THE XPLORER

EDITION #7

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MULTIPLEX TESTING IN MODERN CLINICAL PRACTICE

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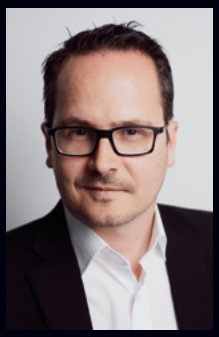


DEAR READERS,

Welcome to a new edition of THE XPLORER – our lucky number seven. Following last year's special issue dedicated to the latest version of our allergy test, ALEX³, we felt it was time to address the many misconceptions surrounding multiplex testing. IgE multiplex testing is often regarded as a "last resort" when other diagnostic methods fall short – but should it be? At MADx, we firmly believe otherwise. In fact, challenging this perception is part of our daily work. In the first part of this edition, we explore the most common myths about IgE multiplexing, from concerns about "data overload" to questions of cost, and demonstrate how ALEX³ supports allergists in moving beyond incomplete diagnoses towards precise, patient-centred allergy management.

In addition, we spoke with two outstanding experts from around the world to gain valuable insights into clinical and laboratory practices using molecular multiplex testing.

We hope you enjoy this edition of THE XPLORER!

Christian Harwanegg
CEO of Macro Array Diagnostics

Common Myths about IgE Multiplexing

by Dr Christian Lupinek and
Mag. Peter Forstenlechner

When bringing the ALEX³ test to allergologists across the globe – currently in more than 90 countries – our team of product managers and specialists commonly encounters similar concerns raised about IgE multiplexing in general and about the ALEX test in particular.

COMPLEXITY OF THE REPORT

The most common concern is the notion that the test report is too complex. Admittedly, a multiplex IgE test yielding 300 data points per patient is a complex diagnostic tool. However, in the hands of a well-trained specialist, the high granularity of the ALEX³ test helps to increase diagnostic accuracy and to speed up the workflow, as shown by many case reports. By analogy, which radiologist would reject MRI technology because of its complexity? In no field of medicine is knowing more about your patient perceived as a disadvantage. However, it is certainly vital to know what to do with that information.

CLAIMS OF REDUNDANCY

Another common objection is that the ALEX³ allergen panel is largely redundant. Again, one specific strength of the ALEX³ test is the broad coverage of specific marker allergens from different and complex allergen sources, and of allergen families with a low degree of cross-reactivity (e.g., nsLTPs, storage proteins). This is one cornerstone of the high sensitivity of ALEX³. For example, omitting Der p 23 as one of the three specific house dust mite markers (Der p 1, 2 and 23) might lead to false-negative results in around 10% of mite-sensitised patients [1].

INTERPRETING COMPLEX CASES

In support of the third frequently voiced concern – that interpreting the report takes too much time – allergologists frequently point to patients with very broad and complex sensitisation profiles. Such complex cases, of course, require more time for interpretation of the lab report. However, this is largely independent of the test system used. One main advantage of a multiplex setup compared to singleplex systems is that only one round of IgE testing is needed, which speeds up the diagnostic work-up. Of note, the fact that ALEX³ contains 300 allergens and extracts does not mean that most patients test positive for many of them. Most test results show well-defined reactivity patterns to only a few allergen families.

POTENTIALLY UNNECESSARY THERAPEUTIC MEASURES

The fourth reservation about IgE multiplexing is that results automatically lead to unnecessary dietary restrictions. As is true for any sIgE test, the ALEX³ test report is not equivalent to an allergy diagnosis. Without proper assessment of the clinical relevance of confirmed allergic sensitisations and proper communication of this relevance to the patient, inappropriate measures may be taken. Thus, interpretation of results from multiplexed sIgE tests must be done in the context of a detailed clinical history. Guidelines from national and international allergology societies provide clear guidance on how to handle clinically silent IgE sensitisations [2]. To further increase the diagnostic specificity of the ALEX system,

CCD-specific IgE antibodies are blocked by default – a unique feature of the ALEX test. Thus, such clinically irrelevant reactivities are eliminated, in contrast to widely used singleplex systems that cannot avoid the CCD trap (i.e., producing clinically irrelevant results) due to their allergen preparation and/or solid phase (e.g., cellulose) [3].

