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Information
FOR HEALTH PROFESSIONALS

Laboratory Investigation of Allergic Diseases

The term 'allergy' is popularly applied to a range of adverse reactions to various environmental exposures, resulting from inhalation, contact or ingestion. However, only a subset of these reactions represent true immunological hypersensitivity when defined as a damaging immune response to a usually innocuous substance, resulting from inappropriate reactivity to that substance.

Diagnosis of a true immunological hypersensitivity is supported by a range of test procedures.

Types of hypersensitivity

Several different types of hypersensitivity occur, reflecting different underlying mechanisms:

- Types I-III are antibody (immunoglobulin) mediated.
- Type IV is cell-mediated.

This document concerns Type I (immediate-type, Immunoglobulin E [IgE] mediated, "true allergy") hypersensitivity, where various investigative procedures exist.

Type IV (delayed-type) hypersensitivity is responsible for allergic contact dermatitis and is investigated by epicutaneous patch testing. It is not considered further in this document.

Although Type II hypersensitivity (antibody-dependent cellular cytotoxicity) and type III hypersensitivity (immune-complex mediated) can be involved in some hypersensitivity reactions to drugs and exogenous triggers, routine testing is not available in most cases and they are not further considered in this document.

IgE-mediated allergy (type I, immediate-type hypersensitivity)

The most well-known IgE-mediated allergy usually leads to rapid-onset symptoms following exposure to the triggering allergen, following contact, ingestion or inhalation. This is mediated by allergen specific IgE-antibodies from prior sensitisation.

Atopy is a genetic predisposition to develop such antibodies against harmless, common environmental or food allergens. The presence of such antibodies is known as "sensitisation", and is necessary, but not sufficient, for the development of clinical symptoms of allergy.

In "allergic" individuals, binding of allergen specific IgE to the allergen activates mast cells whose rapid release of histamine and other mediators causes the symptoms. Inhalation aeroallergens results in allergic rhinitis and allergic asthma while contact with allergens results in allergic conjunctivitis, contact urticaria or exacerbation of atopic dermatitis.

In IgE-mediated food or drug allergy, ingestion of the allergen causes widespread mast cell degranulation, leading to urticaria, angioedema or anaphylaxis. While gastrointestinal symptoms such as vomiting or diarrhoea can be associated with such acute events, chronic gastrointestinal symptoms seldom result from IgE-mediated food allergy.

IgE-mediated insect venom allergy triggers anaphylaxis after stings.

IgE antibodies are produced in tiny quantities compared to other antibody classes, but can be detected by both *in vivo* tests (skin prick tests and intradermal tests) and *in vitro* specific IgE tests (formerly called RAST tests).

IgE antibodies

IgE antibodies are of extremely low abundance compared to other antibody classes. Approximately 50% of IgE antibodies are free, while the other 50% are bound to IgE receptors on a variety of effector cells, with mast cells and basophils being the most important cells involved in Type I allergic responses.

Free IgE has a half-life of only a few days, while IgE bound to cellular receptors has a half-life of about 2 months and it is this bound IgE that is responsible for the effector phase of allergic reactions. When cell-bound

IgE encounters its cognate polyvalent allergen, cross-linking of bound IgE on the cell-surface occurs, leading to a cellular response (e.g. release of histamine, leukotrienes, chemotactic factors, neurokinins and other inflammatory mediators). Cross-linking of only about 1% of IgE molecules on the effector cell surface is required for half-maximal activation of the cell.

Limitations of testing

IgE antibodies raised against even a single allergen are polyclonal and comprise a large population of antibodies with different epitope specificities and binding strengths, none of which is measured by any routine diagnostic test.

A large number of variables have a significant impact on allergen-mediated activation of effector cells, including the total amount of cell-bound IgE, the ratio of allergen-specific to total IgE, the number of epitope-specific antibodies capable of binding (clonality), the binding strength (affinity) of individual IgE antibodies and their cognate allergen, the total number of multivalent IgE binding sites that bind strongly to the allergen (avidity) and the ratio of low-to high-affinity IgE antibodies. IgE antibodies stabilise their own receptors on the cell surface and thus regulate the amount of cell-bound IgE.

