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Newsletter

ALLERGY DIAGNOSTIC TESTING UPDATE

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INTRODUCTION

Allergy and severe allergic reactions are increasing worldwide. Many theories exist for this change in global allergy epidemiology, most commonly variants of the "hygiene hypothesis" and "biome depletion" theory. These theories refer to the impact that modern lifestyles have on the normal microbiological flora (the human biome), as well as decreased exposure to environmental allergens. Research has demonstrated that multiple changes (molecular, genetic, epigenetic and manipulation of external environments in which humans exist) impact human microbiological flora. Animal models and human cohort data have demonstrated some associations with decreased exposures to infectious organism and "hyper-clean" environments (increased antibiotic use and caesarean section rates; decreases in helminth loads), leading to changes in human immune tolerance and ultimately allergic or autoimmune disease.

The immunology of these hypotheses and how disease is caused is complex, and the mechanistic processes that lead to disease (either allergy or autoimmunity) are still being elucidated, but the loss of both cellular and humoral immune-regulatory pathways are involved and the outcome similar with these factors. This, in combination with a multifactorial genetic predisposition to allergy in certain individuals (known as atopy), leads to sensitisation of an individual to various environmental allergens which may or may not manifest as clinical symptoms of allergy.

Allergic reactions manifest clinically as anaphylaxis, allergic asthma, urticaria, angioedema, allergic rhinitis, some types of drug reactions, and atopic dermatitis. These reactions are usually mediated by IgE (Type I hypersensitivity reactions, which occur 2 – 4 hours after exposure to the allergen), which differentiates them from non-IgE-mediated (formerly called anaphylactoid) reactions that involve IgE-independent mast cell and basophil degranulation. Such reactions can be caused by iodinated radiocontrast dye, opiates, or vancomycin, and appear similar clinically to urticaria or even anaphylaxis.

Type IV hypersensitivity reactions, which are the immunological reactions implicated in allergic contact dermatitis, are also called delayed hypersensitivity reactions because they require 24 – 48 hours for signs of inflammation to occur. Type IV reactions are inappropriate or excessive immune reactions that are mediated by specific subsets of CD4+ helper T cells (Th-1 and Th-17 cells) or by CD8+ cytotoxic T cells. These reactions form the basis of contact dermatitis (exposure to nickel, latex) and are also implicated in the pathogenesis of some chronic granulomatous diseases.

DIAGNOSING ALLERGIC DISEASE

The crux of diagnosing common allergic conditions in the laboratory lies with measuring IgE sensitisation to a specific allergen or allergens which cause symptoms in an individual. Most patients who experience symptoms upon exposure to an allergen have demonstrable allergen-specific IgE that specifically recognises that allergen, making these tests essential tools in the diagnosis of allergic diseases.

Test systems have been developed, and are increasingly more sophisticated, that allow accurate measurement of IgE levels, but the trick is knowing how to "translate" these IgE levels and whether they are significant in the clinical situation. In essence the question is: *"Will a patient with detectable levels of IgE sensitisation to a specific allergen have an allergy reaction when exposed to that allergen?"*

Below is a summary of the available methods used in the laboratory to assist in making the diagnosis of an allergy in a patient:

- **Total IgE (T-IgE)**
  Although this test may provide a rough guide as to whether an individual is atopic (i.e. they have the potential to have an allergic reaction), T-IgE is not a specific diagnostic test for allergic sensitisation. It is a crude indicator with a low sensitivity for allergic conditions, especially in adults (< 60%), meaning that almost 40% of individuals with allergy will be missed if only T-IgE is used as a screening test. Many other conditions can also cause raised T-IgE levels, including helminth infestations, viral infections, bacterial infections, atopic dermatitis and hyper IgE syndromes.

- **Specific IgE (s-IgE)**
  This is the most common type of allergy test, and is used to detect sensitisation of an individual to specific foods or environmental allergens. s-IgE can be measured in two ways: *in vivo* using skin prick tests (SPT's) where the patient is present, or *in vitro* using a blood sample sent to the laboratory for specific IgE testing. SPT's and s-IgE testing measure the same thing, but have different advantages and disadvantages (see **Table 1** for a comparison). s-IgE tests on blood were previously known as "RAST" tests, which described a particular laboratory method. Nowadays, s-IgE tests are performed on automated immunoassay platforms, and a vast number of food and aero-allergen tests exist to determine s-IgE sensitisation in individuals. The biggest advantage of the laboratory-based s-IgE test versus the SPT to measure IgE sensitisation is that the s-IgE in a laboratory is a standardised, objective measurement.

