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Individual Recombinant/ Purified Allergen Molecules and Allergen Microarray testing

Previously on skin test or laboratory test systems, we could only measure specific IgE to an allergen source, usually a mixture of many different molecules. In the most important practical advance in laboratory allergy diagnostics, it is now possible to determine the amount of circulating specific IgE to individual allergen molecules. This can be performed for a unique molecule of interest using separate ImmunoCAP's or, for multiple molecules, on a microarray platform called the ISAC® ImmunoCAP.

Work began on isolating individual allergen molecules of interest in the late 1970s and on developing microarray platforms in 2003. Early basic and later clinical research has resulted in mature technologies available to clinicians through specialised laboratories. Since IgE reactivity to some individual molecules can result in potentially serious clinical events, these test systems can provide new diagnostic and prognostic information to clinicians looking after allergic patients.

The ability to identify which molecules are involved in allergic sensitisation is referred to as component-resolved diagnosis.

The Phadia ISAC® ImmunoCAP system presently profiles specific IgE to 103 individual allergen molecules from 47 different allergens, while separate ImmunoCAP reagents are available to test one molecule at a time when the question is more selected. Unfortunately, the Medicare Benefits Schedule has long neglected reimbursement levels for *in vitro* specific IgE testing and the current rebate (\$22.95) for an episode of laboratory allergy testing is considerably less than the reagent cost of these test systems. Our laboratory charges \$40 per recombinant or native individual allergen molecules, with the exception of three purified milk molecules (\$4 each), and \$350 for the ISAC® profile of 103 individual allergens on a microarray. Since ISAC® is a much more cost-efficient approach to such testing, I have called this newsletter 'ISACology' and will provide progressive updates with clinical vignettes.



Individual Recombinant/Purified Allergen Molecules and Allergen Microarray testing (cont.)

In the first table, we list the individual allergen molecules available on separate ImmunoCAPs (Table 1). In general, results of specific IgE testing to these are available within 1-2 days. Interpretation of these results is assisted by co-requesting specific IgE to the whole allergen source (eg. "peanut, ara h1, ara h2, ara h 3,

ara h 8, ara h 9"). Our allergy menu is listed in a separate publication (see back page – available on-line at www.dhm.com.au clinicians/publications). In the second table, we list the individual allergen molecules available on the ISAC® ImmunoCAP. In general, results of these tests are available within 2-4 weeks, as this testing is

performed in batches. A detailed clinical history will help me guide you in interpreting the results, but you may contact me at kbaumgart@dhm.com.au or on 02-98555286.

Karl W Baumgart
Director, Immunology

Table 1

RECOMBINANT AND NATIVE INDIVIDUAL ALLERGEN MOLECULES

n Individual Native (n) \$40 each
r Recombinant (r) allergen molecules \$40 each

ANIMALS		
r	E94	rFel d1 (recombinant cat)
r	E220	nFel d 2 Cat serum albumin Felis domesticus
r	E101	rCan f1 (recombinant dog)
r	E102	rCan f2 (recombinant dog)
n	E221	nCan f 3 Dog serum albumin Canis familiaris
n	E204	nBos d 6 BSA, Cow Bos spp.
n	E222	nSus s Pig serum albumin, Swine Sus scrofa

GRASSES		
r	G205	rPhl p 1 (recombinant timothy)
r	G206	rPhl p 2 (recombinant timothy)
r	G208	rPhl p 4 (native timothy)
r	G209	rPhl p 6 (recombinant timothy)
r	G210	rPhl p 7 (recombinant timothy)
r	G211	rPhl p 11 (recombinant timothy)
r	G212	rPhl p 12 (recombinant timothy)
r	G213	rPhl p1; rPhl p5b (recombinant timothy)
r	G214	rPhl p7;rPhl p12 (recombinant timothy)
r	G215	rPhl p 5b (recombinant timothy)
n	G216	nCyn d 1 Bermuda grass, Cynodon dactylon

FOODS		
c	F76	nBos d 4 a-lactalbumin, Milk Bos spp.
c	F77	nBos d 5 b-lactoglobulin, Milk Bos spp.
c	F78	nBos d 8 Casein, Milk Bos spp.

c Core individual allergens, \$4 each
Note: These allergens are priced as core individual allergens (\$4 each)

FOODS		
n	F232	nGal d 2 Ovalbumin, Egg Gallus spp.
n	F233	nGal d 1 Ovomucoid, Egg Gallus spp.
n	F323	nGal d 3 Conalbumin, Egg Gallus spp.

n	F334	nBos d Lactoferrin, Milk Bos spp.
r	F351	rPen a 1 Tropomyosin, Shrimp <i>Penaeus aztecus</i>
r	F353	rGly m 4 PR-10, Soy Glycine
r	F354	rBer e 1 Brazil nut, Bertholletia excelsa
r	F355	rCyp c 1 Carp Cyprinus carpio
r	F417	rApi g 1.01 PR-10, Celery <i>Apium graveolens</i>
r	F418	rApi g 1.02 (recombinant Celery)
r	F419	rPru p 1 PR-10, Peach Prunus persica
r	F420	rPru p 3 LTP, Peach Prunus persica
r	F421	rPru p 4 Profilin, Peach Prunus persica
r	F416	rTri a 19 Omega-5 Gliadin, Wheat Triticum spp
r	F422	rAra h 1 Peanut <i>Arachis hypogaea</i>
r	F423	rAra h 2 Peanut <i>Arachis hypogaea</i>
r	F424	rAra h 3 Peanut <i>Arachis hypogaea</i>
r	F352	rAra h 8 PR-10, Peanut <i>Arachis hypogaea</i>
r	F427	rAra h 9 LTP, Peanut <i>Arachis hypogaea</i>
r	F425	rCor a 8 LTP, Hazel nut Corylus avellana
r	F426	rGad c 1 Cod Gadus morhua
r	F428	rCor a 1 PR-10, Hazel nut Corylus avellana

TREES		
r	T215	rBet v 1 PR-10, Birch Betula verrucosa
r	T216	rBet v 2 Profilin, Birch Betula verrucosa
r	T220	rBet v 4, Birch Betula verrucosa
r	T221	rBet v 2, rBet v 4 Birch Betula verrucosa
r	T225	rBet v 6 Birch Betula verrucosa
n	T224	nOle e 1 Olive Olea europaea

