checkbox"/> Savinase From <i>Bacillus sp.</i> k206 <p>Venoms</p> <ul style="list-style-type: none"> <input type="checkbox"/> i11: <i>Apis mellifera</i> <input type="checkbox"/> rApi m 1 Phospholipase A2 <i>Apis mellifera</i> t208 <input type="checkbox"/> i13: <i>Vesputia vulgaris</i> <input type="checkbox"/> rVes v 5 Antigen 5 <i>Vesputia vulgaris</i> t209 <input type="checkbox"/> i177: <i>Polistes dominulus</i> <input type="checkbox"/> rPol d 5 Antigen 5 <i>Polistes dominulus</i> t210 <p>r = Recombinant n = Natif</p>	
Doctor: Name:...GADISSEUR Romy Address: CHU de Liège N°RIZIV/ INAMI: .	Date of the request: 28/06/2011 Signature: 	Phadia	

In theory, this is possible...

Practically, ...



- Method

- Selection of 86 sera of patients (26 males/60 females)
 - + sIgE (>0.10 kUA/L) for recombinant allergens (ImmunoCAP[®]250 Phadia).
 - positive and negative sIgE for 55 recombinant or native purified allergen component of different kind of allergenic sources (latex, peanut, birch, Timothy grass, hazelnut, peach, soybean, cat, dog...).
 - In all, we compared the results of 555 sIgE measured in CAP (recombinant allergens) with the ISAC[®] determination.
 - We tested 2 samples with a **high** T_{Tot} IgE rate (> 10.000 kU/L) on ISAC[®].

- Results:

- 384 results out of the 555 measurements were positive in CAP[®] and 171 were found negative.
 - 302/384 positive results were also found Positive with ISAC (concordance 78,65%),
 - 82 discrepant results : mean 0,67 kUA/L.
 - » 52 were below 0,35 kUA/L (former cut-off). New concordance 92,19%.
 - 160/171 negative results were also found negative with ISAC (concordance 93,57%),
 - 11 discrepant results : mean 1,57 ISU.
 - More frequently observed with some allergens (rAsp f 1, rPru p 3, nAna c 2, rApi g 1...)
- Until a rate of 150.000kU/L of T_{Tot} IgE, we did not observe any unspecific binding.

Table 1 Classification of 555 sIgE measured with ImmunoCAP® 250 (CAP) and ImmunoCAP® ISAC (ISAC), including their protein family, occurrence in the study and concordance between the CAP and the ISAC techniques (%).