As a consequence of these complex variables, different types of tests for IgE-mediated allergy measure different things and while various tests correlate qualitatively with one another (are positive or negative), they do not generally correlate quantitatively. Furthermore, the presence of sensitising IgE antibodies to a particular allergen does not imply that that allergen has any clinical relevance.

While specific IgE antibodies can be identified for a wide range of target allergens, the clinical significance of such tests is critically dependent on history. Since specific IgE antibodies are identifiable in many individuals with no evidence of allergic disease, the predictive value of these tests is strongly dependent on a history suggestive of underlying atopic disease with symptoms for which the detected IgE specificity is a plausible trigger.

In patients in whom a history suggestive of IgE-mediated disease is lacking, the predictive value of tests for specific IgE antibodies is low and so such tests should not be used as a method for screening healthy patients for allergy. However, many patients may say they have no food allergies but on specific interrogation say they do not like or avoid certain foods, the basis of which may be food allergy or non-IgE mediated food intolerances as well as simple aversion.

Indications for allergen specific IgE antibody testing

Aeroallergy

IgE antibodies to inhaled allergens mediate allergic rhinitis and allergic asthma. Since such allergens are common in the home and external environment, they may also lead to exacerbation of atopic dermatitis through skin contact. Symptoms triggered by histamine release (eg sneezing, rhinorrhoea, itchy, watery eyes or bronchospasm in response to exposure) are suggestive of underlying IgE-mediated aeroallergy. History might give clues as to the allergen involved (eg seasonal rhinitis in pollen allergy, exacerbation of symptoms from mowing the lawn or exposure to animals), informing the choice of which allergens to include in a panel of tests. The most common triggers for aeroallergy are dust mites, pollens (most often grass pollen, although other wind-borne pollens from trees and weeds can be implicated), animal epithelia and moulds.

Food allergy

IgE-mediated food reactions usually occur within a short time of ingestion of the particular food concerned, ranging from almost immediate to within an hour or two. However, in the case of mammalian meat allergy due to tick-bite induced allergy to alpha-gal (the sugar galactose-α-1, 3-galactose), clinical reactions usually occur 3-6 hours after ingestion and sometimes 8-10 hours later. In some cases, clinical reactions only occur in the presence of cofactors such as exercise, NSAIDs, alcohol or infections, which can often be elucidated from a careful history. Clinical reactions can range in severity and include local tingling or itch of the buccal mucosa, contact urticaria, generalised urticaria, angioedema, vomiting, diarrhoea and in the most severe cases, severe anaphylaxis. Severity of reactions is dependent on many factors, including the type of food allergen (food allergens that are stable to heat and digestion are more likely to lead to severe reactions), the amount

consumed, the presence of cofactors (eg exercise, NSAIDs, ACE inhibitors, beta-blockers) or comorbidity (eg asthma, infection, mastocytosis). Chronic gastrointestinal symptoms (e.g. abdominal bloating or pain) are rarely due to IgE-mediated food allergy, although such symptoms are quite commonly reported in those with alpha-gal allergy. Some food allergens are notorious for inconsistent reactions after ingestion reflecting fragility with cooking, gastric acid or digestive enzymes. These include pollen-cross reactive fruit and nut allergen molecules such as profilins and proteinase-10 (PR-10) proteins although the more stable Lipid Transfer Proteins (LTPs) will commonly elicit oral allergy syndrome symptoms with most exposures. The chemistry of food preparation may also account for variable reactions after exposure such as for the insoluble molecule in wheat, omega-5-gliadin.

Insect venom allergy

A systemic reaction to an insect sting is an indication for testing for hymenoptera venom-specific IgE antibodies. There are a limited number of hymenoptera venoms for which specific IgE-antibody tests are routinely available. In the Australian context, relevant tests are for specific IgE for honey bee venom (*Apis mellifera*), paper wasp venom (*Pollistes spp*), Common wasp (*Vespula spp*) Fire Ant (a whole-body allergen extract) and Jumper Ant (*Myrmecia pilosula*). Jumper ant-specfic IgE testing has not been commercialised and uses a customised method. IgE antibodies recognising these venoms are relatively common in the normal population and in common with other tests for specific IgE antibodies, predictive value is dependent on a history suggestive of IgE-mediated allergy to stings from the relevant insects. Ants have powerful mandibles with a painful bite and some have a stinger they activate after latching onto their target. Mosquito saliva contains many allergenic molecules, some of which are cross-reactive with dust mite, midges, sandflies, bedbugs and scabies. Whole body extracts are sometimes useful for both diagnosis and immunotherapy for difficult mosquito sensitisation, Large local reactions to insect stings are quite common, and only a small minority of such patients will go on to develop systemic allergy to the related venom.