WEEDS		
r	W211	rPar j 2 (recombinant parietaria)
n	W230	nAmb a 1, Ragweed Ambrosia elatior
n	W231	nArt v 1, Mugwort Artemisia vulgaris

MOULDS		
r	M218	rAsp f1 (recombinant aspergillus)
r	M219	rAsp f2 (recombinant aspergillus)
r	M220	rAsp f 3 (recombinant aspergillus)
r	M221	rAsp f4 (recombinant aspergillus)
r	M222	rAsp f6 (recombinant aspergillus)
r	M229	rAlt a 1 (component alternaria)

LATEX		
r	K215	rHev b 1, Latex <i>Hevea brasiliensis</i>
r	K217	rHev b 3, Latex <i>Hevea brasiliensis</i>
r	K218	rHev b 5, Latex <i>Hevea brasiliensis</i>
r	K219	rHev b 6.01, Latex <i>Hevea brasiliensis</i>
r	K220	rHev b 6.02, Latex <i>Hevea brasiliensis</i>
r	K221	rHev b 8, Profilin, Latex <i>Hevea brasiliensis</i>
r	K222	rHev b 9, Latex <i>Hevea brasiliensis</i>
r	K224	rHev b 11, Latex <i>Hevea brasiliensis</i>

VENOM		
n	K203	nApi m 1, Phospholipase A2, Bee Apis mellifera

OCCUPATIONAL ALLERGENS		
n	K205	Alkalase, Bacillus spp.
n	K87	nAsp o 1, a-amylase Aspergillus oryzae
n	K201	nCar p 1, Papain, Papaya Carica papaya
n	K208	nGal d 4, Lysozyme, Egg Gallus spp.
n	K204	Maxatase, Bacillus licheniformis
n	K206	Savinase, Bacillus spp.
n	K213	nSus s Pepsin, Swine Sus scrofa

OTHERS (to exclude CCD reactivity)		
n	Ro214	MUXF3 CCD, Bromelin
n	K202	nAna c 2 Bromelin, Pineapple Ananas comosus

Table 2

IMMUNOCAP ISAC® ALLERGEN COMPONENTS

PLANTS			
nCyn d 1	Bermuda grass	<i>Cynodon dactylon</i>	Grass group 1
rPhl p 1	Timothy	<i>Phleum pratense</i>	Grass group 1
rPhl p 2	Timothy	<i>Phleum pratense</i>	Grass group 2
nPhl p 4	Timothy	<i>Phleum pratense</i>	
rPhl p 5	Timothy	<i>Phleum pratense</i>	Grass group 5
rPhl p 6	Timothy	<i>Phleum pratense</i>	
rPhl p 11	Timothy	<i>Phleum pratense</i>	
rBet v 1	Birch	<i>Betula verrucosa</i>	PR-10 protein
rAln g 1	Alder	<i>Alnus glutinosa</i>	PR-10 protein
rCor a 1.0101	Hazel pollen	<i>Corylus avellana</i>	PR-10 protein
nCry j 1	Japanese cedar	<i>Cryptomeria japonica</i>	
nCup a 1	Cypress	<i>Cupressus arizonica</i>	
nOle e 1	Olive	<i>Olea europaea</i>	
rPla a 1	Plane tree	<i>Platanus acerifolia</i>	
nPla a 2	Plane tree	<i>Platanus acerifolia</i>	
nAmb a 1	Ragweed	<i>Ambrosia artemisiifolia</i>	
nArt v 1	Mugwort	<i>Artemisia vulgaris</i>	
nArt v 3	Mugwort	<i>Artemisia vulgaris</i>	Lipid transfer protein (nsLTP)
rPar j 2	Wall pellitory	<i>Parietaria Judaica</i>	Lipid transfer protein (nsLTP)
nSal k 1	Saltwort	<i>Salsola kali</i>	
nAct d 1	Kiwi	<i>Actinidia deliciosa</i>	
nAct d 2	Kiwi	<i>Actinidia deliciosa</i>	
nAct d 5	Kiwi	<i>Actinidia deliciosa</i>	
nAct d 8	Kiwi	<i>Actinidia deliciosa</i>	PR-10 protein
rApi g 1	Celery	<i>Apium graveolens</i>	PR-10 protein
rDau c 1	Carrot	<i>Daucus carota</i>	PR-10 protein
rMal d 1	Apple	<i>Malus domestica</i>	PR-10 protein
rPru p 1	Peach	<i>Prunus persica</i>	PR-10 protein
nPru p 3	Peach	<i>Prunus persica</i>	Lipid transfer protein (nsLTP)
rAna o 2	Cashew nut	<i>Anacardium occidentale</i>	
nAra h 1	Peanut	<i>Arachis hypogaea</i>	Storage protein, vicilin
nAra h 2	Peanut	<i>Arachis hypogaea</i>	Storage protein, Conglutin
nAra h 3	Peanut	<i>Arachis hypogaea</i>	Storage protein, 11S globulin
rAra h 8	Peanut	<i>Arachis hypogaea</i>	PR-10 protein
rBer e 1	Brazil nut	<i>Bertholletia excelsa</i>	Storage protein, 2S albumin
rCor a 1.0401	Hazelnut	<i>Corylus avellana</i>	PR-10 protein
rCor a 8	Hazelnut	<i>Corylus avellana</i>	Lipid transfer protein (nsLTP)
nCor a 9	Hazelnut	<i>Corylus avellana</i>	Storage protein, 11S globulin
rGly m 4	Soybean	<i>Glycine max</i>	PR-10 protein
nGly m 5	Soybean	<i>Glycine max</i>	Storage protein, b-conglycinin
nGly m 6	Soybean	<i>Glycine max</i>	Storage protein, glycinin
nSes i 1	Sesame seed	<i>Sesamum indicum</i>	Storage protein, 2S albumin
nTri a 18	Wheat	<i>Triticum aestivum</i>	
nTri a gliadin	Wheat	<i>Triticum aestivum</i>	Crude gliadin
rTri a 19.0101	Wheat	<i>Triticum aestivum</i>	Omega-5 gliadin
nTri a aA_TI	Wheat	<i>Triticum aestivum</i>	
rHev b 1	Latex	<i>Hevea brasiliensis</i>	
rHev b 3	Latex	<i>Hevea brasiliensis</i>	
rHev b 5	Latex	<i>Hevea brasiliensis</i>	
rHev b 6	Latex	<i>Hevea brasiliensis</i>	