Allergen source	Component	Occurrence in the study	Concordance between CAP and ISAC, %	Protein family or function
Food				
Peach (<i>Prunus persica</i>)	rDru p 1	26	92,3	PR-10
	rDru p 2	13	61,5	PR-10
	rDru p 3	5	80	PR-10
	rDru p 4	16	81,3	Storage protein, 7S globulin
	rDru p 5	9	77,8	Storage protein, Conalbumin
Kiwi fruit (<i>Actinidia deliciosa</i>)	rAra h 1	10	70	Storage protein, Glycinin
	rAra h 2	15	86,3	PR-10
	rAra h 3	10	100	Storage protein, 2S albumin
Peanut (<i>Arachis hypogaea</i>)	rAra h 1	10	100	PR-10
	rAra h 2	10	100	PR-10
	rAra h 3	10	100	PR-10
Brazil nut (<i>Bertholletia excelsa</i>)	rBer e 1	10	100	PR-10
	rBer e 2	10	100	PR-10
	rBer e 3	10	100	PR-10
Hazelnut (<i>Corylus avellana</i>)	rHae a 1	14	88,3	PR-10
	rHae a 2	10	60	PR-10
	rHae a 3	10	100	PR-10
Celery (<i>Apium graveolens</i>)	rCel f 1	10	100	PR-10
	rCel f 2	10	100	PR-10
	rCel f 3	10	100	PR-10
Soybean (<i>Glycine max</i>)	rGly m 4	11	100	PR-10
	rGly m 5	8	75	PR-10
	rGly m 6	8	75	PR-10
Wheat (<i>Triticum aestivum</i>)	rTri a 19 Gliadin	8	75	Glutelin
	rTri a 10	1	100	Ovomucoid
	rTri a 11	12	91,3	Parvalbumin
Egg white (<i>Gallus domesticus</i>)	rGal d 1	1	100	Parvalbumin
	rGal d 2	12	91,3	Parvalbumin
	rGal d 3	9	83,3	Parvalbumin
Ovomucoid	rGal d 1	1	100	Ovomucoid
	rGal d 2	12	91,3	Parvalbumin
	rGal d 3	9	83,3	Parvalbumin
Carp (<i>Cyprinus carpio</i>)	rCyp c 1	12	91,3	Parvalbumin
	rCyp c 2	9	83,3	Parvalbumin
	rCyp c 3	9	83,3	Parvalbumin
Cod (<i>Gadus callarias</i>)	rGad c 1	9	83,3	Parvalbumin
	rGad c 2	9	83,3	Parvalbumin
	rGad c 3	9	83,3	Parvalbumin
Cow (<i>Bos domesticus</i>)	rBos d 4	7	80	Alpha-lactalbumin
	rBos d 5	1	100	Serum albumin
	rBos d 6	3	66,7	Casain
Alpha-lactalbumin	rBos d 4	7	80	Alpha-lactalbumin
	rBos d 5	1	100	Serum albumin
	rBos d 6	3	66,7	Casain
BSA	rBos d 4	7	80	Alpha-lactalbumin
	rBos d 5	1	100	Serum albumin
	rBos d 6	3	66,7	Casain
Casein	rBos d 4	7	80	Alpha-lactalbumin
	rBos d 5	1	100	Serum albumin
	rBos d 6	3	66,7	Casain
Lactoferrin	rLac f 1	1	0	Transferrin
	rLac f 2	1	0	Transferrin
	rLac f 3	1	0	Transferrin
Shrimp (<i>Penaeus aztecus</i>)	rPen s 1	11	90,9	Tropomyosin
	rPen s 2	11	90,9	Tropomyosin
	rPen s 3	11	90,9	Tropomyosin
Grass pollen				
Timothy grass (<i>Phleum pratense</i>)	rPhl p 1	18	88,9	Grass group 1
	rPhl p 2	11	81,8	Ole c 1-related protein
	rPhl p 3	8	62,5	Profilin
	rPhl p 4	13	92,3	Grass group 2
	rPhl p 5	12	83,3	Barberine bridge enzyme
	rPhl p 6	9	88,9	Grass group 5
	rPhl p 7	12	75	Grass group 6
	rPhl p 8	5	40	Ca Binding protein
	rPhl p 9	5	40	Ca Binding protein
	rPhl p 10	5	40	Ca Binding protein
	rPhl p 11	5	40	Ca Binding protein
	rPhl p 12	5	40	Ca Binding protein
	rPhl p 13	5	40	Ca Binding protein
Occupational allergens				
Latex (<i>Hevea brasiliensis</i>)	rHev b 1	5	80	Rubber elongation factor
	rHev b 2	2	50	Small rubber particle protein
	rHev b 3	2	50	Small rubber particle protein
	rHev b 4	9	88,9	Hevein precursor
	rHev b 5	6	83,3	Profilin
Tree pollen	rBet v 1	1	100	Chitinase
	rBet v 2	1	100	Chitinase
	rBet v 3	1	100	Chitinase
Birch (<i>Betula verrucosa</i>)	rBet v 1	29	100	PR-10
	rBet v 2	14	88,6	PR-10
	rBet v 3	11	77,7	Ca Binding protein
Cypress (<i>Cupressus arizonica</i>)	rCyp a 1	5	100	Pectate lyase
	rCyp a 2	5	100	Pectate lyase
	rCyp a 3	5	100	Pectate lyase
Olive (<i>Olea europaea</i>)	rOle e 1	19	84,2	Common olive group 5
	rOle e 2	19	84,2	Common olive group 5
	rOle e 3	19	84,2	Common olive group 5
Wood pollen				
Mugwort (<i>Artemisia vulgaris</i>)	rArt v 1	7	71,4	Defensin
	rArt v 2	9	66,7	Defensin
	rArt v 3	9	66,7	Defensin
Salswort (<i>Salvia kaly</i>)	rSal l 1	3	66,7	Protein methyltransferase
	rSal l 2	3	66,7	Protein methyltransferase
	rSal l 3	3	66,7	Protein methyltransferase
Venoms				
Honey bee (<i>Apis mellifera</i>)	rApi m 1	2	100	Phospholipase A2
Epidermal and other proteins				
Cat (<i>Felis domesticus</i>)	rFel d 1	18	100	Urotopobin
	rFel d 2	7	71,4	Serum albumin

