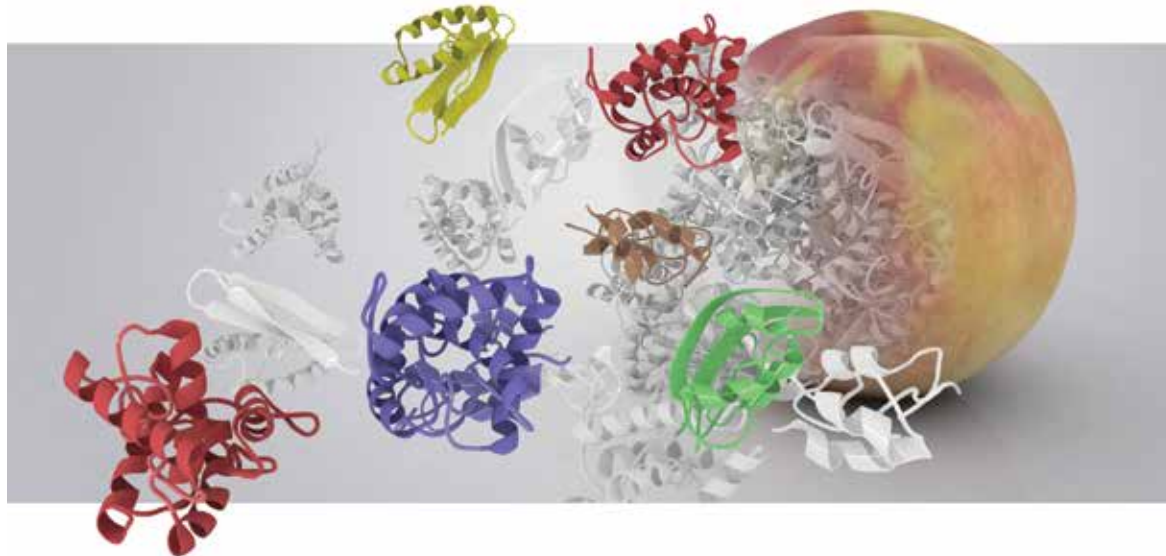


A Clinical Reference Guide to Molecular Allergy

Go Molecular!

1. Molecular Allergy – The Basics

Further information about molecular allergy and the interactive allergy component identification and interpretation tool can be found from **AllergyEducation.co.uk** and **AllergyEducation.ie**



**Allergy
Education**

www.AllergyEducation.co.uk
www.AllergyEducation.ie

 **ImmunoCAP®**
Is it allergy?

Preface

Molecular allergens have been described in scientific literature for well over a decade now, but it has only been in recent years that they have been used more routinely in the allergy clinic.

New technology can be challenging and it often requires a period of adjustment and adaptation. There are many allergen components covering many different sources and their clinical relevance is continually emerging year on year. This can make it difficult to remember their relevance. Many clinicians have commented to me that they could do with a simplified 'all in one guide' so I have tried to simplify molecular allergy based on the components Thermo Fisher Scientific has in its portfolio.

The intention of part 1 in this guidebook series is to give a basic introduction to molecular allergy focusing on plant food allergy, although other molecular sources such as venoms and aeroallergens are also discussed. This guide gives an introductory overview of the important themes within molecular allergy, especially protein families, their clinical relevance and nomenclature. If there is one important aspect to learn in molecular allergy it is the scientific relevance of protein families, as they are the key to understanding clinical molecular allergy.

A straightforward summary of the main allergen components, what ImmunoCAP products are available and interpreting test results can be found in part 2 of this series – 'The Allergen Components'. I hope you find this guidebook series useful.

Neal Bradshaw BSc (Hons)

Molecular Allergy Specialist

Immunodiagnosics

Thermo Fisher Scientific



	Page
Introduction	4
Go Molecular! Molecular allergens tell us more	4
Component families	6
Allergen component nomenclature	8
Other clinical considerations	8
Food allergy, the food matrix and what we eat	10
Specific and cross-reactive allergens	13
Plant components	15
Interpreting results from cross-reactive protein families	17
Summary of plant components	19
Plant proteins in common foods and pollens	21
Other allergen components	22
List of available ImmunoCAP allergen components	25
Common questions regarding molecular components	29
Glossary	30
Educational resources	31
References	32
Recommended literature	33

Go Molecular! Molecular allergens tell us more

Until recently, the main diagnostic tools in IgE mediated allergy have been clinical history, allergen provocation, skin prick and specific IgE blood tests. Molecular allergy brings a new level of understanding which is changing practice as physicians seek to improve on existing diagnostic technologies.

Molecular allergy is the field of allergy diagnosis that investigates protein allergens at molecular level. Therefore instead of investigating the “sum” of all allergen proteins in whole allergens e.g. peanut, important individual proteins within a peanut can be investigated for specific IgE sensitisation. IgE antibody profiles to these molecules vary significantly from patient to patient and they also differ geographically, due to local differences of exposure.

Molecular diagnostics reveal more factual information about what a patient is allergic to, as individual proteins and profiles can indicate different clinical characteristics.

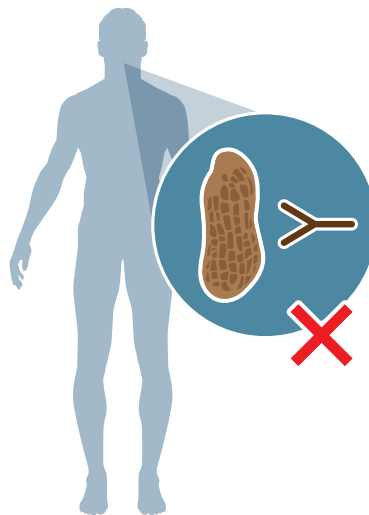


Figure 1: Illustration of the common misconception that there is one IgE antibody produced by the human body for a whole peanut allergen

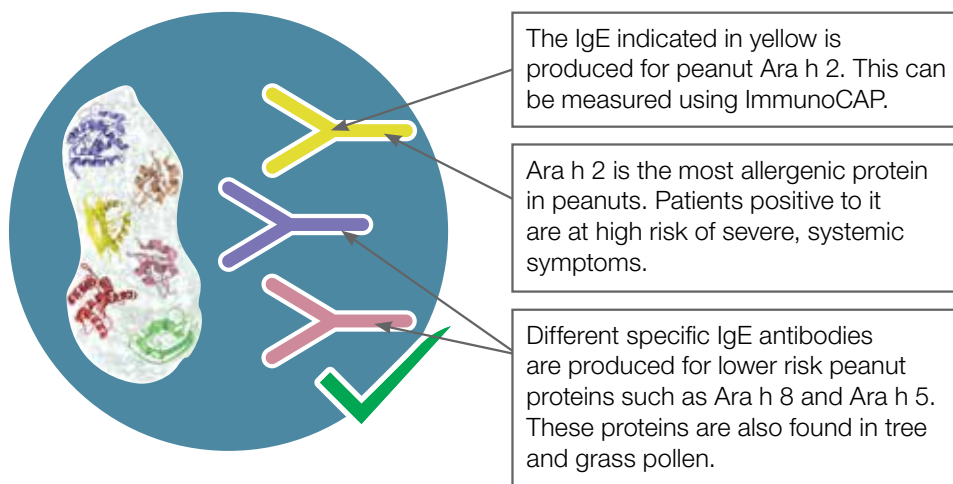


Figure 2: Illustration of the reality that there are lots of different IgE antibodies produced which bind to specific individual molecular proteins in peanut, like Ara h1, Ara h 2 and Ara h 8.

Ara h 2 is an allergen protein resistant to metabolic change and it seems to have the highest allergenic potential from all proteins in peanut. You can measure different antibodies produced by patients in response to different molecular proteins by using ImmunoCAP components. IgE antibodies are useful measurements of a patient's immunological response in their current allergy status. High levels to Ara h 2 will often indicate a patient at high risk of severe, systemic symptoms if peanuts are eaten.

Clinical utility

Allergen component diagnostics measure IgE to particular allergen components, uncovering additional information about an underlying allergy. Not only do they indicate specific allergen reactivity in the way that whole extracts do but they are also indicators for:

1. Understanding patient risk – add confidence to your assessment
2. Selecting patients for immunotherapy – useful for venom and aeroallergy patient selection
3. Understanding cross-reactions between species – help to understand multiple sensitisations e.g. in pollen-food syndrome

The intention of this first guide book is to give the physician, dietician or scientist a background to molecular allergy. A straightforward summary of allergen components and interpretation of the results can be found in part 2 of this series.

Much of the clinical value of molecular allergen testing up to now has been demonstrated within food allergy, especially within plant foods such as nuts, fruits and legumes. Therefore the majority of information in this reference guide focuses on food allergen components, although a brief overview of other allergen components which provide clinical value, such as those from pollens, latex and insect venoms, is included.

Component families

By introducing molecular allergy into your daily practice you will soon change your paradigm of thinking from that of single source allergens to diverse component families.

In molecular allergy, understanding the significance of protein families from protein sources is an important leap of knowledge in terms of scientific understanding.