CROSS REACTIVE MARKERS, PLANTS			
rBet v 4	Birch	<i>Betula verrucosa</i>	Calcium binding protein, Polcalcin
rPhl p 7	Timothy	<i>Phleum pratense</i>	Calcium binding protein, Polcalcin
rBet v 2	Birch	<i>Betula verrucosa</i>	Profilin
rHev b 8	Latex	<i>Hevea brasiliensis</i>	Profilin
rMer a 1	Annual mercury	<i>Mercurialis annua</i>	Profilin
nOle e 2	Olive	<i>Olea europaea</i>	Profilin
rPhl p 12	Timothy	<i>Phleum pratense</i>	Profilin
nAna c 2	Bromelain	<i>Ananas comosus</i>	CCD marker

NON-PLANTS			
nBos d 6	BSA	<i>Bos domesticus</i>	Serum albumin
nBos d 8	Cow's milk	<i>Bos domesticus</i>	Caseins
nBos d	lactoferrin	<i>Bos domesticus</i>	Laktoferrin
nGal d 1	Egg	<i>Gallus domesticus</i>	Ovomucoid
nGal d 2	Egg	<i>Gallus domesticus</i>	Ovalbumin
nGal d 3	Egg	<i>Gallus domesticus</i>	Conalbumin
nGal d 5	CSA (Livetin)	<i>Gallus domesticus</i>	Serum albumin
rCyp c 1	Carp	<i>Cyprinus carpio</i>	Parvalbumin
rGad c 1	Cod	<i>Gadus callarias</i>	Parvalbumin
rDer f 1	House dust mite	<i>Dermatophagoides farinae</i>	
rDer f 2	House dust mite	<i>Dermatophagoides farinae</i>	
nDer p 1	House dust mite	<i>Dermatophagoides pteronyssinus</i>	
nDer p 2	House dust mite	<i>Dermatophagoides pteronyssinus</i>	
rEur m 2	Storage mite	<i>Euroglyphus maynei</i>	
rCan f 1	Dog	<i>Canis familiaris</i>	Lipocalin
rCan f 2	Dog	<i>Canis familiaris</i>	Lipocalin
nCan f 3	Dog	<i>Canis familiaris</i>	Serum albumin
nEqu c 3	Horse	<i>Equus caballus</i>	Serum albumin
rFel d 1	Cat	<i>Felis domesticus</i>	Uterogloblin
nFel d 2	Cat	<i>Felis domesticus</i>	Serum albumin
nMus m 1	Mouse	<i>Mus musculus</i>	Lipocalin
rAlt a 1	Alternaria	<i>Alternaria alternata</i>	
rAlt a 6	Alternaria	<i>Alternaria alternata</i>	
rAsp f 1	Aspergillus	<i>Aspergillus fumigatus</i>	
rAsp f 2	Aspergillus	<i>Aspergillus fumigatus</i>	
rAsp f 3	Aspergillus	<i>Aspergillus fumigatus</i>	
rAsp f 4	Aspergillus	<i>Aspergillus fumigatus</i>	
rAsp f 6	Aspergillus	<i>Aspergillus fumigatus</i>	
rCla h 8	Cladosporium	<i>Cladosporium herbarum</i>	
nApi m 1	Honey bee venom	<i>Apis mellifera</i>	Phospholipase A2
nApi m 4	Honey bee venom	<i>Apis mellifera</i>	Melittin
rBla g 1	Cockroach	<i>Blattella germanica</i>	
rBla g 2	Cockroach	<i>Blattella germanica</i>	
rBla g 4	Cockroach	<i>Blattella germanica</i>	
rBla g 5	Cockroach	<i>Blattella germanica</i>	
rAni s 1	Anisakis	<i>Anisakis simplex</i>	

CROSS REACTIVE MARKERS, NON-PLANTS			
rAni s 3	Anisakis	<i>Anisakis simplex</i>	Tropomyosin
nBla g 7	Cockroach	<i>Blattella germanica</i>	Tropomyosin
rDer p 10	House dust mite	<i>Dermatophagoides pteronyssinus</i>	Tropomyosin
rPen a 1	Shrimp	<i>Penaeus aztecus</i>	Tropomyosin
nPen i 1	Shrimp	<i>Penaeus indicus</i>	Tropomyosin
nPen m 1	Shrimp	<i>Penaeus monodon</i>	Tropomyosin

ImmunoCAP ISAC® (\$350 per profile)

The ISAC® (Immuno Solid-phase Allergen Chip) is a microarray system developed by VBC Genomics and Phadia. ISAC® can now allow laboratories to reliably and simultaneously determine specific IgE levels to a wide range of clinically important individual allergen molecules more economically than using many individual recombinant CAP allergens.

Clinical studies have shown that, for example, even modest levels of serum specific IgE level to ara h 2, one of the nine major peanut allergens, are strongly predictive of more severe reactions and poor tolerance of oral challenges. Similarly, modest levels of serum specific IgE to ovomucoid, an egg allergen which is heat resistant, are strongly predictive of poor tolerance to

even cooked egg in a baked cake; the first challenge usually used by allergy clinics when one is confident that a child might be starting to lose their egg allergy.

The ISAC® profiles are performed in batches, and the results are reported semi-quantitatively and organised into both the individual allergen molecule source, as well as the protein group from which it comes. These protein groups have particular patterns of clinical significance. For example, specific IgE to Lipid Transfer Proteins are often associated with systemic and more severe reactions in addition to oral allergy symptoms and these molecules are usually not altered with food preparation and cooking (enzyme digestion and heat). Similarly, seed storage proteins are also heat resistant

and often associated with significant symptoms with cooked foods. In contrast, PR-10 proteins are heat labile and are associated with oral allergy symptoms with the raw, rather than cooked foods. Similarly, heat sensitive profilin proteins are more associated with nuisance rather than severe symptoms. Tropomyosin proteins account for dustmite-crustacean cross-reactivity. Animal albumins and fish parvalbumins are important causes of other food reactions.