TABLE 1. Comparison between Skin Prick Tests and Specific IgE Tests

Skin Prick Tests (SPT's)	Specific IgE (s-IgE) Tests
• Cheap • Rapid (results within 15 – 20 minutes) • Patient is involved, but it takes time • Antihistamines must be withdrawn for 72 hours prior to testing • Requires normal skin, i.e. no severe eczema • Technique and reporting must be standardised • Storage and care of allergens • Small risk of anaphylaxis • Not to be done in very young infants or in pregnant women	• Relatively more expensive • Larger range of available allergens • Minimal time commitment for the patient • No need to withdraw medication prior to testing • Skin condition irrelevant • Technique always standardised and traceable • Performed in quality-controlled laboratories • Safe, no risk of anaphylaxis • More tests may be added to the same sample after first results • Can test for severity with component resolved diagnostics (CRD)

However, whichever method is ultimately chosen to detect s-IgE, both of these are quantitative tests: SPT's measure a reaction to an allergen in millimetres (mm) and s-IgE measurements in blood give an IgE level against a specific allergen in arbitrary units defined by the test platform used (usually kU/L). The higher the level of s-IgE, either in mm or kU/L, the higher the likelihood that the individual is going to have clinical symptoms of allergy when they are exposed to the specific allergen. This is the positive predictive value (PPV) of the test.

Positive predictive values (PPV's) for both SPT's and s-IgE have been determined for various populations for some common food allergens as outlined in **Table 2.** These clinical decision points must however be used with **CAUTION** as they were derived in specific populations and cannot be directly extrapolated to other groups. Work is in progress to derive similar PPV's for South African and sub-Saharan African populations.

TABLE 2. Positive Predictive Values for common allergens

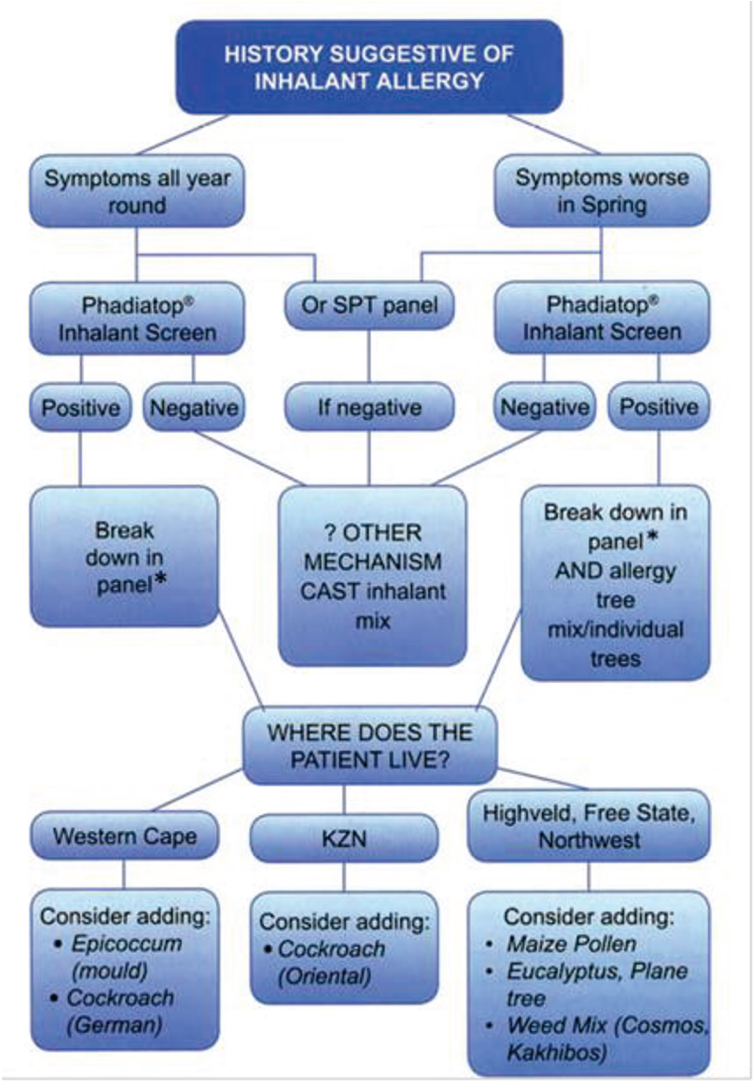
FOOD	95% PPV s-IgE (kU/L)	95% PPV SPT (mm)
Egg	2 - 7	5 - 6
Milk	5 - 15	6 - 8
Peanut	14	8
Fish	20	7
Tree nuts	15	8
Soya	30	9
Wheat	26	7

95% PPV = 95% positive predictive value, i.e. a clinical reaction is likely to occur in 95% of the specified population;
SPT = skin prick test, SPT measurements are wheal diameters in mm;
s-IgE = specific IgE. If a range is given the lower end of the range is applicable in children < 2 years.