Component families referred to in this guide are families with similar functions and structures found in many allergen sources. For example, plants contain storage proteins such as vicilins, transport proteins such as lipid transport proteins and defense proteins such as PR-10s (pathogenesis-related family number 10 proteins).

To begin with, below is an example of a patient's IgE test results with suspect plant food allergy:



The above test results could be interpreted in three different ways:

- Traditional thinking: four different specific IgE reactions to four different plant sources
- On a molecular level: IgE to one protein family group i.e. PR-10 allergy – also indicating cross-reactive IgE
- The patient is also likely to be sensitised to other PR-10 proteins not measured (you couldn't measure them all!). The above is representative of PR-10 sensitisation as a whole and may be relevant to the patient's clinical history to other allergens, e.g. almond contains PR-10 proteins
- This same way of thinking can be applied to profilin or LTP (lipid transfer protein) profiles for example. So you have estimates of results also for sources not tested and this is a fundamental paradigm change in allergy practice

More on protein families and their clinical relevance will be discussed later in this guide.



Interpretation of results

In this guide, interpretation has been simplified as much as possible in terms of presence of IgE. The presence of allergen-specific IgE is usually a risk of allergy symptoms and **a result ≥ 0.1 kUA/L indicates sensitisation**. Traditionally, the higher the IgE level the greater the risk. Some molecular allergens are associated with a higher risk for systemic reactions, whilst some allergens are considered at no or a very low risk for severe reactions. A high level IgE to a high-risk allergen such as Ara h 2 or Cor a 9 would often carry a high risk for patients.

Always consider tests results in association with a clinical history.

Further reading

- Garcia BE, Lizaso MT. Cross-reactivity Syndrome in Food. *Allergy* 2011;21(3):162-170.
- Hauser M, *et al.* – Panallergens and their impact on the allergic patient. *Allergy, Asthma and Clin Immunol* 2010;6:1.
- Terumi Midoro-Horiuti T, *et al.* Pathogenesis-related proteins of proteins of plants as allergens. *Ann Allergy Asthma Immunol* 2001;87:261-271.
- Egger M, *et al.* The role of Lipid Transfer Proteins in Allergic Disease. *Curr Allergy Asthma Rep* 2010;10:326-335.
- Sicherer SH. Clinical implications of cross-reactive food allergen. *J Allergy Clin Immunol* 2001; 108(6):881-890.

Allergen component nomenclature – The WHO/IUIS Committee

Allergen and allergen components are identified and categorised by a joint partnership of The World Health Organization (WHO) and The International Union of Immunological Sciences (IUIS). The WHO/IUIS Allergen Nomenclature Sub-committee is responsible for maintaining and developing a unique, unambiguous and systematic nomenclature for allergenic proteins. The systematic nomenclature is based on the Linnaean system and is applied to all allergens. For further information check the IUIS allergen nomenclature website at www.allergen.org

Allergen components are given an abbreviation based on the Latin name of the allergen source (the first three letters of the first word and first letter of the second). The allergen protein is also given a number based on the order of discovery (when registered/ approved by the IUIS committee). An example of peanut allergen component nomenclature is below, referring to Ara h 2:

Peanut – ***Arachis hypogaea*** – Ara h 2

Phadia AB, the leading manufacturer of allergen components, also gives the test a prefix 'n' for native sourced allergen proteins or an 'r' for recombinant sourced allergen proteins that are used in the IgE tests.

You can look up all identified allergens at www.allergome.org

Other clinical considerations

Allergen load

Of course, always think about the patient's clinical history – the most important part of allergy diagnosis. Taking detailed information from a patient such as their lifestyle and what they eat is essential. Molecular testing will reveal crucial data but it will never replace a good clinical history. From the patient's clinical history you will discover how much of each food allergen they have been eating, for example. Consuming large amounts of allergens at a time such as when quickly drinking a soy milk drink can affect a patient's symptom outcome. Even normally harmless allergens such as PR-10 proteins consumed in great amounts can provoke more serious allergy symptoms in some patients (such as drinking soy milk).

A patient could be sensitised to several allergens, and also to several allergen components within one allergen source. This will contribute to the overall allergen load.¹ For example if a patient is positive to multiple peanut storage components such as Ara h 1, Ara h 2 and Ara h 3, they are likely to have a higher IgE load and are therefore possibly at more risk for severe reactions than someone who is monosensitised.²⁻⁴



Diagnostic performance

ImmunoCAP components contain pure proteins, measure only IgE to single molecules and give easy-to-understand results.

Whole extract-based tests contain all, or at least most, relevant allergen molecules from an allergen source (e.g. peanut) and measure the sum of multiple IgEs which gives high sensitivity but sometimes can create difficulties in interpretation of results.

Components therefore have technical diagnostic superiority at measuring important individual IgEs of interest such as Ara h 2 in peanut or Cor a 9 in hazelnut. They simply measure IgE specific to one protein and offer reliable results in terms of minimal variation – the same as all ImmunoCAP products. However, it must be remembered that a component test only measures one type of IgE and that a patient will often have IgE antibodies to several molecules contained in the specific allergen source.

As such, it is recommended that you request testing for the whole allergens and ask the laboratory to test for components if the whole allergen is positive.

Food allergy, the food matrix and what we eat

Food is made up of complex matrices of natural constituents such as proteins, fats and carbohydrates. The way that the human body processes food creates by-products of the original food structure. The natural state of proteins can be changed even before we eat them, most obviously by cooking but also by storage and processing e.g. liquidising or concentrating (as for fruit juices).

There are many different metabolic processes that occur as soon as food enters the digestive system. Enzymatic action starts straight away in the mouth; heat and gastric juices play a role as food enters the stomach and further activity is then concentrated in the gut until the food is absorbed. Overall – countless interactions between food and biological processes.

Fats are metabolised into products such as fatty acids. Carbohydrates are broken down eventually into sugars. Most allergens are proteins, made up of amino acid chains and peptides, and within these structures are regions called epitopes. It is these recognition sites that specific IgE molecules bind to. This can lead to histamine release and other mediator release, resulting in allergy symptoms.

Molecules of high allergenic potential

Some molecular proteins are more resistant than others to metabolic change, due to strong 3D chemical structures; e.g. storage proteins from peanut (Ara h 1, Ara h 2 and Ara h 3) or ovomucoid from hen's egg (Gal d 1). It is these tough allergens that have higher resistance to digestion. Therefore their allergenic potential is higher as their epitope structures remain intact. As a result, these proteins can cause more systemic symptoms than unstable proteins (Figure 3).

Molecules of low allergenic potential

Cross-reactive allergen molecules such as PR-10s and profilins (present in nuts, fruits and pollen) are more fragile in structure and therefore susceptible to digestive processes of heat and enzyme activity. Fragile proteins start to break down in the mouth which can cause less problematic reactions such as oral allergy syndrome (OAS). As the epitope binding regions in these proteins is destroyed, these molecules don't tend to induce such an aggressive IgE response.

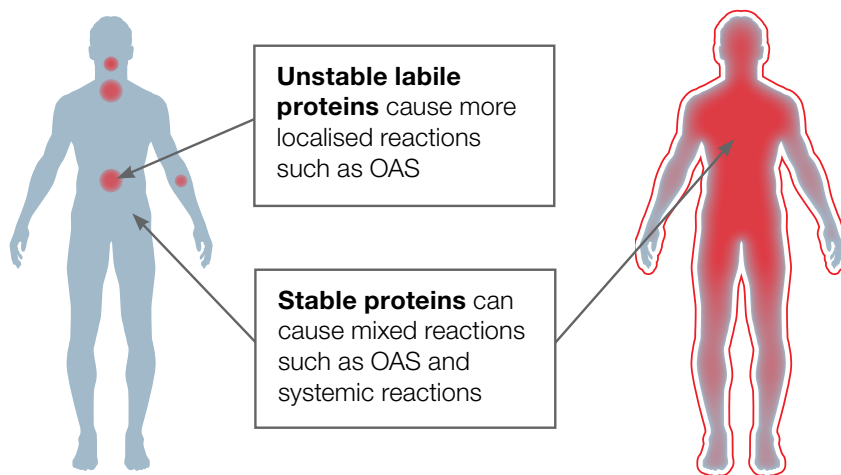


Figure 3: An overview of the biological differences in how proteins can cause different symptoms in the human digestive tract

Allergen profile variability

If molecules vary in their potential to trigger allergy it raises the question:

Q: 'If a patient is IgE tested using a whole extract (the source) how do you know which proteins within the source they are sensitised to?'

A: 'The simple answer is that a whole extract test does not provide all the answers!'

The above question and answer is quite thought-provoking. A whole extract IgE test (the source) is a mixture of lots of individual proteins. It would be impossible to tell which molecular proteins a patient is IgE positive to unless they were separated individually – as they are in ImmunoCAP allergen components. Also, all patients vary in which components they are sensitised to and their molecular profiles therefore vary significantly.

Going molecular by using ImmunoCAP molecular components puts a large portfolio of different allergen components at your disposal. By selecting allergen components you can build individual patient profiles, improving diagnostic clarity and the ability to convey factual information. In ImmunoCAP ISAC you have an allergen profile producer that measures components from 51 sources (representative for approximately 90% of clinical allergen sources). More on ISAC can be found in the third guide book in this series.