Table 2 (Page 3) lists the allergens included in the ISAC® profile.

Component-Resolved Diagnostics

Not all patients with positive skin tests or elevated levels of specific IgE to allergens get symptoms, while some get trivial, and others more serious, symptoms. The size of a skin test correlates with the amount of circulating and local mast cell bound specific IgE present. The level of specific IgE to an allergen in serum correlates with the sum of the concentrations of circulating specific IgE to the individual allergen molecules in the allergen source. Individuals may vary substantially in how much their specific IgE is directed

to individual allergen molecules or components in a whole allergen source. Variability in heat and digestive enzyme stability of some of these components accounts for why some exposures to foods a person may be sensitised to may cause minimal or more substantial symptoms. Since many foods are plant-derived, sensitisation to some molecules in cross-reactive pollen sources may result in substantial reactivity on testing, without significant adverse experiences with exposure. Similarly, some animal-derived proteins may

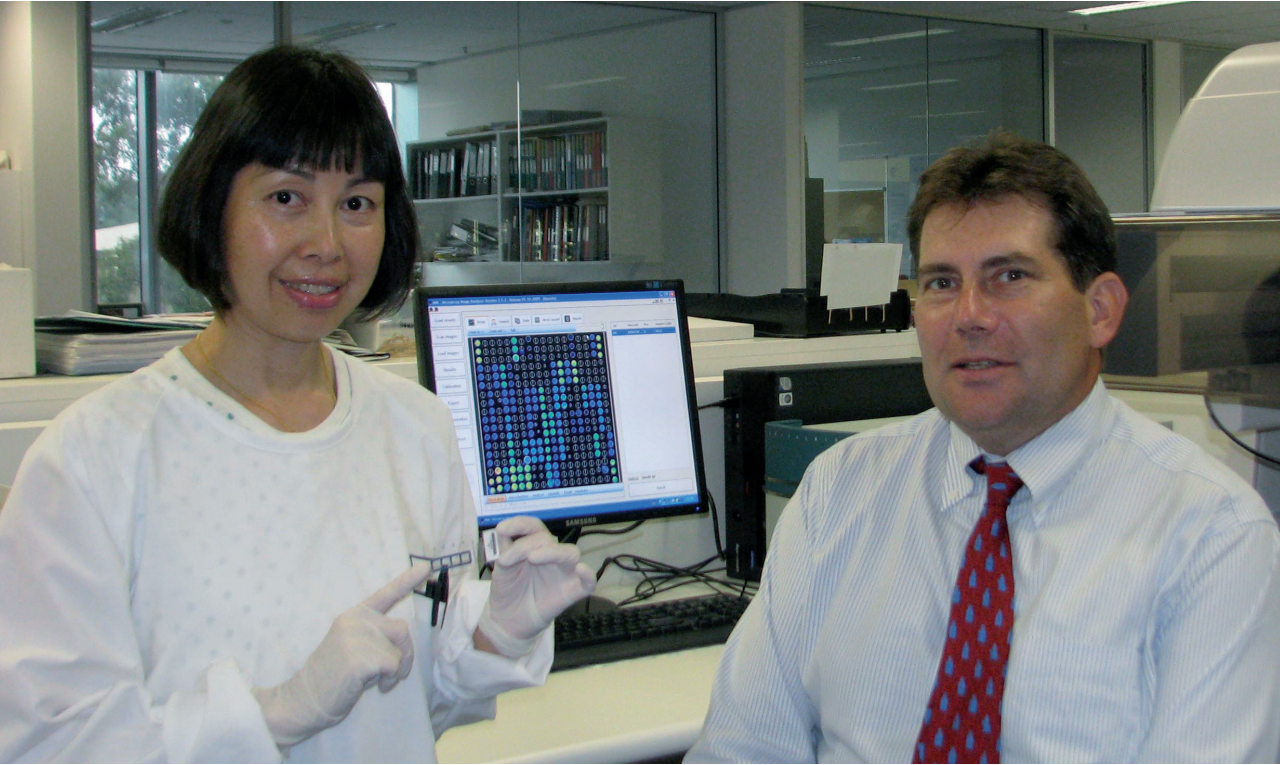
have cross-reactive IgE binding with insect or meat molecules. Component-resolved diagnostics allow us to determine whether IgE binding is directed to one or more classes of proteins and to one or more allergen cross-reactive groups. Consequently, our whole thinking about allergy will start to change from thinking not only about whether a person is allergic to a particular food, to whether the patient is sensitised to particular protein classes common to a variety of foods with class specific characteristics.

Classes of Proteins Involved in Allergy

The profile of individual allergen molecules on the ISAC® ImmunoCAP microarray is provided in Table 2 (Page 3). The third column describes the protein group that these components belong to. The biological and physical properties of these protein groups, with their clinically relevant characteristics, is discussed below.