ECONOMIC CONCERNS

The fifth argument against multiplexed IgE tests is, of course, their cost. Obviously, a test measuring 300 parameters instead of one is more costly. However, considering the whole cascade from the patient's first visit to completion of treatment, the overall cost equation changes. Several studies [4] have shown that the increased specificity and improved differential diagnostics of molecular allergology help avoid unnecessary or inappropriate allergen immunotherapy (AIT) prescriptions. Thus, it is misleading to discuss higher costs of diagnostic tools yielding high-resolution results in isolation from the whole diagnostic and therapeutic procedure.

CONCERNS ABOUT SENSITIVITY

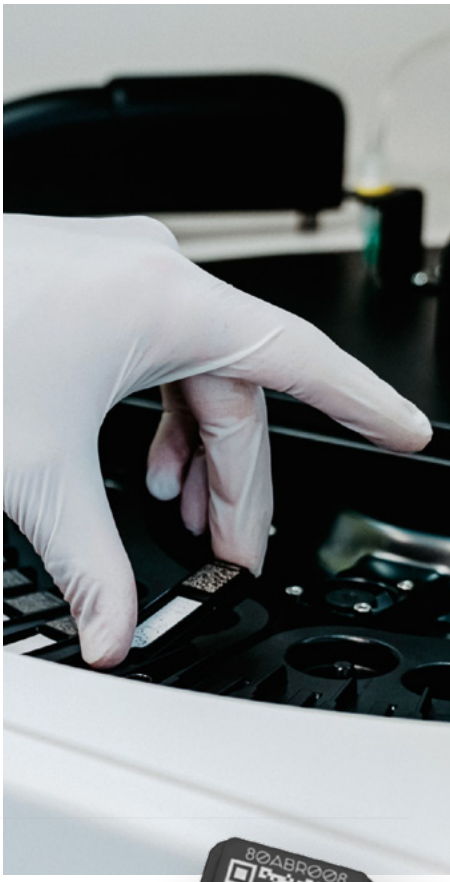
Another objection is that ALEX is less sensitive than singleplex test systems. Clinical studies have demonstrated that the sensitivity ALEX provides for allergens such as house dust mite is comparable to that of singleplex testing [5]. A similar situation has been described for many other important respiratory allergens [6]. Both studies showed that a potentially lower analytical sensitivity is clinically negligible for most patients. Multiplexing is intended to track the breadth and evolution of the 'IgEome'. For example, in paediatric populations, tracking 'epitope spreading' (the expansion of IgE responses to more epitopes of the same allergen source) is a powerful predictor of persistent versus transient allergy [7].

QUESTIONING THE sIgE/tIgE RATIO

Lastly, some believe multiplex results are not 'quantitative' enough to allow the specific IgE activity to be calculated (i.e., the sIgE/tIgE ratio). However, ALEX³ quantifies tIgE between 2 and 1000 kU/L, alongside sIgE to a broad range of clinically relevant molecular allergens, facilitating the calculation of the specific IgE activity in one step [8]. This can be especially useful in 'low IgE producers', where the ratio of specific IgE to total IgE can help to better assess the clinical relevance of very low levels of sIgE [9].

CONCLUSION

These aspects clearly demonstrate that ALEX³ is an effective means of assessing the broad spectrum of a patient's individual IgE sensitisations. By integrating quantitative measurements and CCD inhibitors to block clinically irrelevant results, ALEX³ provides a level of diagnostic granularity that singleplex methods cannot match. ALEX³ allows specialists to move beyond general diagnoses towards precise, patient-oriented allergy management that transforms the diagnostic work-up of allergic disease.