Patients testing positive to a whole extract (e.g. positive skin prick test to peanut or serum IgE to peanut) can be positive to either allergen proteins of high allergenic potential or of low/no allergic potential. By using allergen component diagnostics it is possible to better differentiate between them i.e. put patients into low- and high-risk groups.

Unfortunately, mixed forms of allergy exist as of course a patient can be genuinely sensitised to high-risk allergens and low-risk allergens, and can also have more varied symptoms such as OAS together with systemic symptoms.

Furthermore, in a given situation a lot of other factors such as stress, amount of allergen, ongoing infections etc, have an impact on the actual clinical reaction.

Further reading

- Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010;40(10):1442-1460.
- Hoffman K, *et al.* Food allergen protein families and their structural characteristics and application in component resolved diagnosis: new data from the EuroPrevall project. *Anal Bioanal Chem* 2009; 395:25-35.
- Treudler R. Update on in vitro allergy diagnostics. *J Dtsch Dermatol Ges* 2012;10(2):89-97.
- Breitender H and Mills CEN. Plant food allergens and functional aspects of allergenicity. *Biotechnol Adv* 2005;23:395-399.
- Hauser M, *et al.* Panallergens and their impact on the allergic patient. *Allergy, Asthma Clin Immunol* 2010;6:1.

Specific and cross-reactive allergens

Cross-sensitisation of whole allergens

As you have seen, molecular allergens can be split into allergens of high and low potential for clinical symptoms, and these allergens can be further grouped as being molecules specific to the source or molecules that can be cross-reactive. Identifying these molecules helps us to better understand the characteristics of an individual's allergy.

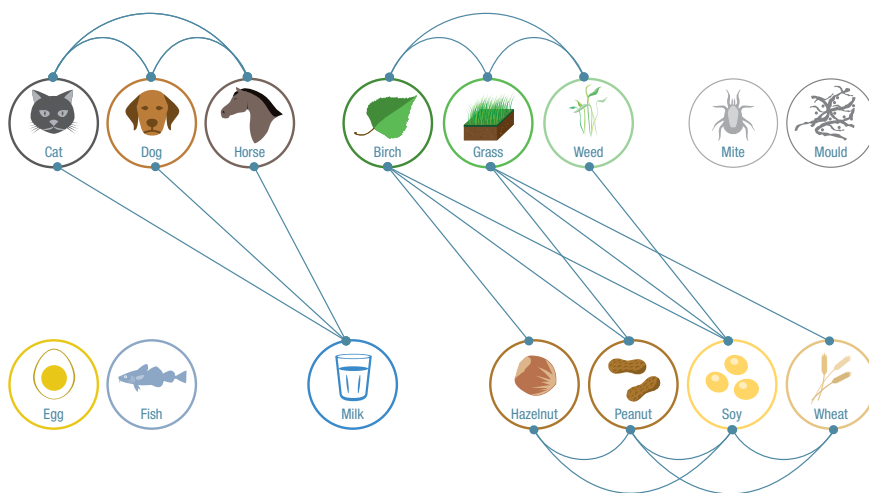


Figure 4: Illustration of a typical UK allergen profile

The above figure demonstrates a typical UK allergen test panel. Many of the allergens could experience IgE cross-reactions. For instance dog, cat and horse all contain the family lipocalin and also serum albumin which is contained in milk. Birch, grass and weed contain profilins, which are also found in the legumes soy and peanut, as well as wheat and hazelnut. IgE cross-reactions can confound results, although ImmunoCAP components can be used to improve diagnostic clarity.

Specific components are more or less unique to their source, whilst cross-reactive allergens can be found in even distantly related species. You can learn more about the significance of these types of allergens at:

AllergyEducation-MA.com

This website contains an educational course which describes the basics of molecular allergy and includes patient case examples.

Specific allergens and primary food allergy

Identifying IgE to specific molecules often indicates the cause of allergy symptoms. In food allergy, the allergens that initially trigger the immune system to produce specific IgE antibodies are often referred to as **primary allergens**. Mostly these are food proteins more resistant to digestion, which therefore are likely to **provoke systemic allergy symptoms**.

Secondary reactions and pollen-food syndrome

Cross-reactive allergens with much conserved structures between different species (so-called “pan-allergens”) can be found in plants, but some are also found in other, not closely related sources, including venoms, fish, mites and shrimp. For example dust mite and shrimp share a cross-reactive risk protein called tropomyosin. In plants in particular, panallergens are very widespread even in distantly related species such as between celery and birch trees.

In plant food allergy, pan-allergens are often the culprits for much asymptomatic or symptomatic sensitisation. IgE antibodies primarily targeted towards proteins in pollen (e.g. Birch Bet v 1) cross-react to similar proteins in food, causing a broad “secondary” sensitisation profile. Within clinical allergy this is often referred to as **pollen-food syndrome** and in the context of latex – **latex-fruit syndrome**.

Cross-reactive allergens can produce allergy symptoms which are **sometimes mild**, but the primary sensitiser should always be sought after. Therefore using a range of specific and cross-reactive allergen component tests it is possible to differentiate primary and secondary reactions.

Further reading

- Garcia BE and Lizaso MT. Cross-reactivity Syndromes in Food Allergy. *J Investig Allergol Clin Immunol* 2011;21(3):162-170.
- Zuidmeer L and van Ree R. Lipid transfer protein allergy; primary allergy/food syndrome in some cases. *Curr Opin Clin Immunol* 2007;7:269-273.
- Santos A and van Ree R. Profilins: Mimickers of Allergy or Relevant Allergens? *Int Arch Allergy Immunol* 2011;155:191-204.
- Fernández-Rivas M, et al. Allergies to fruits and vegetables. *Pediatr Allergy Immunol* 2008;19:675-681.



Plant components

Plant component families are shared between species; the closer the species are related the more similar the components can be. This increases the potential for IgE molecules directed against pollen allergen epitopes to bind to similar allergen epitopes in food. This immunological mechanism is often the cause for broad sensitisation patterns seen in many allergic patients. The two dominant sensitising plant aeroallergens in Northern Europe are pollens from timothy grass and birch trees. Both of these species are culprits for much of the seasonal hay fever symptoms that appear in the UK every spring (birch) and summer (timothy grass). These pollens contain many cross-reacting proteins such as PR-10 proteins and profilin.

There are five main types of plant component groups indicated in allergy. These are PR-10, Profilin, LTPs, Storage Proteins and CCDs which are explained in more detail below. Further references regarding plant food proteins can be found towards the back of this guide.

Storage proteins

Storage proteins are biological reserves of nutrients and amino acids used by organisms to grow. They are found in plants such as seeds and nuts, and proteins with corresponding functions can be found in mammals such as those in egg whites (e.g. ovalbumin) or milk (casein). Storage proteins are structurally complex and commonly regarded as much more stable to heat and proteases compared to allergens such as PR-10s and profilins. There is evidence that the 2S albumin (e.g. Ara h 2) is one of the most stable plant food molecules and therefore the most clinically important. The Ara h 2 molecules are not easily destroyed by gastric fluid and thus will be immunologically functional in the gastrointestinal tract with the potential to trigger systemic reactions such as asthma, urticaria, angioedema or anaphylaxis.⁵ Storage proteins are more or less specific to their source and do not cross-react except for very closely related allergen sources (e.g. between legumes such as soy and peanut).

LTPs (Lipid Transfer Proteins)

LTPs are very stable small molecules widespread in plant food such as fruits, nuts and vegetables. They are found concentrated in the skin of Rosaceae fruits especially in the fuzz of peach – the pulp contains less of the allergen. LTPs are pan-allergens and highly cross-reactive. IgE sensitisation to LTPs has been mostly described in southern Europe, in patients with severe reactions to peach and other fruits belonging to the Rosaceae family (pear, cherry, apple etc.). LTP allergy has also been described in legumes such as peanut, as well as hazelnut.

The LTP sensitisation pattern in northern Europe is not completely understood and is documented more in southern Europe. The protein characteristics of LTPs explain their clinical relevance due to their high resistance to heat and protease digestion. LTP molecules are quite resistant to gastric fluid and thus will be immunologically functional in the gastrointestinal tract with the potential to trigger systemic reactions. LTPs are also associated with local reactions including OAS.

PR-10 (Pathogenesis-Related family number 10) proteins

Plant defence proteins such as PR-10s are concentrated in the pulp of fruit, but they can also be found in pollen. Bet v 1 PR-10 is the major allergen in birch pollen and is highly similar to other PR-10 proteins in other plant food such as Rosaceae fruits (peach, apple and cherry etc.), as well as in nuts and legumes.

In a typical birch allergy scenario, birch pollen causes sensitised patients to become primary sensitised to PR-10 proteins. This can cause typical hay fever-like symptoms such as an itchy/blocked nose, runny eyes etc.