- *Pooled Screening Tests*
  It is cost-effective to screen out potential allergy by using pooled screening tests. For example, the paediatric food mix test (also known as FX5) contains egg, milk, fish, peanut, wheat and soya in one immunoassay. If this pooled test is negative, the patient does not have allergic sensitisation to the foods in the pool, and has been effectively "screened out" for allergy to those foods. However, if the pooled test is positive, each of the foods in the pool need to be tested individually.

  The pooled aero-allergen test (also known as “phadiatop”) similarly contains common environmental allergens like cat and dog dander, dust mite, moulds and limited grasses and trees. It is advisable to follow the South African consensus approach illustrated in **Figure 1** for suspected sensitisation to aero-allergens.

FIGURE 1. South African Inhalant Allergy Consensus Approach



***Allergy Diagnostic Working Group (ADWG) panel:** Bermuda grass, Rye grass, Dermatophygoides pteronyssinus, Blomia tropicalis, Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus, cat, dog.*

- *Component Testing*
  Component-resolved diagnostics (CRD) is a refinement of s-IgE tests, and is another advantage that blood-based s-IgE testing has over SPT's. CRD allows testing for specific molecular epitopes of an allergen to which a patient is sensitised. CRD assists in identifying patients who have a higher risk of anaphylaxis, because they have IgE against major "anaphylactogenic" allergens versus patients with IgE directed against allergens that generally do not elicit anaphylaxis.

  In the case of plant foods, CRD testing may help to clarify which food-allergic patients are likely to experience "oral allergy syndrome” (OAS), and which are at risk for more serious systemic reactions to foods. Such discrimination is not possible using only allergen skin prick testing or conventional allergen-specific IgE assays. For example, in peanut allergy, patients sensitised to pollen-related components, such as the peanut allergen *Ara h 8* (which is related to some birch pollen allergens), usually experience no or very mild oral symptoms. In comparison, those who are sensitised to more stable components, such as seed storage proteins (e.g. *Ara h 2*), are more likely to experience systemic reactions to peanut in any form. CRD for foods in addition to peanut include tree nuts, wheat, vegetables, fruits, milk, and hen's egg. **Table 3** outlines some of the most clinically important component tests in allergy.

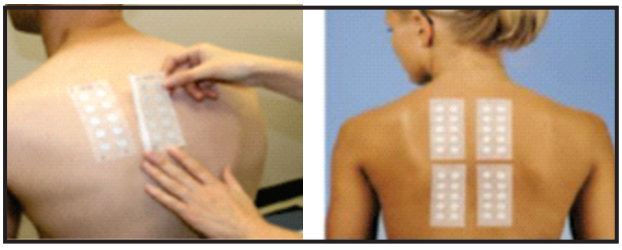
TABLE 3. Clinically Important Component Resolved Diagnostic Tests