PR-10 proteins	These proteins are very similar to a birch tree pollen called Bet v 1. Sensitisation to PR-10 proteins is often due to exposure to birch and related trees in northern Europe and we do see this in patients with prior travel exposure and perhaps more so in Canberra and Melbourne than Sydney residents. In recent years, we have seen more Sydney residents with PR-10 protein reactivity where the mechanism would not be related to birch tree pollen exposure. PR-10 proteins are heat labile, so cooked foods are often tolerated while raw food sources may result in local mouth symptoms that we call the ‘oral allergy syndrome’. Reactivity to PR-10 proteins is often associated with allergic reactions to fruit and vegetables. The ISAC® profile includes PR-10 proteins from birch tree, alder tree and hazel tree pollen, as well as food ones in hazelnut, apple, peach, soybean, peanut, celery, carrot and kiwi. A profile of reactivity to PR-10 proteins could indicate reactivity to PR-10 proteins from other foods not included in this profile.
Non-specific Lipid Transfer Proteins	LTPs are stable to heat and digestion and hence can cause reactions to cooked foods. They are often associated with systemic and more severe reactions in addition to oral allergy syndrome. They are important mechanisms of reactions to fruit and vegetables in southern Europe and some sensitisation is driven by exposure to a weed pollen from wall pellitory, <i>Parietaria judaica</i> , which is also a Sydney problem. The ISAC® profile includes LTPs from peach and hazelnut foods, as well as weed pollens from Parietaria and Mugwort. A profile of reactivity to LTPs could indicate reactivity to LTPs from other food sources not included in this profile.
Profilins	These are referred to as panallergens, due to their great degree of homology (similarity) and cross-reactivity between even distantly related plant species. Reactivity to profilins is not always associated with clinical symptoms. Some apparently large skin test reactions or high <i>in vitro</i> specific IgE levels to inhalant allergens may be predominantly directed to profilins and hence, detection of specific IgE to profilins has been recently found to have clinical importance for rational planning of allergen immunotherapy (desensitisation vaccines). Occasionally, profilin reactivity can be important for a small number of patients with sometimes severe symptoms due to citrus fruits, melons, bananas, lychee and tomato. The ISAC® profile includes profilins in latex, as well as pollens from birch, olive, annual mercury and Timothy grass.
Seed and Other Storage Proteins	These include protein fractions known as 7S and 11S globulins, as well as conglutins. The storage proteins serve as source material during growth of new plants. They are usually species specific. Since they are stable with exposure to digestive enzymes and heat, they are often important in reactions, including to cooked foods. Legumes (soy, peanut and lupins), tree nuts and seeds are important sources of dietary exposure to these storage proteins. The ISAC® profile includes Brazil nut, cashew nut, hazelnut, peanut, soy and sesame storage proteins.

Cross-reactive Carbohydrate Determinants and Other cross-reactive molecules	Patients with specific IgE directed to CCDs only very rarely have allergic reactions mediated by these molecules. Some patients can have cross-reactive IgE binding to carbohydrate determinants that can cause non-clinically relevant positive results in certain laboratory tests for allergy. Several unique molecules have been proposed as specific controls to detect CCD reactivity. The ISAC® profile includes a pineapple molecule called bromelain. However, I have two patients who are truly allergic to pineapple but it is very rare! The other cross-reactive determinant molecules that tend to indicate a lower likelihood of clinical events include profilins (see above) and also Calcium-binding 2-EF hand proteins. The ISAC® profile includes CB2EF hand proteins from birch tree pollen and Timothy grass as a marker for cross-reactivity between pollens which are not relevant to plant-derived food sensitisation.
Lipocalins	These molecules are important in species-specific allergic reactions to animals. Lipocalins are very stable and persist in environment. The ISAC® profile includes cat, dog and mouse lipocalins.
Serum Albumins	These are abundant proteins, present in different animal tissues such as blood, milk and meat. Cross-reactivity between pet animals and animal-derived foods is well recognised, such as between cat and dog and pork! The ISAC® profile includes bovine, cat, dog, horse and chicken albumins.
Parvalbumins	These proteins are the major allergens in fish and amphibians. They are very stable to heat and digestion. The ISAC® includes carp and codfish parvalbumins.
Tropomyosins	These are actin-binding proteins in muscle fibres. The molecules are particularly highly conserved (similar) between dustmite, cockroaches and crustaceans (prawn or shrimp, crab, lobster, Balmain bug and escargot). The ISAC® profile tropomyosins include prawn (shrimp), dustmite rDer p 10, cockroach and a parasite that may affect seafoods, anisakis.



Practical Clinical Applications of ISAC® and Recombinant/Native Individual Allergen Molecule Tests

<p>Case 1</p> <p>A 14 year-old boy had a past history of anaphylaxis to peanut, some other undefined food reactions possibly fruits and hummus and was experiencing progressively more severe allergic rhinitis.</p>	<p>ISAC® Profile</p> <p>On the ISAC® profile he was moderately reactive to the sesame storage protein (rSes i 1), the peanut 7S (rAra h 1) and 11S (rAra h 3) globulins and conglutin (rAra h 2), the couch (Bermuda) grass pollen (rCyn d 1), the Timothy grass pollens (rPhl p1, rPhl p2, rPhl p 5 and rPhlp 6), the peach lipid transfer protein (rPru p 3), cat uteroglobin (rFel d 1), dog lipocalins (rCan f1, rCan f2), an outdoor mould alternaria (rAlt a 1), group one and two dustmite allergens (rDer p1, rDer f 1, rDer p 2 and rDer f 2) but not other important allergens.</p> <p>Interpretation</p> <p>The extent of reactivity to the peanut storage proteins would make it extremely unlikely he would tolerate a peanut challenge. The unanticipated findings were that he was very reactive to sesame at a level likely to result in significant symptoms. Reactivity to the LTP protein rPru p 3 indicates some risk of reactions to some fruits. In fact, he was a careful avoider of several fruits and further testing confirmed allergy to stone fruits. Animal, dustmite, an outdoor mould alternaria and grass pollen allergy, demonstrated on the ISAC® profile, allowed rational planning of avoidance and immunotherapy regimes.</p>
<p>Case 2</p> <p>A 40 year-old woman had had several episodes of anaphylaxis over a two-year period. Three events occurred after finger food at social gatherings. She also suffered from allergic rhinitis.</p>	<p>ISAC® Profile</p> <p>On the ISAC® profile she was markedly reactive to the sesame storage protein (rSes i 1), the couch (Bermuda) grass pollen (rCyn d 1), and the Timothy grass pollens (rPhl p1, rPhl p11, rPhl p 4 and rPhl p 5).</p> <p>Interpretation</p> <p>There was no reactivity to tropomyosins, LTPs, other storage proteins and omega-5-gliadin (a relatively insoluble wheat molecule) effectively excluding other causes of her reactions. Serious sesame allergy was confirmed. The absence of other inhalant allergy and lack of profilin sensitisation confirms a very high likelihood of great benefit for immunotherapy for her grass pollen allergy.</p>
<p>Case 3</p> <p>A 47 year-old man experienced anaphylaxis once every 1-2 years, occasional urticaria when eating out and, sometimes, angioedema when attending the gym for exercise after lunch. He was suspicious about being allergic to lamb and seafood, but skin tests and challenges for these were negative.</p>	<p>ISAC® Profile</p> <p>On the ISAC® profile, he was markedly reactive to omega-5-gliadin (rTri a 19.0101), a relatively insoluble wheat fraction. Lamb and beef allergens (not available on ISAC®) were tested by conventional specific IgE tests and were negative.</p> <p>Interpretation</p> <p>This particular allergy should be always be suspected when there is a history of exercise-induced anaphylaxis. An unusual feature of this particular syndrome is that some patients may have no symptoms, trivial symptoms or severe symptoms after ingestion of wheat without exercise. Standard skin prick test and laboratory test systems using whole wheat extracts can be insensitive for detection of this allergy.</p>

Case 4

A 6 year-old boy had anaphylaxis at an adult family member birthday party where pavlova was served. He had difficult eczema until age 3 and disliked egg and milk, but still ate them.