Multiplex IgE Testing in the Allergy Diagnostic Algorithm

by Constantine Konstantoulas, PhD

INTRODUCTION

Allergic diseases, including food allergy, respiratory allergy, insect venom allergy, and drug hypersensitivity, represent a major and growing global health burden [10] [11] [12]. Accurate diagnosis is fundamental to effective management, guiding allergen avoidance, immunotherapy selection, risk stratification, and patient education [13] [14] [15] [16] [17] [18]. Laboratory diagnostics complement clinical assessment by identifying IgE-mediated sensitisation, yet the optimal placement of these tools within the diagnostic pathway remains under discussion [19] [20].

Conventional diagnostic algorithms typically prioritise targeted testing strategies based on suspected allergens derived from clinical history [2] [13] [14] [15] [16] [17] [18] [21].

However, real-world allergy presentations are frequently complex, involving polysensitisation, cross-reactivity, and incomplete or ambiguous exposure histories [20] [22] [23] [24].

Multiplex IgE testing offers a comprehensive assessment of sensitisation patterns and has the potential to address several limitations inherent in traditional approaches [1] [20] [25] [26] [27]. This review examines the integration of multiplex IgE testing into the allergy diagnostic cas-

cade and evaluates the evidence supporting its use as a first-line diagnostic tool.

THE CLASSICAL ALLERGY DIAGNOSTIC CASCADE

The traditional allergy diagnostic pathway begins with a detailed clinical history and physical examination, followed by first-line testing using skin prick testing (SPT) and/or singleplex serum-specific IgE assays [2] [13] [14] [15] [16] [17] [18] [21]. These tests are typically directed toward a limited number of suspected allergens. When results are inconclusive, discordant with clinical symptoms, or insufficient to guide management, second-line investigations may include expanded IgE testing, molecular allergy diagnostics, oral food challenges, sting challenges, or functional assays such as basophil activation tests [18] [20] [28] [29]. With this stepwise approach, diagnostic accuracy depends heavily on the clinician's ability to pre-select relevant allergens, and sequential testing can increase time to diagnosis, healthcare utilisation, and patient burden [19] [23] [30].

PRINCIPLES OF MULTIPLEX IgE TESTING

Multiplex IgE assays are in vitro tests capable of simultaneously measuring IgE antibodies to multiple allergen extracts and molecular allergens using a single serum sample, often requiring minimal blood volume [1] [20] [25] [26] [27]. Many contemporary multiplex systems incorporate molecular allergy diagnostics, enabling discrimination between genuine sensitisation to primary allergens and sensitisation due to cross-reactive proteins or carbohydrate determinants. The ability to generate a comprehensive sensitisation profile in a single analytical step represents a paradigm shift from sequential, hypothesis-driven testing to a more global and data-rich diagnostic strategy [27] [31] [32].

CURRENT CLINICAL APPLICATIONS

Multiplex IgE testing is increasingly used as a second-line or adjunctive tool in patients with unclear diagnoses, atypical clinical histories, or extensive sensitisation patterns. It is also employed prior to allergen immunotherapy to refine allergen selection and avoid unnecessary or ineffective treatment. In food and venom allergy, multiplex testing contributes to risk stratification by identifying sensitisation to molecular allergens associated with severe or systemic reactions [27] [33] [34].

Despite these applications, restricting multiplex testing to later stages of the diagnostic cascade may limit its clinical value and perpetuate inefficiencies inherent in traditional stepwise approaches.

RATIONALE FOR FIRST-LINE USE OF MULTIPLEX IgE TESTING

REDUCTION OF PRE-TEST BIAS

Targeted first-line testing assumes accurate identification of culprit allergens based on clinical history alone. In practice, this assumption is frequently invalid. Multiplex IgE testing minimises pre-test bias by providing an unbiased overview of sensitisation patterns, particularly valuable in patients with non-specific symptoms, multiple suspected triggers, or limited exposure histories [20] [30] [35].

EARLY IDENTIFICATION OF POLYSENSITISATION AND CROSS-REACTIVITY

Polysensitisation is common among atopic individuals and is associated with increased disease severity and complexity. Multiplex testing enables early detection of multiple sensitisations and cross-reactive profiles, facilitating more accurate interpretation of positive IgE results and reducing the risk of overdiagnosis based on isolated findings [20] [30] [35].