Skin colonising bacterial and fungal allergy

Persons with difficult eczema are often colonised with *Staphylococcus aureus*, *Malassezia*, *Candida* and *Trichophyton*. The level of specific IgE to these agents can assist in management strategies. Skin prick test reagents are only available for *Candida*.

In vivo tests for specific IgE antibodies

Skin prick testing

Skin prick testing is a long-established, sensitive method for the detection of allergen-specific IgE. The method involves the application of drops of solution containing allergens to skin (usually the forearm), with superficial skin pricks being carried out though the drops, introducing a tiny amount of the allergens into the epidermis. This allows the allergen to access specific IgE on the surface of cutaneous mast cells.

If IgE specific for a particular allergen is present, this leads to local mast cell degranulation, with the release of histamine and other inflammatory mediators and the rapid appearance of a wheal and flare response. The wheal response is measured about 15 minutes after the prick and provides a semi-quantitative assessment of specific IgE for each allergen. Results are therefore immediately available at the end of the test procedure.

Since the method is dependent on the release of histamine from cutaneous mast cells, medications which act as H1-receptor antagonists interfere with the test (e.g. antihistamines (often contained in “cold and flu” remedies), tricyclic and tetracyclic antidepressants and phenothiazines).

Most often, standardised commercial allergen extracts are used, but the method can be adapted to use fresh, frozen and dried foods and crude suspensions of environmental allergens. For some allergens (e.g. fruits), clinically relevant allergens are poorly represented in available commercial allergen extracts and prick testing using fresh foods may be necessary. The method has the advantages of high sensitivity, low cost, rapid results and the ability to economically test for reactivity to a wide range of allergens simultaneously.

Furthermore, skin-prick tests detect cell-bound IgE antibodies, which are those involved in allergic responses, although only a small subset of cutaneous effector cells are sampled, and thus responses do not necessarily correlate with responses to allergen exposure at other sites. Disadvantages include false-positive responses,

poor linearity of responses when compared to *in vitro* testing and that the patient has to be exposed to the allergen.

False negatives may occur due to under-representation of some individual allergen molecules due to lability or lack of solubility.

Although skin prick testing is relatively safe, an experienced operator is required for interpretation, and it must be carried out on normal healthy skin, limiting its use in the presence of diffuse dermatological conditions. Dermographism can also confound results and testing is always carried out with both a negative control (with which dermographism can be detected) and a positive (usually histamine) control, mainly to detect interference from drugs.

Occasional systemic reactions occur, although anaphylaxis is extremely rare. It is therefore essential that skin prick tests are conducted by staff that are competent in the management of anaphylaxis and in facilities equipped for such management.

Intradermal testing

Intradermal testing involves the intradermal injection of dilute solutions of allergens. While it is more sensitive than skin prick testing, and can detect sensitisation to an allergen in individuals where a skin prick test for that allergen is negative, it is more likely to generate false positive results and has a higher potential than skin prick tests for systemic allergic reactions, including anaphylaxis.

It is therefore best carried out in specialist facilities, where adequate resources are available for management of anaphylaxis. The main use of intradermal testing in Australia is in the investigation of drug allergy and in the investigation of hymenoptera venom allergy. Test requirements and drug interferences are similar to those for skin prick tests.

Oral food challenges

Skin prick tests and measurement of specific IgE for food allergy generate large numbers of false positive results, which do not correlate with clinical symptoms following ingestion of the food concerned. Such false positive results are often more prevalent than true positive results. The “gold standard” method for diagnosis of food allergy is therefore a supervised, incremental oral food challenge with the suspect food.

Drug provocation tests

Skin prick tests and intradermal tests for IgE-mediated drug allergy are only validated for a few drugs and even then, sensitivity for detection of drug allergy is poor. Negative intradermal test results are therefore usually followed by a drug provocation test, usually by the oral route of administration.