Allergen source	Component	Occurrence in the study	Concordance between CAP and ISAC, %	Protein family or function
Dog (<i>Canis familiaris</i>)				
Dog (<i>Canis familiaris</i>)	rCan f 1	12	100	Lipocalin
	rCan f 2	6	83,3	Lipocalin
	rCan f 3	10	70	Serum albumin
Mites				
House dust mite (<i>Dermatophagoides pteromyssinus</i>)	rDer p 1	13	92,3	Cysteine protease
	rDer p 10	5	100	Tropomyosin
	rDer p 2	12	100	NCF2 family
	rDer p 3	12	100	NCF2 family
Microorganism				
<i>Aspergillus fumigatus</i>	rAsp f 1	14	35,7	Mitogillin family
	rAsp f 2	14	35,7	Mitogillin family
<i>Alternaria alternata</i>	rAlt a 1	16	81,3	Streptolysin
	rAlt a 2	21	95,2	Streptolysin
Carbohydrate determinants				
Bromelin (<i>Ananas comosus</i>)	rAna c 2	11	63,6	Cross-reactive carbohydrate determinants

Gadisseur et al. Clin Chem Lab Med 2011; 49

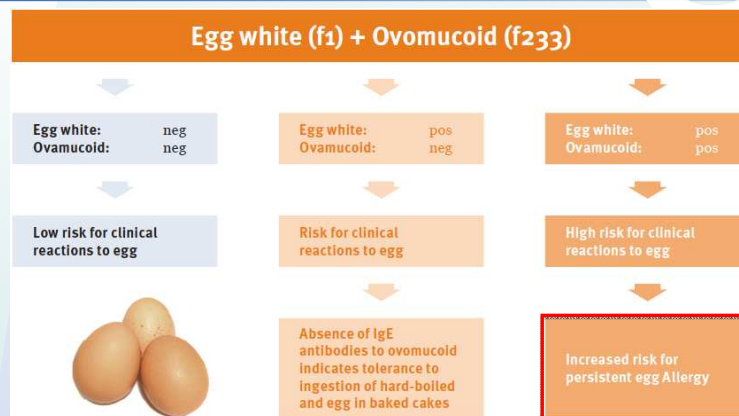
CHU de Liège **Patient cases**
Case n°1

- ♂, 1 year old (Miguel)
 - Anamnesis :
 - Two allergic parents, one allergic sister.
 - Normal diet with cow's milk, fruits and vegetables. Bread with hazelnut pasta.
 - Symptoms :
 - Very severe Atopic Dermatitis,
 - Gastrointestinal pain after peanut ingestion
 - Skin prick tests (SPT) :
 - Positive SPT pneumallergens : Cat, dog.
 - Positive SPT food allergens (crude/cooked) : Cow's milk, egg, peanut, hazelnut,
 - In-vitro tests :
 - Tot IgE : 150 kU/L (+).
 - Eosinophily : 26%.
 - IgE allergen extracts
 - Negative sIgE (<0,10 kUA/L): DPT, Soybean
 - Positive sIgE : egg white (1,16), caseine (11,4).
 - IgE allergen components
 - Negative sIgE (<0,10 kUA/L): rAra h 1, rAra h 2, rGly m 4, rBet v 1.
 - ImmunoCAP ISAC