Food s-IgE	Component Resolved Diagnostic (CRD) Tests			
Egg 	**Gal d 1, Ovomucoid** Heat stable, high allergenicity. Risk for reaction to cooked and raw egg. High s-IgE indicates persistent egg allergy.	**Gal d 2, Ovalbumin** Heat labile. Reactions to raw or slightly cooked eggs. May tolerate cooked egg.	**Gal d 3, Conalbumin** Heat labile. Reactions to raw or slightly cooked eggs. May tolerate cooked egg.	**Gal d 4, Lysozyme** Heat labile. Reactions to raw or slightly cooked eggs. May tolerate cooked egg.
Milk 	**Bos d 8, Casein** Heat stable. Risk for reactions to raw and cooked milk. Can cross-react with other mammal proteins.	**Bos d 4, α-lactalbumin** Whey protein, heat labile. Risk for reactions to raw milk. May tolerate cooked milk.	**Bos d 5, β-lactoglobulin** Whey protein, heat labile. Risk for reactions to raw milk. May tolerate cooked milk.	**Bos d Lactoferrin and Albumin (BSA) Bos d 6** Risk for reactions to raw milk. May tolerate cooked milk. Bos d 6 is the main allergen in beef and may cross-react with other mammal proteins.
Peanut 	**Ara h 1, h 2, h 3** Severe systemic reactions. Stable to heat and digestion. Can cross-react with proteins in other seeds.	**Ara h 8 (PR-10)** Local reactions. Labile to heat and digestion. Can cross-react with plant pollens.	**Ara h 9, lipid-transfer protein (LTP)** Both systemic and local reactions. Oral allergy syndrome (OAS). Stable to heat and digestion.	**Ara h 5 (Profilin)** Marker of grass pollen cross-reactivity. Heat labile. Oral allergy syndrome (OAS).
Wheat 	**Gliadin (α, β, γ, ω)** Risk marker of systemic reactions and persistent wheat allergy.	**Tri a 19, ω-5 Gliadin** Risk marker of systemic reactions and persistent wheat allergy. Marker of wheat-dependent exercise-induced anaphylaxis (WDEIA).	**Tri a 14, lipid-transfer protein (LTP)** Local and systemic reactions. Stable to heat and digestion. Marker of wheat-dependent exercise-induced anaphylaxis (WDEIA).	
Soya 	**Gly m 5, Gly m 6** Storage proteins. Stable to heat and digestion. Severe systemic reactions.	**Gly m 4 (PR10)** Systemic and local reactions. Can cross-react with plant pollen.		
Peach 	**Pru p 3, lipid-transfer protein (LTP)** Cross-reactivity to homologous proteins in Rosaceae fruits (incl. apple, pear, cherry, plum). Exposure can be reduced by peeling the fruit.			
Fish 	**Cip c 1, Gad c 1 (Parvalbumin)** Heat stable. Indicates general fish sensitisation. Large degree of cross-reactivity between fish species.			
Shrimp 	**Pen m 1, Ani s 3, Der p 10, Bla g 7 (Tropomyosin)** Heat stable muscle protein of shrimp, lobster, crayfish, crab, house dust mites, Anisakis simplex (herring worm).			
Red Meat 	**Alpha-Gal** This is a carbohydrate present in red meats (incl. beef, pork, lamb, game and gelatin). Co-factors (tick bites, exercise, alcohol consumption) precipitate allergic reactions in people previously able to tolerate meat.			

- **Allergy Patch Tests (ATP's)**
  APT's are the standard tests for diagnosing allergic contact dermatitis (ACD), and are indicated in any patient with a chronic, pruritic, eczematous, or lichenified dermatitis if underlying or secondary ACD is suspected.

  These tests can be used in patients with atopic dermatitis (AD), because these Individuals are at increased risk of contact sensitisation compared with non-atopic individuals due to their impaired skin barrier. Individuals being prepared for medical or dental implants, joint prostheses or intra-uterine contraceptive devices may also benefit from APT's. Ideally, they should be performed before the surgical intervention. APT's may also be useful if a patient is having an unexplained reaction suggesting an allergic reaction to an implant that has already been done. Guidelines from multiple orthopaedic, dental and allergy societies recommend APT's, rather than the functional MELISA assay, for the assessment of sensitisation to metal and prosthetic implants, due to the standardisation of the APT's compared to the MELISA test.

Figure 2. Typical application of Allergy Path Tests on a patient's back



How APT's are performed and the panels of allergens available are outlined in **Figure 2** and **Table 4,** respectively.

Table 4. Allergy Patch Test Series currently available

Metal/Implant APT	Includes most prosthetic metals, bone cements and glues used in orthopaedic, dental and IUD devices. Iron, Potassium dichromate, Manganese, Platinum, Dimethyl toluidine, Gentamicin bone cement, Cadmium, Titanium, Cobalt, Molybdenum, Polysilicone, Tungsten, Niobium, Tantalum, Gold, Zirconium, Zinc, Nickel.
Standard Series	Extensive series includes some metals (nickel, cobalt), local anaesthetics, fragrances, cosmetics, hair dyes, resins and leather preservatives.
Cosmetic Series	Contains allergens associated with cosmetics applied to the face and body.
Acrylate Nails Series	For artificial nails.

- **Mast Cell Tryptase (MCT)**
  Granules in activated mast cells secrete the enzyme tryptase during anaphylactic reactions. This test is a definitive marker of anaphylaxis. MCT levels peak at 45 - 60 minutes and may remain elevated for several hours (up to 24 hours). Three serial measurements are recommended: the first as soon as possible after the reaction, the second a few hours later, and a final sample after 24 - 48 hours to establish a baseline tryptase level. MCT is also indicated in patients with the complex clinical condition of mastocytosis, reflecting pathological levels in mast cell burden.