As a further consequence, patients who eat PR-10 proteins found in nuts or fruit can react due to IgE cross-reactions. Food allergy caused via cross-reactivity is sometimes referred to as secondary food allergy. Again this is likely to result in local symptoms such as OAS, but depending on the amount of the cross-reactive protein more severe reactions may also occur (e.g. Gly m 4 induced soy milk reactions).

Profilin proteins

Profilin proteins occur in many different plant species and cause broad sensitisation patterns. They are found for example in pollen (e.g. birch or grass), fruit (Rosaceae: apple, cherries) and vegetables, nuts and latex. It has been proposed that just one profilin from one plant species is enough for testing IgE sensitisation to profilin, due to the large similarity and cross-reactivity of this protein group. Profilins from birch (Bet v 2) and/or timothy grass (Phl p 12) are often used in measuring IgE to profilin. Profilins are sensitive to heat and proteases and will thus primarily give rise to OAS as the clinical manifestation of food allergy. It is widely accepted that profilins have less clinical relevance than PR-10 proteins, although in some cases profilin sensitisation may cause severe reactions.

CCDs (Cross-reactive Carbohydrate Determinants)

Some molecular structures such as CCDs are shared between many species and can be found in insect venoms, pollens and plant foods. CCDs are not proteins but carbohydrate chains (glycan side chains attached to amino acid structures). The clinical impact of IgE to CCDs is considered very low although positive IgE test results are frequent. Again, by using a molecular approach this can be easily established and patients' management tailored to their results.

Equally CCDs help us to understand polysensitisation to multiple plant foods and latex or double positivity between bee and wasp venoms. It is also worth noting that most plant allergen extract preparations from natural sources contain CCD molecules (as they do at the source), whilst recombinant sources are CCD-free and hence more specific.

Interpreting results from cross-reactive protein families

Example 1

You could use a variety of ImmunoCAP component tests when resolving a birch-food allergic patient. Is it a true food allergy? Bet v 1 PR-10 is a dominating primary allergen of a birch-allergic patient. Bet v 1 could produce cross-reactions between other plant food species. The example below demonstrates a patient profile of PR-10 sensitisation with a suspected case of IgE-mediated peanut allergy. For the purposes of this example all other risk allergens such as Ara h 2 in peanut or Cor a 9 from hazel nut were IgE-negative.



Like all ImmunoCAP specific IgE tests, allergen components give results in kU_A/L (ImmunoCAP ISAC gives results in ISU). Primary sensitising allergens from within the same protein family (in this example PR-10 protein family) will normally give the highest specific IgE level. Other secondary IgE sensitisations will give similar specific IgE readings but normally lower levels than the primary sensitising allergen due to reduced protein homology (and therefore reduced IgE binding).

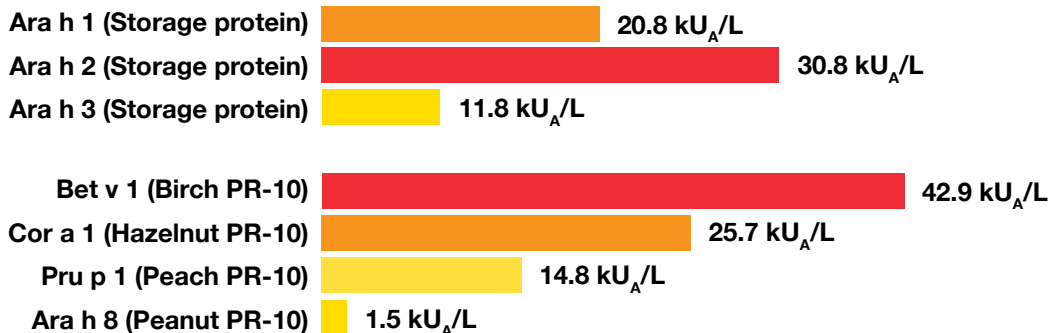
Clinical interpretation:

- Food-pollen syndrome caused by a primary PR-10 birch-related pollen allergy
- Likely symptoms local/mild or none e.g. oral allergy to hazelnut, peach and peanut

“Secondary” reactions due to cross-reactivity can occur via plant allergens such as CCDs and profilins. On the other hand if a patient is allergic to primary molecular proteins that don't cross-react (such as storage proteins) then this serves as a diagnostic marker of risk which is covered further in this guide.

Example 2

Using the previous example of suspected peanut allergy the IgE results could have looked like this:



Clinical interpretation:

- Primary sensitisation to peanut allergens Ara h 1, Ara h 2 and Ara h 3
- Ara h 2 is the most important peanut allergen; the patient is at high risk of severe, systemic symptoms
- The patient also has concomitant birch sensitisation and perhaps other allergy symptoms such as rhinitis, asthma and oral itching
- Food pollen syndrome – caused from a primary PR-10 birch-related pollen allergy. Likely reactions to these foods are local/mild or none e.g. oral allergy
- Overall mixed allergy might occur – both systemic and local symptoms

References and further reading on the plant proteins can be found at the back of this book.

Always use the results in combination with a clinical history. IgE presence always implies risk.

Summary of plant components

Plant protein families are shared between species; the closer the species are related the more similar the proteins can be. This increases the potential for IgE molecules directed against pollen allergen epitopes to bind to similar allergen epitopes in food. There are five main types of plant protein groups indicated in allergy. These are storage proteins, nsLTP, PR-10, profilin proteins and CCDs (cross-reactive carbohydrate determinants):

Protein family	Risk for systemic reactions?	Do I have to consider many different allergen sources?
Storage proteins	Yes. Storage proteins are heat and digestion stable which explains their ability to more often cause systemic reaction in addition to OAS.	No. Storage proteins are not cross-reactive, except for very closely related allergen sources (e.g. between legumes such as soy and peanut).
nsLTP	Yes. nsLTPs are heat and digestion stable which explains their ability to more often cause systemic reaction in addition to OAS.	Yes. Partly cross-reactive (the degree of structural similarity varies between nsLTPs in plant food and pollen).
PR-10	Low. Often cause only local symptoms such as OAS due to their sensitivity to heat and digestion, but a few cases with systemic reactions have been reported e.g. for soy Gly m 4 and Celery Api g 1.	Yes. Partly cross-reactive (the degree of structural similarity varies between PR-10 in plant food and birch-related pollen).
Profilin	Low. Often have little clinical relevance in allergic diseases. However, profilins may cause local reactions in some patients allergic to plant foods including citrus fruits, banana and tomato, and a few cases with systemic reactions have been reported e.g. for melon and lychee.	Yes. Highly cross-reactive (high degree of structural similarity between profilins in pollen, plant food and latex).
CCD	Very low. Usually not associated with clinical reactions but may induce IgE antibody responses in some patients.	Yes. Highly cross-reactive (same CCD structure in pollen, plant food and venoms).

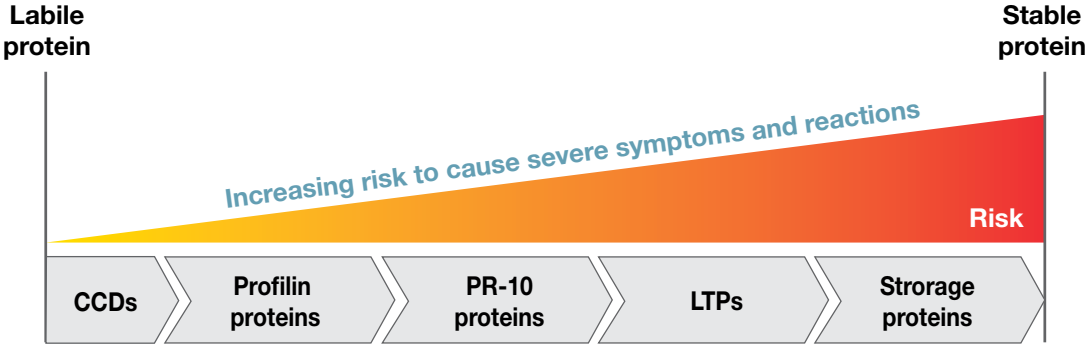


Figure 5: Illustration of level of risk associated with common plant component families

Plant components in common foods and pollens

Component family/ allergen source	Prolifin	PR-10	LTP	Storage proteins				
Birch	Bet v 2	Bet v 1						
Timothy grass	Phl p 12							
Latex	Hev b 8		Hev b 12					
Apple	Mal d 4	Mal d 1	Mal d 3					
Cherry	Pru av 4	Pru av 1	Pru av 3					
Almond	Pru d 4	Pru du 1	Pru du 3	Pru du 2s	Pru du 11S			
Apricot		Pru ar 1	Pru ar 3					
Peach	Pru p 4	Pru p 1	Pru p 3					
Pear	Pyr c 4	Pyr c 1	Pyr c 3					
Raspberry	Rub i 4	Rub i 1	Rub i 3					
Strawberry	Fra a 4	Fra 1	Fra a 3					
Peanut	Ara h 5	Ara h 8	Ara h 9	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 7
Soy	Gly m 3	Gly m 4		Gly m 5	Gly m 6			
Hazelnut	Cor a 2	Cor a 1	Cor a 8	Cor a 9	Cor a 14			
Brazil Nut				Ber e 1	Ber e 2			
Walnut	Jug r 5		Jug r 3	Jug r 1	Jug r 2	Jug 4		
Pistachio				Pis v 1	Pis v 2	Pis v 3	Pis v 5	
Cashew				Ana o 1	Ana o 2	Ana o 2	Ana o 3	
Sesame	Des i 8			Ses i 1	Ses i 2	Ses i 3	Sesi 6	Ses i 7
Wheat*	Tri a 12		Tri a 14	Tri a 19	Gliadin			
Barley	Hor v 12		Hor v 14	Hor v 21	Hor v 36			
Maize	Zea m 12		Zea m 14	Zea m G1	Zea m G2			
Rice	Ory s 12		Ory s 14	Iry s 19kd	Ory s 36	Ory s GLP52	Ory s GLP63	
Carrot	Dau c 4	Dau c 1	Dau c 3					
Cabbage	Bra o 8		Bra o 3					
Tomato	Lyc 1	Lyc 4	Lyc e 3	Lyc e 7S	Lyc e 11S			
Melon (musk)	Cuc m 2	Cuc m 3						
Celery	Api g 4	Api g 1	Api g 2 and 6					