Requirements for oral food challenge and drug provocation tests

These tests are time consuming, labour intensive and are associated with a significant risk of systemic allergic reactions, including anaphylaxis. They must therefore only be carried out by experienced clinicians in facilities with resuscitation equipment available.

Provocation tests for aeroallergens

Skin prick tests and measurement of specific IgE in the context of allergic rhinitis also generate many false positive results and various methods have been developed to assess the functional effect of inhalant allergens in these conditions. These include nasal provocation testing, specific inhalational challenge and the use of environmental challenge chambers.

Such methods have not been standardised, require specialist experience and specialised equipment, are time-consuming and carry a significant risk of severe bronchospasm. Their use is therefore largely confined to research, although specific inhalational challenge is used in occupational medicine for investigation of workplace-induced asthma.

In vitro tests for specific IgE antibodies

Routine measurement of circulating allergen specific IgE antibodies in patient sera was initially accomplished using Radio-Allergo-Sorbent Tests (RAST), which used a radioisotope detection method. This has been superseded for many years, with detection now taking place using enzyme and fluorescence based methods, but the RAST nomenclature for such tests has persisted.

The methods used employ allergens or mixtures of allergens, coupled to a solid phase support. These are incubated with patient serum, allowing free specific IgE to bind to the allergen on the solid matrix. This is then washed to remove unbound IgE and a second labelled anti-IgE antibody is used to detect bound IgE. These methods are robust and fully quantitative. Sensitivity for detection of specific IgE may be less than that of skin prick tests or intradermal tests for some allergens, while for other allergens they can be more sensitive. These methods provide linear quantitation over a much more extended range than skin prick tests. Unlike skin-prick tests and intradermal tests, these methods detect free specific IgE, not the mast-cell bound fraction, and it is assumed that these are proportionate.

These *in vitro* methods have several advantages over skin prick tests:

- The patient is not exposed to the allergen, preventing any likelihood of allergic reactions during testing.
- There is no interference due to the patient taking antihistamines or other medications.
- Tests are not contraindicated by the presence of diffuse dermatological disease or confounded by dermographism.
- Standardisation of test systems and quantitation allows more robust comparison in an individual over time as well as between testing locations.
- Specific IgE can be assessed to a wide range of allergens from a single blood sample and a larger test menu is available.

Disadvantages include:

- The need for venepuncture, which can be undesirable in young children.
- Compared to skin prick tests, there is some delay in obtaining results.
- Tests using mixtures of allergens can give misleading results, since the amount of any single allergen in a mixture can be limiting (leading to binding of only a fraction of specific IgE for that allergen in the tested patient serum and giving a low or negative result) or alternatively, false positive results can occur when the patient serum contains low levels of specific IgE antibodies recognising multiple components of the mixture.
- Limiting allergen amounts can also lead to falsely low results for allergens that are poorly represented in the allergen extract used in the assay.
- Some individuals have IgE antibodies to non-pathological cross-reactive carbohydrate determinants (CCD) resulting in apparent false positive results.
- IgG antibodies to the allergen, especially when present in high titre (such as are found in allergen immunotherapy patients), can compete with IgE antibodies for binding to the allergen, giving rise to falsely low results for specific IgE.

Role of total IgE

Total (free) IgE serum levels within a normal population have a log-normal distribution (ie logarithmic transformation yields a Gaussian distribution). Geometric means show significant variation with age, sex, parasite burden and geographic location and are higher in atopic populations. Given this variation, reference intervals and cutoff values should ideally be established for local populations, and should be age-range specific. However, this is seldom the case, with most laboratories reporting reference intervals stated by the assay manufacturer, which are often based on decades-old data, based on subsets of a single geographically-restricted population.

If optimal cutoffs are selected for local populations, there is moderate specificity (>70%) for detection of atopy, with high values of total IgE being associated with atopy. However, there is poor sensitivity, and thus low

values of total IgE are poor at excluding atopy, unless cutoffs are selected that are around or below the population median, which limits usefulness.

Due to the log-normal distribution, very high levels of total IgE can be found in a substantial proportion of atopic patients, especially those with atopic eczema. Monitoring of total IgE is useful when patients are being treated with immunosuppression for severe eczema. Nevertheless, determination of the total IgE can be useful in interpreting specific IgE tests (see below).