ISAC® Profile

On the ISAC® profile, he was moderately reactive to one of the three kiwi fruit molecules (nAct d 2), a heat stable egg molecule ovomucoid (nGal d1), a heat labile egg molecule ovalbumin (nGal d 2) and several milk molecules, including alpha-lactalbumin (nBos d 4), beta-lactalbumin (nBos d 5), casein (nBos d 8) and lactoferrin (nBos lactoferrin). He was also found to be reactive to an outdoor mould alternaria (rAlt a 1) and a bee venom allergen, melittin (nApi m 4).

Interpretation

These results demonstrated persistent significant sensitisation to eggwhite, milk and unrecognised sensitisation to kiwi fruit. For this boy, the pavlova was a dangerous dietary experience. Peanut, treenut, sesame and other significant food sensitisations were excluded. Egg, within a baked muffin, was tolerated but a scrambled egg challenge was not. Restriction in egg, other than baked cakes, and restriction in milk, except occasional yoghurt and cheese, resulted in resolution of his eczema. Reactivity to alternaria indicates a high likelihood of some inhalant allergy, although the absence of dustmite and grass sensitisation at this age was encouraging. Some persons with alternaria allergy may have intermittent or more difficult asthma due to its small particle size. Although many persons with kiwi allergy may have cross-reactive allergy to latex, there was no evidence of latex allergy on his ISAC® profile. Additional history of a large local reaction to a bee sting was obtained.

Case 5

A 13 year-old girl had past serious reactions to egg, milk, peanut and fish. She was a fussy eater with ongoing allergic rhinitis and asthma, but previous eczema had resolved. Her mother was keen for her to have a peanut challenge, since serial skin tests over the last several years had now become nearly negative (4 mm diameter wheal). The girl was most reluctant to have a peanut challenge.

ISAC® Profile

On the ISAC® profile, she was markedly reactive to the peanut storage protein conglutin (rAra h 2). She was markedly reactive to almost all of the PR10 proteins in the ISAC® profile (birch pollen rBet v 1, hazel tree pollen rCor a 1.0101; hazelnut rCor a 1.0401, apple rMal d 1, peach rPru p 1 and soybean rGly m 4) and one LTP from *Parietaria judaica* (rPar j 2). She was also strongly reactive to the group 1 and 2 dustmite allergens (rDer p1, rDer f 1, rDer p 2 and rDer f 2) and a storage mite allergen (rEur m 2). She was strongly reactive to the fish parvalbumins from carp (rCyp c 1) and codfish (rCod c 1) and to the dustmite tropomyosin (rDer p 10), but not to the tropomyosins of prawn (shrimp), cockroach or anisakis.

Interpretation

Her degree of reactivity to the serious peanut allergen rAra h 2 would make a challenge highly likely to be positive. Her dislike of fruits is explained by her PR10 protein sensitisation, but cooked fruits should be better tolerated and explored. Her strong reactivity to the Parietaria LTP has direct implications for her asthma and rhinitis, given its local prevalence, and has potential implications for some fruit and vegetable reactions. Although she will not likely tolerate fish, she could well tolerate calamari and crustaceans, apart from the risk of cross-contamination with food handling and preparation.

Case 6

A 20 year-old woman wishes to enrol in nursing, but had had a past reaction to kiwi fruit and a skin test was strongly reactive to latex and kiwi fruit. She had not any past reactions to latex exposure with dental and gynaecological examinations.

ISAC® Profile

On the ISAC® profile, she was strongly reactive to latex profilin (rHev b 8), birch tree pollen profilin (rBet v 2) and Timothy grass pollen profilin (rPhl p 12), as well as the kiwi allergen (nAct d 2). She was not reactive to the other latex allergens, rHev b1 (rubber elongation factor), rHev b 3 (small rubber particle), rHev b 5 or rHev b 6 (Hevein).

Interpretation

Reactivity to profilins is rarely associated with clinical problems. Although kiwi fruit may pose a clinical problem for her, she can be reassured that her apparent latex reactivity is confined to reactivity to the latex profilin and she should not have any IgE-mediated clinical issues with latex exposure.

Case 7

An 18 year-old man had longstanding allergic rhinitis and allergic conjunctivitis. There was no history of asthma or eczema. He had past ENT surgery for removal of his adenoids and reduction of inferior turbinates. His skin prick tests showed moderate reactivity to dustmite, marked reactivity to grasses, with mild reactivity to olive tree pollen and moderate birch tree pollen reactivity. He was commenced on subcutaneous immunotherapy with a combined vaccine for dustmite, grasses and oleaceae after limited benefit from dustmite control measures and long-term inhaled nasal corticosteroids. After two years, he had limited benefit from desensitisation, despite good compliance and adequate dose.

ISAC® Profile

On the ISAC® profile, he had significant reactivity to group 1 and 2 dustmite allergens as well as rDer p 10 (dustmite tropomyosin, which can cross-react with crustaceans). His pollen reactivity was confined to that of the profilins of grasses (rPhl p 12), birch (rBet v 2) and olive tree pollen (nOle e 2).

Interpretation

His desensitisation vaccine was consolidated to only dustmite at a higher dose, with rapid clinical improvement. In some patients, apparent pollen allergy may be directed only to the relatively clinically insignificant profilins.