	Plant food not associated with storage proteins unless found in the seed
	Grasses do not contain PR-10 proteins
	Available plant food ImmunoCAP allergen components
	Protein not formally identified but likely
	No storage proteins associated with this allergen source

*Wheat – Tri a 19 and gliadin are storage proteins but belong to a different family to, for example, LTPs or 2S albumins – they belong to the cereal prolamin family

Other allergen components

Molecular allergens also provide useful information from non-plant sources such as venoms from stinging insects. Below is a brief overview, although further information on clinical interpretation and what ImmunoCAP components are available can be found in guidebook 2 – ‘The Allergen Components’. The below is intended as an introduction to other allergen component areas, including a few references for further reading.

Allergens from non-plant sources

Egg and milk

Dairy products such as milk and egg are associated more with paediatric allergy which children tend to outgrow at a young age. However, in a recent longitudinal egg allergy study in the UK, Clark *et al.* showed that many children don't outgrow their egg allergy until well past 5 years old, in fact the median age in this study was 10 years of age for egg allergy resolution.⁶

Egg and milk contain allergen components that are likely to be linked to more severe symptoms; these allergens are resistant to metabolic change (hen's egg Gal d 1 Ovomucoid; cow's milk, Bos d 8 Casein). Therefore patient groups negative to these tests have been observed to tolerate cooked forms of the allergen. Conversely persistent allergy is associated with the same epitopes, which again can be used as risk markers.

Further reading

- Ando H, *et al.* Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol* 2008; 122:583-588.
- Alessandari C, *et al.* Ovomucoid (Gal d 1) specific IgE detected by microarray system predict tolerability to boiled hen's egg and an increased risk to progress to multiple environmental sensitisation. *Clin and Exp Allergy* 2012;42(3):441-450.
- Fiocchi A, *et al.* Molecular diagnosis of cow's milk allergy. *Curr Opin Allergy Clin Immunol* 2011;11:216-221.
- Nowak-Węgrzyn A, *et al.* Tolerance to extensively heated milk in children with cow's milk allergy. *J Allergy Clin Immunol* 2008;122:342-347.

Parvalbumins

Cyp c 1 (carp, oily fish) and Gad c 1 (cod, white fish) are both major fish allergen proteins and markers for fish IgE sensitisation. Parvalbumins are expressed in lower levels in certain fish species such as tuna, swordfish and some mackerel and this perhaps explains why fish-allergic patients can sometimes tolerate these species. Using ImmunoCAP Cyp c 1 and Gad c 1 gives broad spectrum coverage of the parvalbumins family and IgE analysis of fish. A negative result to both tests in a patient investigated for food allergy would inform the clinician of potential low risk of oral challenge and/or lead to further investigations for other possible culprit allergens.

Further reading

- Garcia BE and Lizaso MT. Cross-reactivity Syndromes in Food Allergy. *J Investig Allergol Clin Immunol* 2011;21(3):162-170.
- Sharp MF and Lopata AL. Fish Allergy in Review. *Clin Rev Allerg Immunol* 2013; Epub ahead of print.
- Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010;40(10):1442-1460.



Tropomyosins

Tropomyosin proteins are highly cross-reactive actin-binding proteins located in muscle fibres amongst many invertebrate species such as shrimps (Pen a 1), dust mite (Der p 10), cockroach (Bla g 7) and other crustacean foods such as crab, lobster and mollusc. Therefore tropomyosin is an allergen that can be both inhaled and ingested. About 10% of dust mite-allergic patients have IgE to tropomyosin. Some studies suggested that exposure to dust mite tropomyosin may sensitise against shrimp tropomyosin.

Further reading

- Leung NYH, *et al.* Current Immunological and Molecular Biological Perspectives on Seafood Allergy: A comprehensive review. *Clin Rev Allerg Immunol* 2012; Epub ahead of print.
- Gamez C, *et al.* Tropomyosin IgE positive results are a good predictor of shrimp allergy. *Allergy* 2011;66:1375-1383.

Latex

True latex allergy can be identified using specific markers as much cross-reactivity is caused by the profilin allergen Hev b 8 and also CCDs. The association of latex allergy and allergy to plant-derived foods is called latex-fruit syndrome. An increasing number of plant sources, such as avocado, banana, chestnut, kiwi, peach, tomato, potato and bell pepper have been associated with this syndrome.

IgE antibodies to Hev b 1 and Hev b 3 are considered specific markers for latex allergy especially in multi-operated children/patients. IgE to Hev b 5 and Hev b 6 are mainly associated with occupational exposure to latex e.g. in healthcare workers and food-handling personnel using latex gloves. Hev b 8 the profilin and Hev b 6.02 can be used for examining cross-reactivity. If an exclusive sensitisation to latex profilin (Hev b 8) occurs, then clinically relevant allergic symptoms are hardly to be expected. Further information on latex markers can be found in part 2 of this series.

Further reading

- Garnier L, *et al.* Molecular allergens in the diagnosis of latex allergy. *Eur Ann Allergy Clin Immunol* 2012;44(2):73-79.
- Schuler S, *et al.* Microarray-based component-resolved diagnosis of latex allergy: isolated IgE-mediated sensitization to Latex profilin Hev b 8 may act as confounder. *Clin Transl Allergy* 2013;3:11.

Immunotherapeutics – Aeroallergens

Understanding cross-sensitisation and identifying the right allergen source

Clinically it is obviously important to select the correct patients for the right aero-immunotherapy and this is not always easy. Patients can be cross-sensitised to several plant species; therefore sometimes it is not clear what the disease-eliciting source is. Molecular allergen tests can help streamline the identification process. Specific molecules from, for example, grass species can differentiate and identify true grass-allergic patients.

Determining a patient's molecular profile will also help to indicate if they are likely to respond satisfactorily to immunotherapy. Immunotherapy products vary from manufacturer to manufacturer; they contain molecules from the allergen source but which ones and in what quantity? Most immunotherapies contain larger quantities of the major allergens such as Bet v 1 in birch and Phl p 1 and Phl p 5 in timothy grass. Much lower quantities of the minor allergens are included. Patients who are positive only to the minor allergens are less likely to respond to immunotherapy satisfactorily since the treatment extracts sometimes contain low amounts of these allergens. For example the patient might be sensitised to Phl p 7 or Phl p 12 which are present in low and variable amounts in grass immunotherapy products.

Sander *et al.*⁷ explored the relationship between Phl p 5 content of grass skin prick extracts and different sublingual immunotherapies.⁷ The study data showed quite a variance of content of Phl p 5 in skin prick test solutions. Phl p 5 content varied from 15-427 µg/mL. Whereas Phl p 5 content in immunotherapeutics ranged from 0.2-21.6 µg/mL. There is more information regarding immunotherapy in the second part of this mini-series of guidebooks.

Further reading

- Focke M, *et al.* Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy* 2008;38(8):1400-1408.
- Focke M, *et al.* Molecular composition and biological activity of commercial birch pollen allergen extracts. *Eur J Clin Invest* 2009;39(5):429-436.
- Schmid-Grendelmeier P. Recombinant Allergens – Routine diagnostics or still only science? *Der Hautarzt* 2010;61(11):946-953.

Immunotherapeutics – Venoms

Many patients with suspected venom allergy can be positive for both bee and wasp whole allergens. Using specific markers for wasp (Ves v 1 and Ves v 5) and bee (Api m 1) it is possible to differentiate patients before selecting the right immunotherapeutic solution. Double positivity can be caused by CCDs. ImmunoCAP recombinant venom components are CCD-free which enables an allergist to distinguish between positivity from cross-reactions and true venom allergy.

Further reading

- Mittermann I, *et al.* Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *J Allergy Clin Immunol* 2010;125(6):1300-1307.
- Muller U, *et al.* IgE to recombinant allergens Api m 1, Ves v 1 and Ves v 5 distinguish double sensitisation from cross-reaction in venom allergy. *Allergy* 2012;67:1069-1073.

Available ImmunoCAP allergen components

The term 'allergen component' is used for products based on molecular allergens purified from either their natural source (native) or biotechnologically produced as recombinant proteins.