Extremely high levels of total IgE are found in IgE myeloma, a very rare myeloma subtype. When such levels are evident, it is prudent for the laboratory to carry out serum electrophoresis, with immunofixation for IgE, which is not usually included in standard immunofixation protocols.

Specific IgE Index (ratio of specific IgE to total IgE)

There are no internationally accepted calibration standards for specific IgE antibodies, which are therefore usually measured in arbitrary units. However, there is an official WHO standard for total IgE, and a reference curve calibrated to this standard is usually incorporated into each specific IgE assay run, which allows measurement signals for specific IgE antibodies to be converted into standardised IgE units (expressed in kU/L). This is known as heterologous calibration, and assumes that the binding strength of the allergen for specific IgE is similar to the binding strength between anti-IgE antibodies and total IgE used to create the calibration curve. While this introduces an error, it is usually less than 10%, if the methods used for the specific- and total-IgE assays are comparable. This allows for the calculation of a specific IgE to total IgE ratio, also known as antibody-specific activity or specific IgE index, which can improve interpretation of results.

This is particularly helpful in the interpretation of specific IgE levels in patients with very low levels of total IgE, in non-atopic patients with IgE-sensitisation to particular allergens such as hymenoptera venoms or occupational allergens or in patients with extremely high total IgE levels (e.g. as is found in atopic dermatitis). It has been demonstrated that the serum specific IgE index is reflected in the ratio observed bound to effector cells and if specific IgE is expressed as a ratio, it allows for normalisation of the specific IgE values to the individual patient's total IgE. This leads to better concordance between the specific IgE level and other tests for IgE sensitisation such as skin prick tests and the Basophil Activation Test. The specific IgE index may also have predictive value in relation to the outcome of allergen immunotherapy.

Measurement of specific IgE antibodies to allergen extracts

Most *in vitro* specific IgE tests use standardised aqueous extracts of macromolecules obtained from individual foods, inhalants (eg pollens, mites, animal epithelia, moulds) or environmental allergens (eg latex). Such allergen extracts comprise thousands of different macromolecules and despite best attempts at standardisation, some allergen molecules may be poorly represented in the extracts and composition varies between manufacturers and even between batches. Hundreds of allergens are available, but due to cost constraints and utility, only a proportion of these are routinely available in testing laboratories. In common with allergen extracts used for skin prick testing, RAST tests using allergen extracts can detect IgE antibodies to cross-reactive determinants, which may have no clinical correlation and therefore give rise to false positive results.

Choice of allergen-specific IgE to be tested should be determined by a careful clinical history in all cases. Both *in vitro* and *in vivo* test systems can be complementary in allergy diagnosis.

Measurement of specific IgE antibodies to allergen molecules (allergen components, single allergens or component resolved diagnosis) in singleplex assays

In recent years, many individual molecular allergen components have been identified which are recognised by IgE antibodies in patients with allergies. This has allowed identification of dominant allergen components which are commonly recognised by IgE antibodies from allergic patients, as well as characterisation of the physicochemical properties of individual allergen components.

A number of these allergen components are now available in commercial tests for specific IgE antibodies. Some of these are purified from natural sources, when the purified product usually contains multiple naturally-occurring isoforms of the allergen concerned and are normally glycosylated.

While this means that the purified components contains a range of epitopes which can bind to specific IgE from sensitised patients, certain N-glycan carbohydrates (CCD, cross-reactive carbohydrate determinants) present on such preparations can lead to cross-reactive IgE antibody binding, which is usually of no clinical relevance and gives rise to false positive results. Also, some allergen components are present in very small quantities in the natural allergen source, making purification free of impurities very difficult. These difficulties have led to the increasing use of recombinant allergens as single protein molecules, produced in *Escherichia coli*, where glycosylation does not take place and where the protein can be produced in large quantities. While this circumvents the problem of CCD, it provides an allergen component which lacks the natural variant isoforms and may not possess all the epitopes recognised by specific IgE in patient sera.