- ♂, 1 year old (Miguel)
 - ImmunoCAP ISAC results :
 - Respiratory allergens
 - Sensitization to Hazelnut : Storage protein (11S-Globulin), nCor a 9 (Verwij et al., Ped All Immunol, 22, 2011).
 - Sensitization to birch pollen : rBet v 1 (PR10-protein).
 - Sensitization to egg (ovomucoid, nGal d 1) and cow's milk (casein, nBos d 8).
 - » Increased risk for persistent allergy to egg and cow's milk
 - Sensitization to dogs: rCan f 1 and rCan f 2.

Aliment d'origine végétale			
Noisette	nCor a 9	Storage protein, 11S globulin	1,3 ISU
Pollens d'arbres			
Bouleau	rBet v 1	PR-10 protein	4,7 ISU
Aliment d'origine animale			
Oeuf, Ovomucoïde	nGal d 1	Ovomucoid	2,2 ISU
Lait, Caséïnes	nBos d 8	Casein	0,6 ISU
Animal			
Chien	rCan f 1	Lipocalin	6,1 ISU
	rCan f 2	Lipocalin	16 ISU
Composants avec une réactivité croisée limitée			
Protéïnes PR10			
Bouleau	rBet v 1	PR-10 protein	4,7 ISU



Young infants with atopic dermatitis can display sensitization to Cor a 9, an 11S legumin-like seed-storage protein from hazelnut (*Corylus avellana*)
Verwij et al., Ped All Immunol, 22, 2011

- ♂, 3 years old (Amine)
 - Anamnesis :
 - Mother and brother allergic.
 - Symptoms :
 - Severe Atopic Dermatitis
 - Often dyspnea, asthma after some meals
 - Perannual rhinitis
 - Skin prick tests (SPT) :
 - Positive SPT food allergens : crab, hazelnut, kiwi, egg white.
 - » Suspicious : peanut, cod.
 - » Negative SPT pneumallergens : wheat.
 - In-vitro tests :
 - Tot IgE : 1379 kU/L +++
 - Eosinophily : 6.6%.
 - IgE allergen extracts :
 - » Positive sIgE : cod (0,24), DPT, peanut (0,6), hazelnut (3,22), soybean (1,53)
 - » Negative sIgE : cat, latex
 - IgE allergen components
 - » Positive sIgE : rAra h 2 (0,11)
 - » Negative sIgE : CCD, rAra h 1, rAra h 3, rAra h 8, rAra h 9, rCor a 8
 - ImmunoCAP ISAC



- ♂, 3 years old (Amine)
 - ImmunoCAP ISAC results :
 - Respiratory allergens
 - Sensitization to different grass pollens : Quack grass (nCyn d 1), Timothy grass (major rPhl p 1).
 - Sensitization to cats : major allergen rFel d 1.
 - Sensitization to HDM and Storage mites : NPC2 allergen family (nDer f 2 and rDer p 2) and Cystein protease family (nDer p 1 and nDer f 1), to Eur m 2.
 - Sensitization to moulds : *Aspergillus fumigatus*, (rAsp f 6).

Grass pollens

Pollens de graminées			
Chiendent digité	nCyn d 1	Grass group 1	0,7 ISU
Phléole	rPhl p 1	Grass group 1	5,8 ISU

Cat

Animal			
Chat	rFel d 1	Uteroglobine	20 ISU

Moulds

Moississure			
Aspergillus	rAsp f 6	Mn superoxide dismutase	0,3 ISU

HDM

Acarien			
Acariens domestiques	nDer p 1	Cysteine protease	34 ISU
	nDer f 1	Cysteine protease	20 ISU
	nDer p 2	NPC2 family	25 ISU
	rDer f 2	NPC2 family	30 ISU
Acariens de stockage	rEur m 2	NPC2 family	4,5 ISU

• ♂, 3 years old (Amine)

– ImmunoCAP ISAC results :

▪ Food allergens.

– Sensitization to a Storage Proteins:

» Soybean nGly m 5...