DIAGNOSTIC EFFICIENCY AND STREAMLINED CARE PATHWAYS

By consolidating multiple potential tests into a single investigation, multiplex IgE testing can shorten diagnostic pathways and reduce the need for repeated clinic visits and sequential laboratory requests. This approach has the potential to improve patient experience and optimise resource utilisation within healthcare systems [20] [33] [35] [36].

EARLY ACCESS TO MOLECULAR INFORMATION

Multiplex platforms incorporating molecular allergens provide clinically actionable data early in the diagnostic process. In food allergy, identification of stable storage proteins versus labile cross-re-

active components informs risk assessment and challenge decisions. In insect venom allergy, molecular-level data can distinguish primary sensitisation from cross-reactivity, directly influencing immunotherapy selection [18] [20] [35].

SUITABILITY FOR SPECIAL POPULATIONS

Multiplex IgE testing also offers advantages in populations where skin testing is contraindicated or impractical, including young children, patients with extensive eczema, individuals receiving antihistamines, and those with a history of severe anaphylaxis. The standardised nature of laboratory testing and the low sample volume requirements further support its role as an initial diagnostic investigation [20] [33] [35].

ALIGNMENT WITH PRECISION MEDICINE

Modern allergy care increasingly emphasises precision medicine, integrating detailed immunological data to guide individualised management. Multiplex IgE testing aligns with this approach by providing high-resolution sensitisation profiles early in the diagnostic pathway, enabling more tailored and evidence-based clinical decisions [20] [33] [35].

CLINICAL AND ECONOMIC CONSIDERATIONS

Although multiplex IgE testing may involve higher upfront costs compared with singleplex assays, cost-effectiveness should be assessed in terms of total diagnostic expenditure, avoidance of downstream testing, and the impact on clinical outcomes. When strategically deployed as a first-line investigation, multiplex testing may reduce cumulative costs by preventing unnecessary testing, misdiagnosis, and inappropriate treatment [33] [37].

Interpretation of multiplex results requires appropriate clinical expertise, as sensitisation does not equate to clinical allergy. Integration of results with clinical history and training of healthcare professionals are essential to maximise diagnostic accuracy and clinical utility [20] [35].

CONCLUSION

Multiplex IgE testing represents a significant advancement in allergy diagnostics, offering comprehensive and clinically informative insights into allergic sensitisation. While traditionally positioned as a second-line tool, multiplex IgE testing has a strong rationale for its use as a first-line laboratory investigation in various patient populations. Adoption of a multiplex-first strategy has the potential to reduce diagnostic uncertainty, streamline care pathways, and support precision-medicine approaches in allergy management.

Not So Simple: Cases That Defy First Impressions

by Julia Sinner, MSc

At first glance, a patient's clinical history may appear straightforward, prompting the attending physician to limit testing to a few suspected allergens. These two cases illustrate why a 'multiplex-first' strategy is a game-changer: by revealing hidden sensitisations that standard tests might miss, the ALEX test ensures a comprehensive diagnostic picture from the very first blood sample, ultimately improving patient outcomes.

CASE #1

CLINICAL HISTORY

The patient is a 39-year-old male living in the Netherlands. For years, he struggled with classic symptoms of rhinoconjunctivitis (RC) peaking during the spring and summer months. His medical history pointed towards a seasonal pollen allergy, a common enough presentation that typically suggests a straightforward diagnostic path. To confirm the triggers, a standard skin prick test (SPT) was performed. The results showed broad sensitisation:

- Trees: birch, hazel, alder
- Grasses/weeds: timothy grass, nettle, ribwort, ragweed, mugwort
- Perennials: house dust mites

Allergen	Reaction	Allergen	Reaction
Histamine	+	Alder	+
Control	-	Birch	+
House dust mites	+	Hazel	+
Timothy grass	+	Horse	-
Perennial ryegrass	-	Cat	-
Rye	-	Dog	-
Nettle	+	Rabbit	-
Ribwort	+	Alternaria alternata	-
Ragweed	+	Aspergillus fumigatus	-
Mugwort	+	Cladosporium herbarum	-

Table 1: Standard SPT results (case #1).