By using tests for single allergenic components as a complement to more traditional IgE antibody tests, further clinically relevant information can be gained.

ImmunoCAP allergen components are useful tools when investigating and explaining allergic reactions more in detail and to determine if they are caused by cross-reacting IgE antibodies to different allergens.

Allergen components, native and recombinant

Product		Code	Size	Art. No.	Barcode
Grass pollens					
nCyn d 1 Bermuda grass	<i>Cynodon dactylon</i>	g216	10	14-4972-01	CFA
rPhl p 1 Timothy	<i>Phleum pratense</i>	g205	10	14-5234-01	BSU
rPhl p 2 Timothy	<i>Phleum pratense</i>	g206	10	14-5235-01	C0K
nPhl p 4 Timothy	<i>Phleum pratense</i>	g208	10	14-5288-01	C0L
rPhl p 6 Timothy	<i>Phleum pratense</i>	g209	10	14-5289-01	BSV
rPhl p 7 Timothy	<i>Phleum pratense</i>	g210	10	14-5290-01	BSW
rPhl p 11 Timothy	<i>Phleum pratense</i>	g211	10	14-5291-01	BSX
rPhl p 12 Profilin, Timothy	<i>Phleum pratense</i>	g212	10	14-5292-01	BSY
rPhl p 1, rPhl p 5b Timothy	<i>Phleum pratense</i>	g213	10	14-5312-01	BU1
rPhl p 7, rPhl p 12 Timothy	<i>Phleum pratense</i>	g214	10	14-5313-01	BU2
rPhl p 5b Timothy	<i>Phleum pratense</i>	g215	10	14-5338-01	BV3
Weed pollens					
nAmb a 1 Ragweed	<i>Ambrosia elatior</i>	w230	10	14-4969-01	CF8
nArt v 1 Mugwort	<i>Artemisia vulgaris</i>	w231	10	14-4970-01	CF9
nArt v 3 LTP, Mugwort	<i>Artemisia vulgaris</i>	w233	10	14-4983-01	CJ2
rPar j 2 LTP, Wall pellitory	<i>Parietaria judaica</i>	w211	10	14-5311-01	C2M
nSal k 1 Saltwort	<i>Salsola kali</i>	w232	10	14-4978-01	CFE
rPla l 1 Plantain	<i>Plantago lanceolata</i>	w234	10	14-5751-01	D1H

Product		Code	Size	Art. No.	Barcode
Tree pollens					
rBet v 1 PR-10, Birch	<i>Betula verrucosa</i>	t215	10	14-5225-01	BPV
rBet v 2 Profilin, Birch	<i>Betula verrucosa</i>	t216	10	14-5226-01	BR1
rBet v 4 Birch	<i>Betula verrucosa</i>	t220	10	14-5287-01	BT7
rBet v 6 Birch	<i>Betula verrucosa</i>	t225	10	14-5345-01	CF1
rBet v 2, rBet v 4 Birch	<i>Betula verrucosa</i>	t221	10	14-5310-01	BU0
nCup a 1 Cypress	<i>Cupressus arizonica</i>	t226	10	14-4977-01	CFD
rOle e 1 Olive	<i>Olea europaea</i>	t224	10	14-5705-01	CTC
nOle e 7 LTP, Olive	<i>Olea europaea</i>	t227	10	14-4993-01	CKT
rOle e 9 Olive	<i>Olea europaea</i>	t240	10	14-4999-01	CTZ
rPla a 1 London plane	<i>Platanus acerifolia</i>	t241	10	14-5957-01	D2H
Microorganisms					
rAlt a 1	<i>Alternaria alternata</i>	m229	10	14-5346-01	CE0
rAsp f 1	<i>Aspergillus fumigatus</i>	m218	10	14-5293-01	BPL
rAsp f 2	<i>Aspergillus fumigatus</i>	m219	10	14-5294-01	BPM
rAsp f 3	<i>Aspergillus fumigatus</i>	m220	10	14-5295-01	BT4
rAsp f 4	<i>Aspergillus fumigatus</i>	m221	10	14-5296-01	BPH
rAsp f 6	<i>Aspergillus fumigatus</i>	m222	10	14-5297-01	BPP
Epidermals and animal proteins					
nBos d 6 BSA, Cow	<i>Bos spp.</i>	e204	10	14-5009-01	BRV
rCan f 1 Dog	<i>Canis familiaris</i>	e101	10	14-4955-01	CBN
rCan f 2 Dog	<i>Canis familiaris</i>	e102	10	14-4956-01	CBP
nCan f 3 serum albumin Dog	<i>Canis familiaris</i>	e221	10	14-5241-01	C14
rCan f 5 Dog	<i>Canis familiaris</i>	e226	10	14-4998-01	CMZ
rFel d 1 Cat	<i>Felis domesticus</i>	e94	10	14-4905-01	BY0
rFel d 4 Cat	<i>Felis domesticus</i>	e228	10	14-5702-01	CT9
rEqu c 1 Horse	<i>Equus caballus</i>	e227	10	14-5700-01	CN7
nFel d 2 serum albumin Cat	<i>Felis domesticus</i>	e220	10	14-5240-01	BRX
nSus s Pig albumin, Swine	<i>Sus scrofa</i>	e222	10	14-5242-01	C36
Mites					
nDer p 1 House dust mite	<i>Dermatophagoides pteronyssinus</i>	d202	10	14-4966-01	CFG
rDer p 2 House dust mite	<i>Dermatophagoides pteronyssinus</i>	d203	10	14-4967-01	CG2
rDer p 10 Tropomyosin, House dust mite	<i>Dermatophagoides pteronyssinus</i>	d205	10	14-4985-01	CG5



Product		Code	Size	Art. No.	Barcode
Venoms					
rApi m 1 Phospholipase A2, Honey bee	<i>Apis mellifera</i>	i208	10	14-4987-01	CJ7
rVes v 1 Phospholipase A1, Common wasp	<i>Vespula vulgaris</i>	i211	10	14-4995-01	CMR
rVes v 5 Common wasp	<i>Vespula vulgaris</i>	i209	10	14-4992-01	CJ8
rPol d 5 Paper wasp	<i>Polistes dominulus</i>	i210	10	14-4994-01	CJ09
Occupational					
rHev b 1 Latex	<i>Hevea brasiliensis</i>	k215	10	14-5324-01	C20
rHev b 3 Latex	<i>Hevea brasiliensis</i>	k217	10	14-5326-01	C2A
rHev b 5 Latex	<i>Hevea brasiliensis</i>	k218	10	14-5327-01	C1Z
rHev b 6.01 Latex	<i>Hevea brasiliensis</i>	k219	10	14-5328-01	C28
rHev b 6.02 Latex	<i>Hevea brasiliensis</i>	k220	10	14-5329-01	C22
rHev b 8 Profilin, Latex	<i>Hevea brasiliensis</i>	k221	10	14-5330-01	C1V
rHev b 9 Latex	<i>Hevea brasiliensis</i>	k222	10	14-5331-01	C2C
rHev b 11 Latex	<i>Hevea brasiliensis</i>	k224	10	14-5333-01	C29
Occupational / Enzymes					
Alkalase	<i>Bacillus spp.</i>	k205	10	14-5126-01	C1F
nAna c 2 Bromelain, Pineapple	<i>Ananas comosus</i>	k202	10	14-5127-01	BT1
nAsp o 21 alpha-amylase	<i>Aspergillus oryzae</i>	k87	10	14-5370-01	595
nCar p 1 Papain, Papaya	<i>Carica papaya</i>	k210	10	14-5130-01	BT0
nGal d 4 Lysozyme, Egg	<i>Gallus spp.</i>	k208	10	14-5128-01	C0T
Maxatase	<i>Bacillus licheniformis</i>	k204	10	14-5128-01	C2F
Savinase	<i>Bacillus spp.</i>	k206	10	14-5132-01	C2R
nSus s Pepsin, Swine	<i>Sus scrofa</i>	k213	10	14-5258-01	C3B