» Hazelnut rCor a 9...

» Sesame seed rSes i 3...

» Major allergenicity of this protein family (heat stability and stability to digestion).

» If positive oral food challenge or a clear history : nuts and soybean eviction is recommended.

– Sensitization to kiwi fruit via (Act d 2).

– Sensitization to Tropomyosins.

» Positive sIgE against 6 proteins of this family (from various sources: shrimps, HDM, cockroach, anisakis) are found at significant levels.

» Stability to heat and digestion causing reactions also to cooked foods. Often associated with severe reactions.

» Seafood avoidance is required !

Kiwi	nAct d 2	Thaumatococin-like protein	0,4 ISU	■
Noisette	nCor a 9	Storage protein, 11S globulin	0,6 ISU	■
Graine de sésame	nSes i 1	Storage protein, 2S albumin	0,5 ISU	■
Soja	nGly m 5	Storage protein, Beta-conglycinin	0,4 ISU	■

HDM

Tropomyosines				
Crevette	rPen a 1	Tropomyosin	12 ISU	■
	nPen i 1	Tropomyosin	15 ISU	■
	nPen m 1	Tropomyosin	19 ISU	■
Acariens domestiques	rDer p 10	Tropomyosin	21 ISU	■
Blatte	nBla g 7	Tropomyosin	11 ISU	■
Anisakis	rAni s 3	Tropomyosin	11 ISU	■

• ♂, 8 years old (Cyril)

– Anamnesis :

▪ Symptoms :

- 1-year of age : Milk allergy, Asthma after eating a peach => cow milk and peach avoidance
- November 2011 : Chronic nausea, gastrointestinal trouble, headaches after each meal.
- December 2011 :

- » Cyanosis after eating 4 olives,
- » Angioedema after eating cooked apple with bread.

▪ Gastroscopy : no Eosinophilic oesophagitis.

▪ Skin prick tests (SPT) :

- Positive SPT pneumallergens : Cat dander, Mugwort, House Dust Mites.
- » Negative SPT pneumallergens : grass pollens, birch.
- Positive SPT food allergens (crude/cooked) : apricot, plum, peach, hazelnut, peanut, soybean, apple, orange, wheat, ...
- » Negative SPT food allergens : tomato, fish, meat, milk, vanilla, buckwheat, rice.

▪ In-vitro tests :

- Tot IgE : 392 kU/L ++
- Eosinophily : 1,9 %.
- IgE allergen extracts
 - » Positive sIgE : birch (0,39), egg white (0,15), barley (5,32), rice (1,37), potato (0,31), wheat (0,76), soybean (6,74), hazelnut (1,51), tomato (1,81), kiwi (4,62), peanut (10,1), orange (3,46), apple (16,8), banana (0,55), peach (16,3), plum (16,8), peas (4,25)...
 - » Negative sIgE : latex, cod, DPT
- IgE allergen components
 - » Negative sIgE : CCD

– ImmunoCAP ISAC



- ♂, 8 years old (Cyril)
 - ImmunoCAP ISAC results :
 - Respiratory allergens
 - Sensitization to different grass pollens : Quackgrass (nCyn d 1), **Timothy grass** (rPhl p 1 & 2).
 - Sensitization to House Dust Mites : NPC2 allergen family (nDer f 2)
 - Sensitization to moulds : *Aspergillus fumigatus*, (rAsp f 3 & 6).

Timothy Grass

Aspergillus
fumigatus

Pollens de graminées			
Chiendent digité	nCyn d 1	Grass group 1	1,6 ISU
Phléole	rPhl p 1	Grass group 1	1,6 ISU
	rPhl p 2	Grass group 2	7,2 ISU
Moisissure			
Aspergillus	rAsp f 3	Peroxisomal protein	0,4 ISU
	rAsp f 6	Mn superoxide dismutase	0,4 ISU
Acarien			
Acariens domestiques	rDer f 2	NPC2 family	0,3 ISU

- ♂, 8 years old (Cyril)
 - ImmunoCAP ISAC results :

Peach LTP →








Food allergens.