Measurement of specific IgE antibodies for individual allergen components can be useful for the following reasons:

- Analytical sensitivity can be increased, especially when important allergens are poorly-represented in allergen extracts or are unstable in the extracts.
- Analytical specificity can be increased, especially when the specific IgE antibodies to an allergen component provide information relevant to risk stratification (e.g. in food allergy), cross-reactivity on allergens from other sources or primary (species-specific) sensitisation.

However, successful utilisation of these tests requires careful choice of the allergens used for specific IgE testing, based on individual patient history, test results using crude allergen extracts and a knowledge of the allergen components available for testing.

In latex allergy, testing for specific IgE to a range of latex allergen components provides a more sensitive means of detection of sensitisation than other approaches and can also provide information on relative risk.

In food allergy, sensitisation to certain allergen components can be associated with an increased or decreased relative risk of severe reactions to the food from which it is derived (e.g. multiple peanut allergen components), can identify an increased risk from cooked versions of the food (e.g. ovomucoid-specific IgE in egg allergy) or can be useful in identifying sensitisation to allergen components involved in cofactor-dependent food allergy. However, no reliable prediction of future tolerance of the food concerned can be made solely on the basis of these tests. Once again, relevance is dependent on correlative symptoms in the patient's history or the results of allergen provocation testing.

When symptoms occur with multiple, seemingly unrelated plant foods, especially in the context of pollen or latex allergy, the possibility of sensitisation to cross-reactive proteins is suggested and can be confirmed by measurement of specific IgE antibodies to potential cross-reactive allergen components.

In the case of respiratory allergy, sensitisation to individual pollen-allergen components is useful in identifying responses to cross-reactive allergen components, which do not usually correlate with respiratory symptoms, or species-specific components which are more relevant to disease and this can in some situations inform the choice of allergens used in allergen immunotherapy. Within the Australian context, the utility of dissecting grass-pollen reactivity in this way is limited, because of the high prevalence of subtropical grass pollens such as Bermuda grass or Bahia grass in warmer locations, for which only a single allergen component is available for Bermuda grass (Cyn d 1) and none is available for Bahia grass. Furthermore, in contrast to Europe, none of the allergen extracts used for pollen immunotherapy in Australia provides information on the representation of particular allergen components in the extracts.

It should also be borne in mind when requesting RAST tests for allergen components that test reagents for these tests are much more expensive than those used for crude allergen extracts. This may result in significant out-of-pocket expense for the patient and such tests are best used judiciously, on a case-by-case basis, to answer particular questions.

Application of skin prick tests and Singleplex RAST Tests to the investigation of allergy

Since the detection of allergen-specific IgE is often not clinically relevant, current guidelines discourage the random screening of patients for IgE-mediated sensitisation, since the number of positive results by far exceeds the number of clinically-relevant allergies.

Instead, it is recommended that the choice of diagnostic tests is guided by clinical symptoms. A careful and comprehensive history is obtained in the first instance and used to choose allergen extracts for skin prick testing, RAST testing or both. This usually results in one of two clinical scenarios:

1. Limited numbers of positive allergen-extract based sensitisation results.

When one or only a few positive results are obtained using skin prick tests, intradermal tests or RAST tests for inhalant allergens or selected foods and this correlates with the history, the analytical specificity of the allergen extract based tests might be sufficient to identify or exclude the clinically-relevant allergen and no further testing would be required. Exceptions to this are potentially false negative sensitisation tests to under-represented or unstable allergens, which is particularly relevant to certain foods, when an oral food challenge might be required to exclude the food as a trigger for symptoms.

2. Multiple allergen-extract based sensitisation results.

More frequently, multiple positive tests are obtained with allergen extracts, suggesting cross-reactive specific IgE antibodies. In this context, further specific IgE testing using allergen components may provide useful additional information. This requires a knowledge of allergen components which might be useful in further investigation. A detailed discussion of these is beyond the scope of this article. Several examples of scenarios where such investigation is useful are given below.

(a) Sensitisation to multiple unrelated pollen species.

In this context, checking for sensitisation to cross-reactive “panallergens” and for species-specific “marker” allergens allows discrimination between genuine sensitisation and cross-reactivity. This can be useful in informing the choice of allergen extract used for immunotherapy.

(b) Multiple sensitisations to different furry animals.

Measurement of specific IgE antibodies for animal allergen components can identify responses to cross-reactive components as opposed to multiple, species-specific sensitisations.