Product		Code	Size	Art. No.	Barcode
Foods					
rAct d 8 PR-10, Kiwi	<i>Actinidia deliciosa</i>	f430	10	14-4984-01	CG7
rAna o 3 Cashew nut	<i>Anacardium occidentale</i>	f443	10	14-5760-01	D0W
rApi g 1.01 PR-10, Celery	<i>Apium graveolens</i>	f417	10	14-4957-01	CBR
rAra h 1 Peanut	<i>Arachis hypogaea</i>	f422	10	14-4963-01	CDF
rAra h 2 Peanut	<i>Arachis hypogaea</i>	f423	10	14-4964-01	CDG
rAra h 3 Peanut	<i>Arachis hypogaea</i>	f424	10	14-4965-01	CDH
rAra h 8 PR-10, Peanut	<i>Arachis hypogaea</i>	f352	10	14-5341-01	CEZ
rAra h 9 LTP, Peanut	<i>Arachis hypogaea</i>	f427	10	14-4980-01	CFC
rBer e 1 Brazil nut	<i>Bertholletia excelsa</i>	f354	10	14-5343-01	CDS
nBos d 4 alpha-lactalbumin, Milk	<i>Bos spp.</i>	f76	10	14-4522-01	CTP
nBos d 5 beta-lactoglobulin, Milk	<i>Bos spp.</i>	f77	10	14-4523-01	CTR
nBos d 8 Casein, Milk	<i>Bos spp.</i>	f78	10	14-4524-01	CTS
nBos d Lactoferrin, Milk	<i>Bos spp.</i>	f334	10	14-5253-01	C16
rCor a 1 PR-10, Hazel nut	<i>Corylus avellana</i>	f428	10	14-4981-01	CFB
rCor a 8 LTP, Hazel nut	<i>Corylus avellana</i>	f425	10	14-4968-01	CDP
nCor a 9, Hazel nut	<i>Corylus avellana</i>	f440	10	14-5758-01	D0M
rCor a 14, Hazel nut	<i>Corylus avellana</i>	f439	10	14-5754-01	CZP
rCyp c 1 Carp	<i>Cyprinus carpio</i>	f355	10	14-5344-01	CF0
rGad c 1 Cod	<i>Gadus morhua</i>	f426	10	14-4971-01	CEY
nGal d 1 Ovomucoid, Egg	<i>Gallus spp.</i>	f233	10	14-4805-01	904
nGal d 2 Ovalbumin, Egg	<i>Gallus spp.</i>	f232	10	14-4804-01	903
nGal d 3 Conalbumin, Egg	<i>Gallus spp.</i>	f323	10	14-5222-01	C18
rGly m 4 PR-10, Soy	<i>Glycine max</i>	f353	10	14-5340-01	CDR
nGly m 5 beta-conglycinin, Soy	<i>Glycine max</i>	f431	10	14-4990-1	CLV
nGly m 6 Glycinin	<i>Glycine max</i>	f432	10	14-4991-01	CLU
rJug r 1 Walnut	<i>Juglans regia</i>	f441	10	14-5762-01	D0T
rJug r 3 LTP, Walnut	<i>Juglans regia</i>	f442	10	14-5954-01	D11
rMal d 1 PR-10, Apple	<i>Malus domestica</i>	f434	10	14-5703-01	CWR
rMal d 3 LTP, Apple	<i>Malus domestica</i>	f435	10	14-5704-01	CWS
rPen a 1 Tropomyosin, Shrimp	<i>Penaeus aztecus</i>	f351	10	14-5335-01	C11
rPru p 1 PR-10, Peach	<i>Prunus persica</i>	f419	10	14-4960-01	CBV
rPru p 3 LTP, Peach	<i>Prunus persica</i>	f420	10	14-4961-01	CBW
rPru p 4 Profilin, Peach	<i>Prunus persica</i>	d421	10	14-4962-01	CBX
rTri a 14 LTP, Wheat	<i>Triticum aestivum</i>	f433	10	14-5701-01	CN6
rTri a 19 Omega-5 Gliadin, Wheat	<i>Triticum aestivum</i>	f416	10	14-4954-01	C8H
Gliadin		f98	10	14-5752-01	CXG
Miscellaneous					
MUXF3 CCD, Bromelain		214	10	14-5339-01	CJU



Common questions regarding molecular components

What is a molecular allergen-specific IgE test and does it differ technically from normal specific IgE tests that I request from my laboratory?

Technically they work in the same way and give results in kUA/L the same as normal whole extract sources such as cat, peanut etc.

How many ImmunoCAP components are available?

There are currently just over 100 component allergens in the product range. There is a list included in this guide.

For each allergen source how do I know that the allergen components available represent the whole allergen extract?

The components that are selected are generally considered the most clinically important ones, as defined by current scientific studies. For example there are over 30 proteins reported in the peanut extract, many not clinically relevant or with unknown relevance. For technical reasons we cannot produce all the components that are needed but the number will increase. Thermo Fisher Scientific develops between 4-8 new components every year. Since today all components are not available as single tests it is suggested to use the available components together with the whole extract to cover the spectrum of patients' sensitisations. Like genetic science, the field of molecular allergy is ever-expanding as we gain further scientific information and knowledge.

What is ImmunoCAP ISAC?

ImmunoCAP ISAC is a microarray chip which tests for 112 allergen components simultaneously. It is a multiplex test and produces a report on a patient's allergen component profile. It has been found useful for the following but this list is not exhaustive: complex allergy, OAS, and cases of multi-sensitisation, idiopathic anaphylaxis and high total IgE patients. Further information on ImmunoCAP ISAC is in the third mini guide of this series.

Where can I get access to ImmunoCAP ISAC?

Your local immunology laboratory should be able to refer your sample for testing, therefore contact your local lab to find out what is possible.

Is it possible to have a whole extract test negative and component to be positive?

This is possible in some cases. The whole extract is a mass of mixed proteins represented in the test as it is within its natural composition at the source. The allergen component is a pure protein of only one type with no interference from other proteins. Overall we consider the component tests as an advancement of technology over whole extract tests giving more specificity and sometimes even more sensitivity. Using a combination of both whole extract and components (where possible) is currently considered the best strategy for diagnosis.

Glossary

Allergen component – single allergy-provoking molecular protein from an allergen source e.g. Ara h 2 from a whole peanut extract.

Cross-reactivity/Cross-sensitisation – IgE antibodies produced to one allergen may cross-react to other allergens from botanically related and/or structurally similar sources. Cross-reactive antibodies can cause a variety of different clinical outcomes.

Epitope – the amino acid sequence of a protein corresponding to the allergen-binding part of the IgE antibody (Fab). Determinant is another name.

ImmunoCAP – an *in vitro* test for the measurement of IgE antibodies. ImmunoCAP is the most used blood test in this area and is regarded as the gold standard. ImmunoCAP is also available for testing for other immunoglobulins (e.g. IgA/ IgG).

Panallergen – evolutionarily conserved and widely distributed allergen, ubiquitous component of several complex sources of allergens. IgE antibodies to a panallergen may cross-react with homologous allergens and thus also give rise to symptoms to many different allergens in a patient.

Primary sensitising allergen – an allergen originally triggering the immune system to produce specific IgE antibodies. For example Bet v 1 from birch or Ara h 2 from peanut.

Minor and major allergens – often you find references and descriptions of major and minor allergens. Major allergen components are allergens that account for over 50% of sensitisation within an allergy. Minor allergens are often less prevalent in triggering allergy. For instance in birch allergy the major allergen is Bet v 1 (PR-10), whilst a minor allergen is Bet v 2 (profilin).

Secondary allergen – an allergen that has a similar structure to a primary sensitising allergen and that cross-reacts with IgE. This occurs in food-pollen syndrome for example, when an individual is sensitised to birch PR-10 (Bet v1) and the IgE antibodies then cross-reacts to peanut PR-10 (Ara h 8).

Whole allergen extract – refers to the crude mixture of proteins that is obtained from an allergen source (e.g. birch pollen or peanut)



Educational resources

- Website: **AllergyEducation.co.uk** – Thermo Fisher Scientific educational website explaining the basics of Molecular Allergy and interactive tool to help with identification of relevant components and interpretation
- Website: **AllergyEducation-MA.com** – Thermo Fisher Scientific educational training course exploring the basics of Molecular Allergy
- Canonica GW, *et al.* A WAO – ARIA – GA2LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013;6(1):17.
- Thermo Fisher Scientific – Cross-reactivity in plant food allergy – A focused book on cross-sensitisation
- Thermo Fisher Scientific – Native and cross-reactive allergen components – A more detailed book giving an overview of allergen components
- Thermo Fisher Scientific – Individual literature packs on various components are available. Educational PowerPoint slide sets are also available. Please contact Thermo Fisher Scientific if you would like a set:

– Egg	– Milk	– Birch
– Grass	– Hazelnut	– Peanut
– Wheat	– Soybean	– Venoms
– Apple	– Walnut	– Cashew

References

1. Wickman M. When allergies complicate allergies. *Allergy* 2005;60(S79):14-18.
2. Nicolau N, *et al.* Allergy or intolerance in children sensitised to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol* 2010;125:191-197.
3. Sicherer DH, *et al.* US prevalence of self-reported peanut, tree nut and sesame allergy: 11 year follow up. *J Allergy Clin Immunol* 2010;125:1322-1326.
4. Rona RJ, *et al.* The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007; 120:638-646.
5. Sen M, *et al.* Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. *J Immunol* 2002;169(2):882-887.
6. Clark A, *et al.* A longitudinal study of resolution of allergy to well-cooked and uncooked egg. *Clin Exp Allergy* 2011;41:706-712.
7. Sander I, *et al.* Allergen content of grass pollen preparations for skin prick testing and sublingual immunotherapy. *Allergy* 2009;64(10):1486-1492.