Indications for ISAC® Profiles

These cases illustrate the utility of ISAC® profiles in well-selected patients. At present, the ISAC® profiles can be very useful in the following circumstances:

- 1 When clarification is needed of multiple sensitisations in broadly atopic persons with food safety issues.
- 2 When patients have had significant birch pollen exposure and have multiple allergic sensitivities.
- 3 When skin prick tests and *in vitro* specific IgE (RAST) results have been found to be discordant with clinical outcomes or history.
- 4 When serious food allergy, especially to multiple food groups, such as eggs, milk, seeds, nuts, legumes, seafoods, fruit and vegetables, is suspected.
- 5 When there is unexplained anaphylaxis.
- 6 For clarification of latex allergy.
- 7 When progress with pollen immunotherapy is disappointing.

Indications for selected individual/recombinant individual allergen molecule tests

At present, use of selected individual allergen molecule testing can be informative without the need to resort to the ISAC® profile.

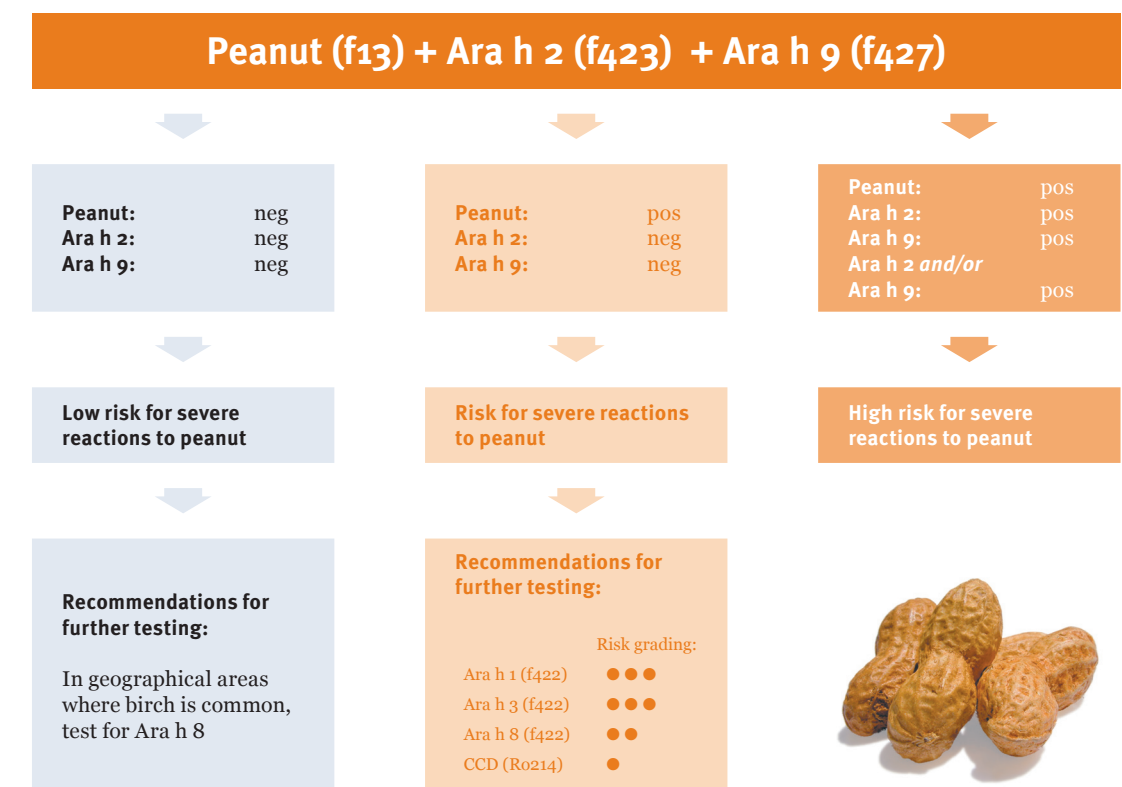
These circumstances include:

- 1 Clarification of progression/resolution of food allergy prior to challenge (certain results make challenge unlikely to be successful).
- 2 Confirmation of allergy to Omega-5-gliadin in wheat.

Usually in these situations it is advisable to request both a conventional whole allergen as well as a selected individual allergen molecule. Examples of clinical algorithms or pathways that have been proposed for egg, peanut and wheat testing are shown below.

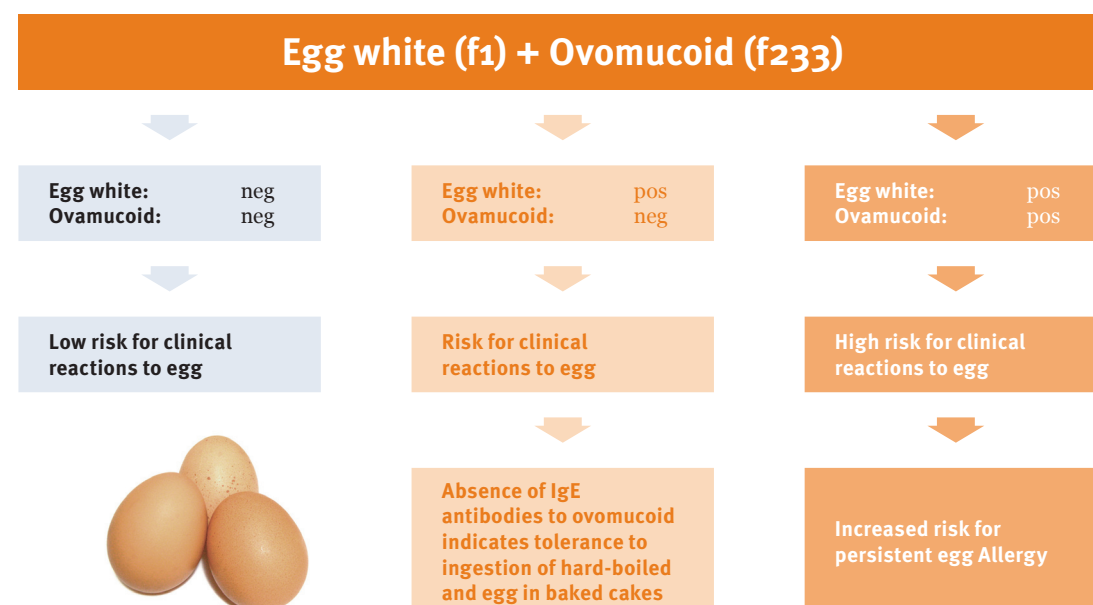
Suspicion of Peanut allergy

Is it allergy? Risk of severe reactions?