Based on these results, the clinical picture seemed clear.
The patient was diagnosed with a combined grass and tree pollen allergy.

THE CLINICAL PUZZLE

Following the diagnosis, the patient began subcutaneous immunotherapy (SCIT) using a tree mix (birch, hazel, alder) and a 10-grass mix.

After three years of consistent treatment, the results were frustratingly mixed. While the patient showed a significant clinical response to grass AIT, the tree pollen-related symptoms remained persistent. The 'simple' case had become complicated: Why was the tree pollen immunotherapy failing despite a positive SPT for those allergens?

A re-evaluation of the Dutch botanical landscape revealed a critical oversight: ash. Despite its high prevalence and clinical importance in the Netherlands, ash had not been included in the initial SPT.
The medical team was faced with a dilemma: Should they order a long, expensive list of individual sIgE tests to check for birch, ash, plane tree, Alternaria alternata, and various weeds?

To resolve the ambiguity without ordering dozens of singleplex tests, an ALEX multiplex test was performed. This provided a comprehensive molecular map of the patient's IgE profile in one step.

EVALUATION & RESULTS

The multiplex test confirmed the known sensitisations and added the 'missing piece' of the puzzle. The patient showed strong positivity for:

- Betulaceae family: birch, hazel, beech
- Oleaceae family: ash and olive

Allergen source	IgE level [kU_A/L]
Birch	2.05
Hazel	0.99
Beech	0.41
Ash	1.10
Olive	1.76

Table 2: Multiplex allergy test results (case #1).

The ALEX test revealed that the 'simple' diagnosis of birch allergy was only half the story. The patient's poor response to tree mix SCIT was likely due to a significant, undiagnosed sensitisation to ash. In much of northern Europe, ash is a major contributor to spring symptoms, yet because it belongs to the Oleaceae family, it does not cross-react with the Betulaceae mix the patient was receiving. The molecular data showed that the patient was not simply 'allergic to trees' – he was allergic to two distinct botanical families, but his immunotherapy was only covering one of them. By pinpointing the specific involvement of ash, the clinical team finally explained his 'poor responder' status and could adjust the therapeutic strategy accordingly.

SUMMARY

- Standard test panels may miss regionally significant allergens (like ash in the Netherlands). If the panel is incomplete, the diagnosis is incomplete.
- Overlapping pollination seasons can mask the presence of multiple, distinct sensitisations.
- SCIT success depends on targeting the primary sensitiser. In this case, the patient was being treated for allergy to birch and alder, but his ash allergy was left untreated.
- Multiplex testing allowed for a 'wide-angle' view of the patient's IgE profile, identifying the Oleaceae sensitisation that the initial SPT missed.
- When a patient does not respond to immunotherapy as expected, look beyond the standard panel. Molecular diagnostics can reveal the hidden triggers that skin prick tests often miss.

CASE #2

CLINICAL HISTORY

The patient is an 18-month-old male with no prior history of allergic reactions or chronic health issues. Until recently, he had been thriving without any dietary restrictions or regular medication. His family history was also unremarkable, with no reported atopic diseases among his immediate relatives.

THE CLINICAL CHALLENGE

The 'simple' case began when the toddler experienced two episodes of severe systemic reactions within a short period. Both incidents occurred after consuming a multi-ingredient sandwich containing tuna, ham, egg, lettuce, and butter. The symptoms were alarming: angioedema (acute facial swelling) and dyspnoea (shortness of breath).

At first glance, the diagnosis seemed straightforward: a clear-cut food allergy. Given the alarming nature of the reactions, the primary focus naturally fell on the common high-risk allergens such as wheat, fish, egg, milk, and meat. Yet, this initial assumption was quickly challenged when singleplex sIgE testing failed to identify a single trigger.

The clinical team found themselves at a diagnostic standstill; the symptoms were undeniable, but the true culprit remained elusive, hidden behind a mask of negative test results.