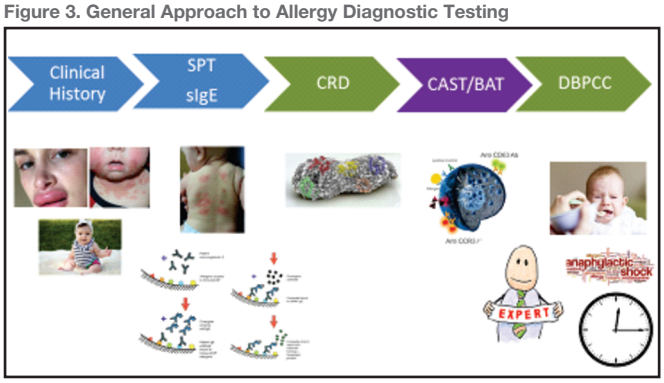
- **Specific IgG4**
  This test measures IgG4 to specific allergens. IgG4 antibodies are biomarkers of antigen exposure and immune tolerance. The ratio of allergen-specific IgG/IgG4 can be used to measure response in patients receiving allergen immunotherapy (AIT). Increased levels of IgG4 antibodies are typically seen in patients receiving AIT for aero-allergens and bee venoms. They are not used in the diagnosis of allergic disease.

- **Eosinophil cationic protein (ECP)**
  Eosinophils are responsible for producing inflammation in asthmatics. ECP is a cytotoxic protein found in eosinophilic granules and increased levels of this protein indicate activation of eosinophils, which leads to epithelial damage and desquamation, which in turn leads to increased bronchial hypersensitivity and chronic inflammation.

- **Immuno solid-phase allergen chip (ISAC) test**
  This is a microarray assay system designed to detect s-IgE against multiple allergens, including recombinant allergen proteins for component resolved diagnostics, at the same time. There are more than 100 targets in this multiplex assay and it requires specialised equipment to be read. It uses a small volume of serum, but even so the serum is in excess of the allergens and thus high affinity IgE antibodies are the ones which predominantly bind. This is in contrast to normal s-IgE tests, which detects binding of both high and low affinity antibodies. The current evidence base has demonstrated that the ISAC test lacks sensitivity and it is extremely costly. If used at all, it should be reserved for complex cases for which all other tests have not been able to assist in the diagnosis.

- **Allergy IgG**
  These tests are mentioned here for completion and information. These tests are being marketed directly to the public and health professionals, claiming to be more effective than traditional skin prick tests or serum specific IgE tests. They are not offered by private or academic laboratories in South Africa. The manufacturers and suppliers of IgG tests (sometimes referred to as "ALCAT") claim that the tests have diagnostic value in identifying substances responsible for allergic and intolerance reactions. Consensus statements from allergy associations throughout the world, including South Africa, after thorough evaluation, unanimously do NOT recommend these tests to be used in the assessment of allergy and food intolerance.

Figure 3. General Approach to Allergy Diagnostic Testing



IN CONCLUSION

Because of the range of tests available for allergy and what they measure exactly, approaching a patient with symptoms suggestive of allergy may be confusing. The general approach is outlined in **Figure 3.** Invariably the starting point is a good clinical history, followed by a specific IgE test (SPT or laboratory-based immunoassay) and only then resorting to more specialised tests like CAST/BAT testing and APT's, if the symptoms and signs direct to these specialised tests. However, CAST/BAT testing is usually the first line allergy test in suspected drug allergy (including non-steroidal anti-inflammatory drugs, anaesthetics and antibiotics), and APT's the first line in allergic contact dermatitis or dental/orthopaedic implants.

SPT = skin prick test; sIgE = specific IgE; CRD = component resolved diagnostics; CAST/BAT = cellular antigen stimulation test/basophil activation test; DBPCC = double-blind placebo-controlled challenge.

SUMMARY – Take away 10 Points

1. **There has been an increase in the prevalence of allergic conditions globally.**
2. **Allergic sensitisation occurs either as Type I (IgE mediated or non-IgE mediated) OR Type IV hypersensitivity reactions.**
3. **Clinical history must direct allergy testing.**
4. **Laboratory methods measure sensitisation, the relevance of which has to be determined clinically before restricting patients or treating them for allergy.**
5. **Component resolved diagnostics are useful to determine prognosis and severity of certain allergies.**
6. **CAST/BAT testing is indicated in complicated allergy cases, and as first line tests for drug allergies.**
7. **Allergy patch tests should be used for allergic contact dermatitis or sensitivity to orthopaedic and dental implants.**
8. **Mast cell tryptase is a test for anaphylaxis or mastocytosis syndromes.**
9. **Specific IgG4 is used to determine tolerance in patients on allergen immunotherapy.**
10. **IgG testing (also known as “ALCAT”) is not supported by allergy societies globally, and patients should be discouraged from using this expensive test which has no clinical relevance.**

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