- Sensitization to a **Storage Protein of soybean (nGly m 5)**.
 - » Major allergenicity of this protein (heat stability and stability to digestion).
 - » If positive oral food challenge or a clear history : soybean avoidance is recommended. Cross-reactions !
- Sensitization to birch pollen (rBet v 1). Low rates of sIgE for **PR-10 proteins of peach and peanut** (respectively rPru p 1 and rAra h 8). Not very relevant...no clinical symptoms (no rhinitis, nor conjunctivitis...)
- Sensitization to Lipid Transfer Proteins (LTP)**.
 - » Positive sIgE against 3 proteins of this family (from various sources: peach, hazelnut, mugwort) are found at significant levels.
 - » Stability to heat and digestion causing reactions also to cooked foods. Often associated with systemic and more severe reactions in addition to Oral Allergy Syndrome (OAS). Often associated with allergic reactions to fruit and vegetables in Southern Europe (rare in the Northern Europe).
 - » It is very important to maintain a total food avoidance of foods containing LTP when clinical symptoms are associated with their consumption. Indeed, allergy to LTP is associated with the occurrence of severe symptoms year if consumed raw or cooked food.

Soja	nGly m 5	Storage protein, Beta-conglycinin	0,9 ISU
Protéines PR10			
Pêche	rPru p 1	PR-10 protein	0,3 ISU
Arachide	rAra h 8	PR-10 protein	0,4 ISU
Protéines de transfert des lipides			
Pêche	nPru p 3	Lipid transfer protein (nsLTP)	6,1 ISU
Noisette	rCor a 8	Lipid transfer protein (nsLTP)	0,8 ISU
Armoise	nArt v 3	Lipid transfer protein (nsLTP)	2,7 ISU

- ♂, 10 years old (Marty)
 - Anamnesis :
 - Symptoms :
 - Consultation for hazelnut eviction (sIgE hazelnut>20 kUA/L) !
 - Chronic Ulcero Hemorrhagic Colitis,
 - Atopic Dermatitis,
 - Rhinitis during tree pollinosis
 - OAS
 - » Mild after eating crude peaches, apples.
 - » Important after drinking soybean milk. Asthma ?
 - Rem : No OAS after cooked fruits.
 - Skin prick tests (SPT) :
 - Children refused !
 - In-vitro tests :
 - Tot IgE : 336 kU/L ++
 - Eosinophily : 7,2 %.
 - IgE allergen extracts
 - » Positive sIgE : hazelnut (20,4), grass pollens (1,69), tree pollens (21,6).
 - » Negative sIgE : Barley, meat, thuna, caseine, DPT, cat, dog, pollens of weeds.
 - IgE allergen components
 - » Positive sIgE : rBet v 1.
- ImmunoCAP ISAC

- ♂, 10 years old (Marty)
 - ImmunoCAP ISAC results :
 - Respiratory allergens
 - Sensitization to different grass pollens : Quackgrass (nCyn d 1), Timothy grass (rPhi p 1).
 - Sensitisation to tree pollens : Olive tree (nOle e 1) and Birch (rBet v 1), Alder (rAln g 1), Hazeltree (rCor a 1.0101).
 - Sensitization to moulds : *Aspergillus fumigatus* (rAsp f 2)

Pollens de graminées			
Chiendent digité	nCyn d 1	Grass group 1	0,7 ISU 
Phléole	rPhi p 1	Grass group 1	1,1 ISU 
Pollens d'arbres			
Bouleau	rBet v 1	PR-10 protein	32 ISU 
Aulne	rAln g 1	PR-10 protein	10 ISU 
Pollen de noisetier	rCor a 1.0101	PR-10 protein	5,2 ISU 
Olivier	nOle e 1	Common olive group 5	59 ISU 
Moississure			
Aspergillus	rAsp f 2	Fibrinogen Binding Protein	0,4 ISU 

Represents sensitization to Fraxinus, Lygustrum, forsythia...