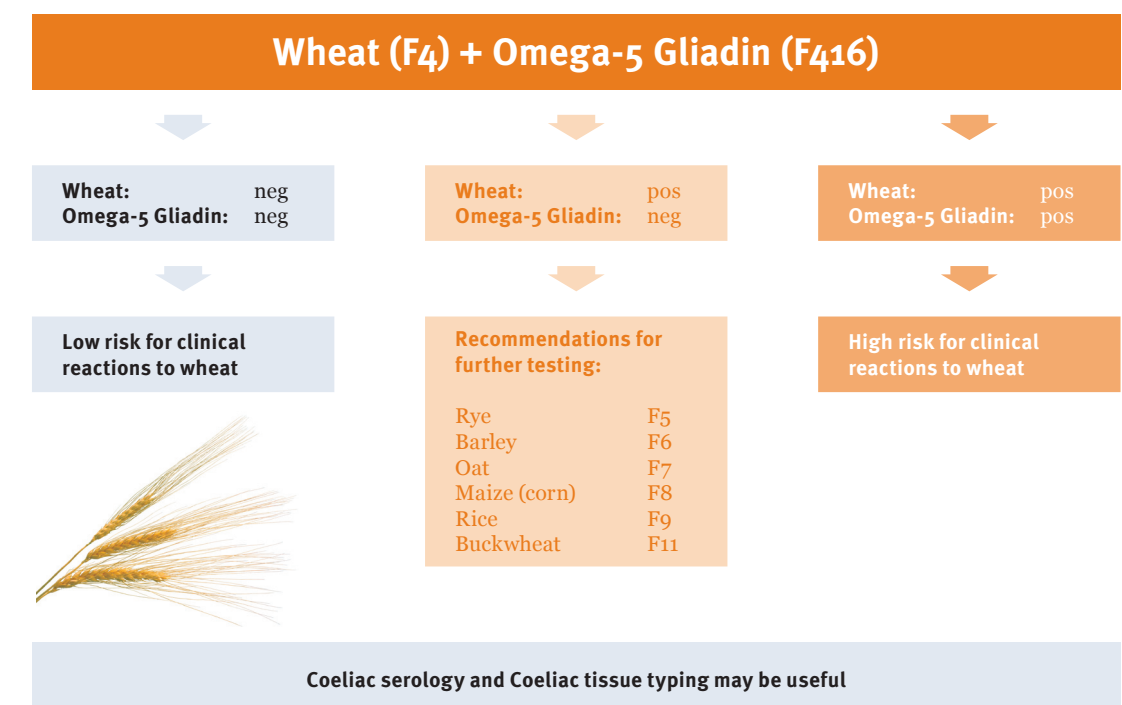
Suspicion of Egg allergy

Is it allergy? Risk of clinical reactions?



Suspicion of Wheat allergy

Is it allergy? Risk of clinical reactions?

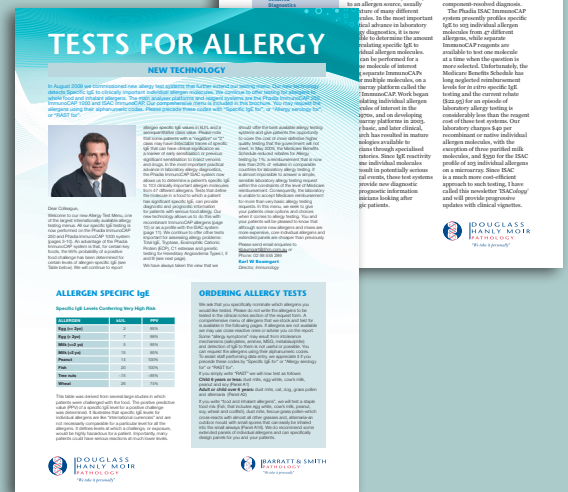


Our Information on Allergy Testing

Brochures are available to be ordered from stores or you may view on our website www.dhm.com.au or www.bsp.com.au clinicians/publications.

Allergy may be viewed directly using [http://www.dhm.com.au/media/297726/allergy\(2010\).pdf](http://www.dhm.com.au/media/297726/allergy(2010).pdf).

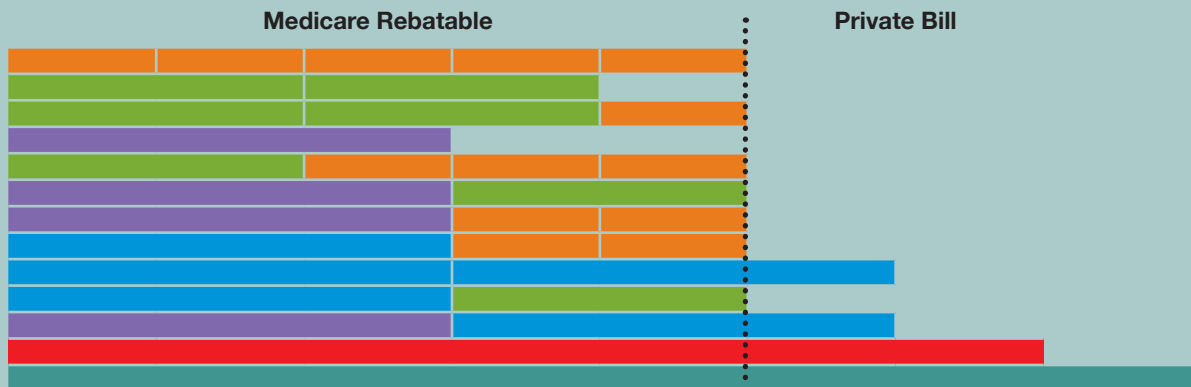
ISACology may be viewed using http://www.dhm.com.au/media/8605737/isacology_newsletter.pdf



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