The family was left in a difficult position: a child at risk of experiencing life-threatening reactions, but no identifiable cause. The resulting fear that any meal could be a risk created an urgent need for answers. To avoid a tedious trial-and-error approach or putting the child on an unnecessarily restrictive diet, a broader diagnostic approach was required. An ALEX multiplex IgE test was performed to screen for a wide array of potential triggers simultaneously.

EVALUATION & RESULTS

The multiplex results revealed a specific sensitisation pattern that the initial tests had missed:

- Pumpkin seed: 2.47 kU_A/L
- Sesame: 1.69 kU_A/L
- Poppy seed: 0.83 kU_A/L

Allergen source	IgE level [kU_A/L]
Pumpkin seed	2.47
Poppy seed	0.83
Sesame	1.69
Total IgE	<20

Table 3: Multiplex allergy test results (case #2).

While the reactions were triggered by a sandwich, the culprits were not the main fillings like ham or tuna, but rather the seeds often found in artisanal or multi-grain breads.

The presence of IgE against 2S albumin storage proteins from poppy seed and sesame was particularly significant. These proteins are known to be heat-stable and resistant to digestion, often leading to severe systemic reactions even in small quantities.

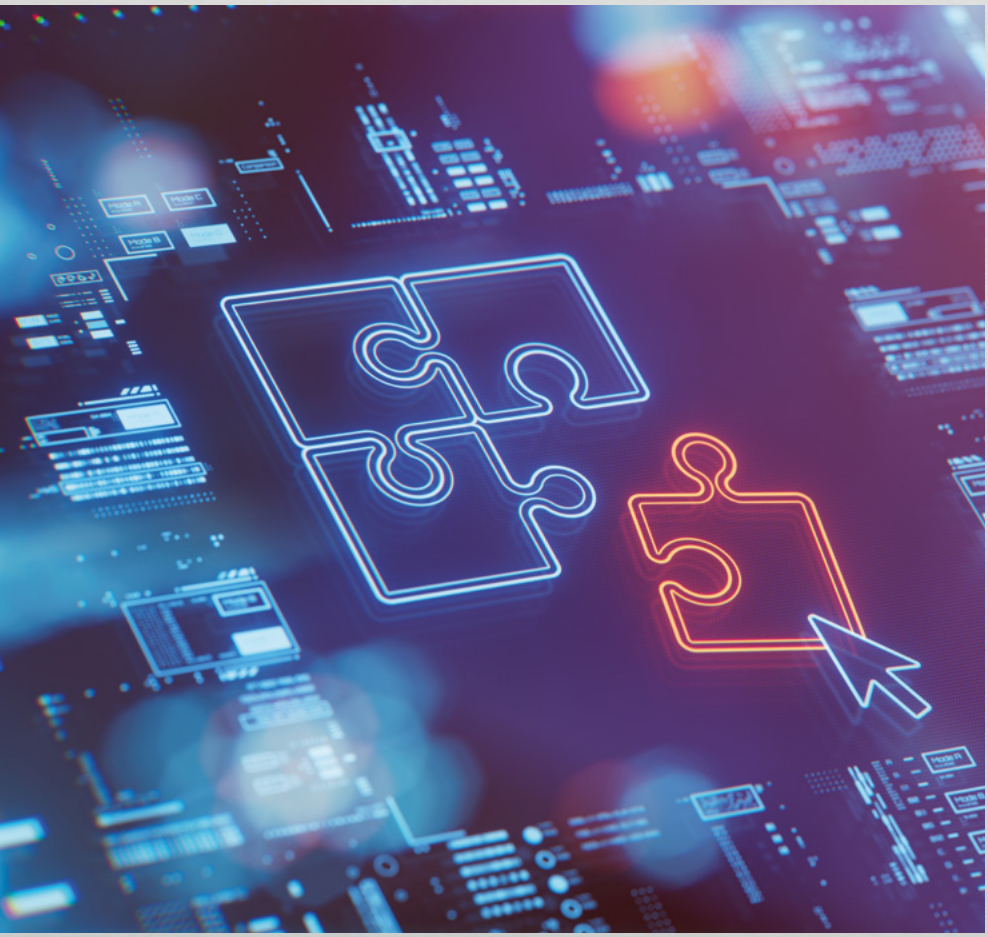
SUMMARY

- The case demonstrates that in paediatric anaphylaxis, the most obvious suspects are not always the real culprits.
- Traditional singleplex testing for common allergens produced negative results, leaving the patient at risk.
- Multiplex testing identified three specific triggers (sesame, poppy seed, and pumpkin seed) in a single step.
- By identifying the exact molecular triggers, the family avoided a blanket diet that would have unnecessarily excluded milk, egg, fish, wheat, or meat.
- Instead, targeted avoidance of specific seeds provided both safety and a good quality of life for the child.

Decoding the Allergy Puzzle

Navigating Complex Results with RAVEN²

by Eszter Sarzsinszky, MSc



The moment a modern allergy report lands on a desk in a busy practice, it often brings a mix of diagnostic promise and practical challenges. We have successfully transitioned from traditional, single-allergen testing to the era of molecular multiplex diagnostics. Today, a single blood sample analysed through a comprehensive panel like ALEX³ provides an incredibly high-resolution picture of a patient's immune system, testing for hundreds of allergen extracts and molecular allergens simultaneously.

However, this technological leap introduces a distinct obstacle. When you open a report and find positive hits scattered across pollens, foods, and animal dander, the immediate task is difficult: how do you separate the true clinical signal from the biological background noise during a standard 15-minute appointment?