- ♀, 10 years old (Marty)
 - ImmunoCAP ISAC results :

Protéines PR10			
Bouleau	rBet v 1	PR-10 protein	32 ISU
Aulne	rAln g 1	PR-10 protein	10 ISU
Pollen de noisetier	rCor a 1.0101	PR-10 protein	5,2 ISU
Noisette	rCor a 1.0401	PR-10 protein	2,5 ISU
Pomme	rMal d 1	PR-10 protein	9,7 ISU
Pêche	rPru p 1	PR-10 protein	19 ISU
Soja	rGly m 4	PR-10 protein	1,9 ISU
Arachide	rAra h 8	PR-10 protein	9,8 ISU
Kiwi	nAct d 8	PR-10 protein	0,7 ISU
Céleri	rApi g 1	PR-10 protein	2,1 ISU

PR10 of celery and soybean heat stable, resistant to digestion

- Food allergens.
 - Sensitization to birch pollen (rBet v 1) and Bet v 1-homologues.
 - » sIgE for PR-10 proteins of hazelnut (rCor a 1.0401), apple (rMal d 1), peach (rPru p 1), soybean (rGly m 4), peanut (rAra h 8), kiwi (nAct d 8) and celery (rApi g 1).
 - » Heat labile protein, cooked food are often tolerated.
 - » Often associated with local symptoms such as OAS.
 - » Often associated with allergic reactions to fruit and vegetables in northern Europe.

- ♀, 43 years old (Charlotte)
 - Anamnesis :
 - Consultation in the aim to understand the severity of her food allergy !!
 - Symptoms :
 - Rhinitis, conjunctivitis, asthma during pollen period.
 - Asthma after eating a pear.
 - Skin prick tests (SPT) :
 - Positive SPT pneumallergens : grass pollens, cat, mugwort, birch.
 - Positive SPT food allergens : banana, apricot, plum, melon, peach.
 - Negative SPT : apple, strawberry, House Dust Mites.
 - In-vitro tests :
 - Tot IgE : 209 kU/L
 - Eosinophily : 13.1%
 - IgE kiwi (1.02), banana (1.61), birch (4.95).
 - ImmunoCAP ISAC
 - ImmunoCAP ISAC Results
 - Pneumallergens :
 - Sensitization to grass pollen, including Timothy Grass (rPhl p 1, rPhl p 2, nPhl 4, rPhl p 5, rPhl p 6, rPhl p 11 and rPhl p 12) and Bermuda grass (nCyn1).
 - Cat's Uteroglobin (rFel d 1).
 - Protein families :
 - different profilins, rBet v 2, nOle e 2, rHev b 8, rMer a 1 and rPhl p 12.
 - » Profilins are panallergens rarely associated with symptoms but may have clinical significance, and even cause severe reactions in a small minority of allergic patients including lemon, melon, banana, lychee and tomato.

1. Résumé des résultats des dosages d'IgE spécifiques positifs

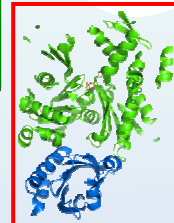
Composants spécifiques d'espèce

Pollens de graminées			
Chiendent digité	nCyn d 1	Grass group 1	1,3 ISU
Phléole	rPhl p 1	Grass group 1	6,2 ISU
	rPhl p 2	Grass group 2	7,6 ISU
	rPhl p 4	Berberine bridge enzyme	1,2 ISU
	rPhl p 5	Grass group 5	11 ISU
	rPhl p 6	Grass group 6	3,7 ISU
	rPhl p 11	Ole e 1-related protein	4,4 ISU

Animal			
Chat	rFel d 1	Uteroglobine	1,3 ISU

Composants avec une réactivité croisée large

Profilines			
Bouleau	rBet v 2	Profilin	1,8 ISU
Olivier	nOle e 2	Profilin	0,8 ISU
Latex	rHev b 8	Profilin	2,3 ISU
Mercuriale	rMer a 1	Profilin	3,1 ISU
Phléole	rPhl p 12	Profilin	1,2 ISU



Profilin rPhl p 12

– ImmunoCAP ISAC

– ImmunoCAP ISAC Results

▪ Pneumallergènes :

- Sensitization to grass pollen, including Timothy Grass (rPhl p 1, rPhl p 2, nPhl 4, rPhl p 5, rPhl p 6, rPhl p 11 and rPhl p 12) and Bermuda grass (nCyn1).
- Cat's Uteroglobine (rFel d 1).