Recommended literature

PR-10 proteins

- Hauser M, *et al.* Panallergens and their impact on the allergic patient. *Allergy, Asthma Clin Immunol* 2010;6:1.
- Midoro-Horiuti T, *et al.* Pathogenesis-related proteins of proteins of plants as allergens. *Ann Allergy Asthma Immunol* 2001;87:261-271.
- Bohle B. The impact of pollen-related food allergens in pollen allergy. *Allergy* 2007;62:3-10.
- Moverare R, *et al.* Change in the pattern of IgE reactivity to Timothy grass and birch pollen allergens over a 20-year period. *J Investig Allergol Clin Immunol* 2006;16:274-278.
- Cudowska B, *et al.* Immunoblotting in the diagnosis of cross-reactivity in children to birch. *Rocz Akad Med Mialymst* 2005;116:1327-1333.
- Mittag D, *et al.* A novel approach for investigation of specific and cross-reactive IgE epitopes on Bet v 1 and homologous food allergen in individual patients. *Mol Immunol* 2006;43:268-278.
- Mittag D, *et al.* Birch Pollen-related food allergy to legumes; identification and characterisation of the Bet v 1 homologue in mungbean (Vig radiate), Vig r 1. *Clin Exp Allergy* 2005;35:1049-1055.
- Ricci G, *et al.* Relationship between Bet v 1 and Bet v2 specific IgE and food allergy in children and grass pollen allergy. *Mol Immunol* 2005;42:1251-1257.
- De Amici M, *et al.* Recombinant birch allergens (Bet v 1 and Bet v 2) and oral allergy syndrome in patients allergic to birch pollen. *Ann Allergy Asthma Immunol* 2003;91:490-492.
- Fernandez-Rivas M, *et al.* Allergy to Rosaceae fruits without related pollinosis. *J Allergy Clin Immunol* 1997;100: 728-733.
- Asanoj A, *et al.* Peanut component Ara h 8 sensitisation and tolerance to peanut. *J Allergy Clin Immunol* 2012;130(2):468-472.

LTPs

- Egger M, *et al.* The role of Lipid Transfer Proteins in Allergic Disease. *Curr Allergy Asthma Rep* 2010;10:326-335.
- Asero R. Lipid Transfer Proteins Cross-reactivity assessed *in vivo* and *in vitro* in the office: pros and cons. *J Investig Allergol Clin Immunol* 2011;21(2):129-136.
- Pascal M, *et al.* Lipid transfer protein syndrome: clinical pattern, co-factor effect and profile of molecular sensitization to plant-food and pollens. *Clin Exp Allergy* 2012;42:1529-1539.
- Quercia O, *et al.* Allergy to beer in LTP-sensitized patients: beers are not all the same. *Allergy* 2012;67:1186-1189.
- Fernandez-Rivas M. The place of lipid transfer proteins (LTPs) in the cross-reactivity of plant foods. *Rev Fr Allergol* 2009;49:433-436.
- Cudowska B, *et al.* Lipid transfer proteins in diagnosis of birch-apple syndrome in children. *Immunobiology* 2008:213:89-96.
- Flinterman AE, *et al.* Lipid transfer proteins-linked hazelnut allergy in children from a non-Mediterranean birch-endemic area. *J Allergy Clin Immunol* 2008;121:423-428.
- Borges JP, *et al.* Lipid transfer proteins from Rosaceae fruits share consensus epitopes responsible for their IgE binding cross-reactivity. *Biochem Biophys Res Commun* 2008;365:685-690.
- Palacin A, *et al.* Wheat lipid transfer protein is a major allergen associated with bakers asthma. *J Allergy Clin Immunol* 2007;120:1132-1138.
- Pastorello EA, *et al.* Wheat mediated food allergy in European patients; alpha-amylase inhibitors, lipid transfer proteins and low-molecular-weight glutenins. Allergenic molecules recognised by double blind, placebo-controlled food challenge. *Int Arch Allergy Immunol* 2007;144:10-22.

Profilin proteins

- Ebo DG, *et al.* Sensitisation to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of Allergy. *Clin Exp Allergy* 2004;34:137-144.
- Radauer C, *et al.* Species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006;36:920-929.
- Ghunaim N, *et al.* Antibody profiles and self-reported symptoms to pollen-related food allergens in grass-pollen allergic patients from Northern Europe. *Allergy* 2005;60:185-191.
- Asero R, *et al.* Detection of clinical markers of sensitisation to profilin patients allergic to plant-derived foods. *J Allergy Clin Immunol* 2003;112:427-432.
- Nieto A, *et al.* Assessment of profilin as an allergen for latex-sensitised patients. *Allergy* 2002;57:776-84.
- Ballmer-Weber BK, *et al.* Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. *J Allergy Clin Immunol* 2002;110:167-73.
- Reindl J, *et al.* IgE reactivity to profilin in pollen-sensitised subjects with adverse reactions to banana and pineapple. *Int Arch Allergy Immunol* 2002;128:105-14.
- Ganglberger E, *et al.* Hev b 8, the Hevea brasiliensis latex profilin, is a cross-reactive allergen of latex, plant foods and pollen. *Int Arch Allergy Immunol* 2001;125:216-227.
- Benitez D, *et al.* Specific immune response to Phleum pratense plant profilin in atopic patients and control subjects. *Allergol Immunopathol (Madr)* 2001;29:9-15.
- Diez-Gomez ML, *et al.* Fruit-pollen-latex cross-reactivity: implications of profilin (Bet v 2). *Allergy* 1999;54:951-961.
- Santos A and Ven Ree R. Profilins: Mimickers of Allergy or Relevant Allergens? *Int Arch Allergy Immunol* 2011;155:191-204.

Storage proteins

- Holzhauser T, *et al.* Soyabean (Glycinemax) allergy in Europe: Gly m 5 (beta-conglycin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. *J Allergy Clin Immunol* 2009;123(2):452-458.
- Verweij MM, *et al.* Young infants with atopic dermatitis can display sensitization to Cor a 9 an 11S legumin-like seed storage protein from hazelnut (Corylus avellana). *Pediatric Allergy and Immunology* 2011;22:196-201.
- Dang TD, *et al.* Increasing the accuracy of peanut allergy diagnosis by using Ara h 2. *J Allergy Clin Immunol* 2012;129(4):1056-1063.
- Nicolaou N, *et al.* Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. *J Allergy Clin Immunol* 2011;127(3):684-685.
- Eller E and Bindslev-Jensen C. Clinical value of component-resolved diagnostics in peanut-allergic patients. *Allergy* 2013;68(2):190-194.
- Nicolaou N and Custovic A. Molecular diagnosis of peanut and legume allergy. *Curr Opin Allergy Clin Immunol* 2011;11(3):222-228.
- Verweij MM, *et al.* Young infants with atopic dermatitis can display sensitisation to Cor a 9, an 11S legumin-like seed-storage protein from hazelnut (Corylus avellana). *Paediatr Allergy Immunol* 2011; 22:196-201.
- Pedrosa M, *et al.* Peanut seed storage proteins are responsible for clinical reactivity in Spanish peanut-allergic children. *Paediatr Allergy Immunol* 2012;23(7):654-659.
- Codreanu F, *et al.* A Novel Immunoassay Using Recombinant Allergens Simplifies Peanut Allergy Diagnosis. *Int Arch Allergy Immunol* 2011;154:216-226.
- Lieberman JA, *et al.* The Utility of Peanut Components in the Diagnosis of IgE-Mediated Peanut Allergy Among Distinct Populations. *J Allergy Clin Immunol Pract* 2013;1(1):75-82.
- Masthoff LJ, *et al.* Sensitisation to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. *J Allergy Clin Immunol* 2013;132(2):393-399.
- Robotham JM, *et al.* Ana o 3, an important cashew nut (Anacardium occidentale L.) allergen of the 2S albumin family. *J Allergy Clin Immunol* 2005;115(6):1284-1290.



CCDs

- Wisniewska M, *et al.* Cross-reactive carbohydrate determinants in diagnostics of occupational allergy-preliminary results. *Allergy* 2010;66:665-666.
- Paschinger K, *et al.* Definition of immunogenic carbohydrate epitopes. *Acta Biochimica Polonica* 2005;52:629-632.
- Ebo DG, *et al.* Sensitisation to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of Allergy. *Clin Exp Allergy* 2004;34:137-144.
- Jin C, *et al.* Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. *J Allergy Clin Immunol* 2008;121:185-190.
- Malandain H, *et al.* The influence of carbohydrate structures present in common allergen sources on specific IgE results. *Eur Ann Allergy Clin Immunol* 2007;39:216-220.
- Mahler V, *et al.* Natural rubber latex and hymenoptera venoms share Immunoglobulin E-epitopes accounting for cross-reactive determinants. *Clin Exp Allergy* 2006;36:1446-1456.
- Jappe U, *et al.* *In-vitro* hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinates and reciprocal inhibition. *Allergy* 2006;61:1220-9.
- Kochuyt AM, *et al.* Prevalence and clinical relevance of specific immunoglobulin E to pollen caused by sting-induced specific immunoglobulin E to cross-reacting carbohydrate determinants in Hymenoptera venoms. *Clin Exp Allergy* 2005;35:441-447.
- Malandain H. Widening sensitisation spectrum through carbohydrate pan-epitopes-a hypothesis. *Allerg Immunol (Paris)* 2004;36:297-299.
- van der Veen MJ, *et al.* Poor biological activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997;100:327-34.