(c) Symptoms occur in response to ingestion of multiple unrelated plant foods, due to sensitisation to cross-reactive panallergens.

In this scenario, certain clusters of foods causing symptoms can suggest which class of panallergens might be involved. Specific IgE testing using examples of each class can confirm sensitisation and can be useful for risk stratification: for example, sensitisation to the Bet-v1 cluster and profilin groups is usually associated with mild “oral allergy syndrome” symptoms which are often absent when the relevant foods are cooked, since the allergens are heat-and digestion-labile. On the other hand, sensitisation to the lipid transfer-protein (LTP) group can be associated with more severe symptoms, which can be exacerbated by cofactors such as exercise.

(d) Severe reactions to certain plant foods.

When severe reactions have occurred to ingestion of plant foods such as peanut, soy, tree nuts and seeds and multiple positive sensitisations are found using tests for specific IgE using allergen extracts, assessment of specific IgE for allergen components can help identify sensitisation to high-risk, species-specific heat and digestion-stable food allergen components and distinguish this from sensitisation to lower risk, low abundance or heat-and digestion-unstable allergens. This information is useful for risk-stratification.

(e) Systemic reactions to hymenoptera stings.

When a systemic reaction occurs to hymenoptera sting, sensitisation may be found to more than one species of insect, using tests for specific IgE antibodies using venom allergen extracts. Determination of sensitisation to certain venom allergen components can help distinguish between true sensitisation to one of the species and cross-reactivity. This can be helpful in choosing the most appropriate allergen extract for immunotherapy and can also identify sensitisation to cross-reactive components (eg CCD) which are not associated with clinical allergy, but which give rise to positive test responses using allergen extracts.

Measurement of specific IgE antibodies to a large array of allergen components in multiplex assays

Multiplex assays have become available, which allow for the determination of specific IgE-sensitisation to a large number of allergen components (e.g. Immunocap ISAC test, for 115 allergen components and Allergy Explorer MacroDXTM tests).

Screening for sensitisation using Multiplex testing irrespective of the clinical history is of little clinical utility for the following reasons:

- Only a limited number of allergen components are present in these multiplex assays compared to the entire “allergome”.
- Multiplex tests are expensive and yield a lot of information, which requires careful interpretation.
- Multiplex tests are often less sensitive than single RAST tests using either allergen extracts or selected allergen components.
- The range of allergens tested is biased by the availability of allergens on the array.
- The number of positive IgE sensitisations detected on such arrays may exceed the number of clinically relevant allergies, yielding a large proportion of positive test results that are clinically irrelevant.

However, the targeted use of such multiplex testing can be helpful in certain situations. Various clinical scenarios where multiplex testing may or may not be useful are summarised in the following table.

Green – ISAC test recommended	Orange – Consider other investigations prior to ISAC test	Red – ISAC test not recommended
Investigation of Oral allergy Syndrome due to cross-reaction between pollen and plant food allergens	Anaphylaxis • Is there a suspected food or venom/sting allergen trigger than can be measured by specific IgE / RAST?	Suspected drug allergy – no drugs are tested in the ISAC test.
Investigation of potentially cross-reactive allergens in a poly-allergic patient for determination of thermally labile versus non-labile allergen reactivity to determine systemic reactivity risk	Asthma • Has the patient had measurement of RAST / specific IgE for common inhalant allergens? (e.g. house dust mite; grass pollens, animal danders, moulds)	Suspected food chemical sensitivity – this is not an IgE antibody mechanism.
De-labelling of allergy in children or adults on highly restrictive diets	Suspected food allergy • Is there a suspected food allergen trigger that can be measured by specific IgE / RAST?	Assessment of potential stinging insect allergy venoms no longer included in ISAC.
Investigation of patient with anaphylaxis to unknown triggers when other tests are exhausted	Suspected mammalian meat allergy • Consider measurement of specific IgE for alpha-gal and milk, beef, lamb and pork.	Diagnosis or progress assessment of allergy to single common food allergens, e.g. milk, peanut, egg. Specific IgE measurement is preferred.
Detailed assessment of the known poly-allergic patient		Atopic dermatitis – for investigation of allergic contributions. Consider measurement of RAST/ specific IgE for common inhalant allergens, skin colonisers and selected foods

In vitro tests for significance of IgE-mediated sensitisation - Basophil Activation Test (BAT) and Flow Cytometry BAT

The basophil activation test (BAT) is a functional test resembling an *in vitro* provocation test. In common with mast cells, basophils have high affinity receptors on their surface which bind IgE (FcεR1), and cross-linking of this surface-bound IgE by allergen leads to degranulation of the basophil, with release of histamine and other inflammatory mediators.