Protein families :

- different profilins, rBet v 2, nOle e 2, rHev b 8, rMer a 1 and rPhl p 12.

Profilins are panallergens rarely associated with symptoms but may have clinical significance, and even cause severe reactions in a small minority of allergic patients including lemon, melon, banana, lychee and tomato.

- Currently available standard products for allergy testing based on allergen extracts prepared from biological raw materials
 - Natural mixtures of allergenic and non-allergenic molecules
 - not fully standardized referring to their content of major/minor allergen components.
- Increasing availability of allergen components
 - purified from their natural source or biotechnologically produced as recombinant proteins
 - **Revolution in allergy diagnosis : Molecular Allergology, CRD.**
- Microarrays allow us to investigate IgE reactivity to a large number of different molecules or allergenic components with a single and rapid test.
 - With the traditional methods, impossible to analyse the panel of natural and recombinant allergens ensuring the presentation of a significant number of epitopes.
- Allergen panel can be expanded and improved continuously, depending on the availability of new components, with the incorporation of the most appropriate profiles defined by the clinical studies.
 - Version 103 components >>> next version, some allergen removed or added.

- Based on modern biochip technology
 - sIgE antibodies >100 purified natural or recombinant allergen components, >40 common sources.
 - The amount of patient serum consumed is far smaller than in the case of conventional immunoassaying =30 µl serum is enough to determine the sIgE panel.
 - Interest of the technique in paediatric patients
 - Capillary blood sampling can be used, enabling a less invasive procedure for testing young children.
 - Highly reliable results versus venous serum sampling, according to the studies of Ott et al.
 - High reliability by intrinsic replicate testing and quality controls.
 - Good sensitivity, good correlation with ImmunoCAP.
 - Diagnostic tool
 - Understanding of the sensitization profile.
 - Some components are unavailable with CAP.
 - Unexpected sensitizations.
 - As this technique provides potentially unexpected results, the microarray determination has to be used in conjunction with the clinical findings.
- CRD !!**
- Personalised medicine, contribute to the diagnosis, prognosis and staging of disease and to treatment selection, facilitating preventive medicine, predictive diagnosis and individualised curative therapies.

- **The way for the discover of sensitization profiles is open...**
 - **CRD is in our hands.**
 - Sensitizations can cause diagnosis errors (and therapeutic errors too).
 - Protein families have been described, similarities of structure (homology of amino-acids or structural homology)
 - Allergy to one protein can cause cross-reactions to other proteins of the same family.
 - Recombinant allergens permit to focus (one or more) allergen(s) of one source:
 - Available in CAP (and/or in ISAC) or to be developed...
 - **When should we perform an ISAC ?**
 - Suspicion of multiple allergies, to determine the “complete” sensitization profile...Help for the diagnostic.
 - To predict the different cross-reactivities in a polysensitized patient.
 - Symptoms that are not well defined when there is a history of possible allergy but negative in-vivo and in-vitro tests.
 - To persuade a patient that he is not allergic when the different traditional test remain negative.
 - To test some components unavailable on CAP

- Vast amount of data that will be generated by future systems
 - Interpreted and managed in a structured and well-organised manner.
 - Data base structure,
 - Support the clinical scientist who needs to move the medical diagnostic frontier forward
 - Help the practicing physician both to treat the patient and cut healthcare costs more effectively.
 - Phadia Xplain software
 - Data interpretation to support clinical scientists.
- Difficult interpretation of the results :
 - Should be limited to specialists !



Standard de Liège

Winner of the Belgian Cup 2011



Department of Clinical Chemistry
- Endocrinology, Allergology -



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