Unlike mast cells, basophils are present in peripheral blood, where they have a half-life of about a week. Basophil granules contain CD63 on their inner surface and when degranulation of the basophil occurs, CD63 becomes translocated to the cell membrane, acting as a convenient activation marker that can be detected by flow cytometry. Other activation markers have been characterised (e.g. CD203c), but CD63 is the best clinically-validated marker.

To carry out the BAT, peripheral blood is obtained from the patient and incubated in the presence of various concentrations of allergen. Whole blood flow-cytometry is then used to identify the basophil fraction and the fraction of these which are CD63-positive at each allergen concentration. This allows determination of the maximum response (basophil reactivity) and the concentration of allergen which gives the half-maximal response (basophil sensitivity).

Ideally, blood should be fresh, but it is possible to use for up to 24 hours after collection to document sensitisation to an allergen, although basophil reactivity may deteriorate significantly over this time frame. It is preferable for blood to be collected within one year of the patient's last exposure to the test allergen, since sensitised basophils decrease with time, especially for drugs.

BATs have been used to identify responses to intact inhalant allergens, food allergens, hymenoptera venoms and various drugs and in general, correlate better with clinical reactions than simple detection of IgE-sensitisation. In food allergy, the need for oral food challenge has been reduced significantly by the use of BATs in some studies. BATs can also be used with allergen components, which in some studies, has correlated perfectly with the outcome of Oral Food Challenge.

In the case of drug allergy, BATs have been demonstrated to have predictive value for clinical reactions to drugs where no other investigative modality is available, other than provocation testing.

Modified BATs using blood from non-sensitised donors incubated with patient sera have been used for investigation of autoreactivity in chronic urticaria and suppression of basophil responses has been used to monitor the response of patients to allergen immunotherapy.

It would therefore appear that BATs are promising tools for investigation of allergy. However, currently BATs are not routinely available in Australia for a number of reasons, including the substantial cost of such tests (which are not Medicare-rebated), a requirement for fresh blood for assays, sampling of patient basophils within one year of last exposure, lack of well characterised allergen sources and a lack of current test standardisation.

Additional tests in assessment of suspected allergy

Eosinophil count

An elevated eosinophil count is a relatively non-specific finding. In Australia, the most common cause for eosinophilia is atopy, but a wide range of other conditions can be associated with eosinophilia including parasitosis (both endoparasites and ectoparasites such as scabies), various autoimmune diseases, neoplasia, asthma and a number of eosinophilic syndromes. Discussion of these is outside the scope of this article.

Eosinophilic cationic protein (ECP)

Eosinophilic cationic protein (ECP) is produced by activated eosinophils. High levels of ECP occur with more eosinophil activation such as with fungal upper and lower respiratory tract disease, eosinophilic oesophagitis, asthma and primary hyper-eosinophilic disorders.

Serum tryptase

Tryptase is an enzyme present in mast cell granules and is released during mast cell degranulation. During an anaphylaxis, serum levels increase, rising to a peak concentration between one and two hours after the anaphylaxis and return to baseline within six hours. Monitoring the tryptase time course following the onset of symptoms can help distinguish anaphylaxis from other causes of the presenting symptoms. In this context, **an acutely elevated tryptase is defined as a level greater than: 1.2 x baseline tryptase (µg/L) + 2µg/L**

Baseline tryptase is a marker for mast cell number and is elevated in mastocytosis, which can lead to severe anaphylaxis in response to various drugs and insect stings, as well as creating a risk of osteoporosis. When found to be elevated, tryptase should be periodically checked and bone marrow examination should occur if the baseline level exceeds 20µg/L. Allergic persons with elevated baseline tryptase levels may be susceptible to relatively more severe reactions to hymenoptera stings and with certain food and drug allergies.