DEALING WITH DATA OVERLOAD

To understand this data overload, we must look at the biological reality of cross-reactivity. Nature is highly efficient and frequently recycles successful structural designs. This means that certain protein families are highly conserved across different botanical or animal species. When a patient develops a primary allergy, their immune system produces IgE antibodies against a specific protein. However, those same antibodies will often bind to structurally similar proteins in completely different sources. On paper, the patient appears allergic to a list of diverse triggers. In reality, they may tolerate most of those sources perfectly well. Misinterpreting these cross-reactions as primary, dangerous allergies is a common pitfall that can force patients into anxiety-inducing and unnecessary dietary restrictions.

This is the exact gap that RAVEN² was designed to bridge. Available through the RAPTOR Server, RAVEN² acts as a digital clinical companion that is automatically included in the ALEX report upon request. It is a deterministic, rule-based interpretation tool grounded strictly in peer-reviewed molecular allergology. Instead of leaving the physician to manually cross-reference dozens of molecular allergens against textbook protein families, the software does the heavy analytical work. The physician simply inputs the patient's specific allergy symptoms and their seasonal occurrence.

RAVEN² then integrates this real-world clinical history with the raw molecular data to synthesise a clear, actionable narrative. With the latest software update, the report also includes the original RAVEN interpretation alongside RAVEN², ensuring that foundational textbook knowledge remains easily accessible.

RAVEN²: A PRACTICAL EXAMPLE

To truly understand how this transforms the diagnostic workflow, consider a highly realistic scenario from a general practitioner's perspective. You are preparing for a consultation with a 43-year-old male patient. His clinical history notes mild eye irritation and moderate nasal symptoms during both the spring and summer months. The patient suspects his summer symptoms are caused by rye pollen. Furthermore, he complains of similar perennial symptoms that he firmly believes are triggered by exposure to dogs. Notably, he reports no allergic reactions to any foods. Seeking diagnostic clarity, you order an ALEX³ multiplex test. Out of the 300 parameters tested, nine are positive.

Below is a snapshot of the identified sensitisations before any clinical interpretation is applied:

Allergen category	Relevant findings (selected molecules)
Tree pollen	Birch (Bet v 1: 5.96 kU_A/L) Alder (Aln g 1: 6.68 kU_A/L) Oak (Que a 1: 6.42 kU_A/L)
Grass pollen	Timothy grass (Phl p 1: 6.26 kU_A/L) Maize (Zea m 1: 1.39 kU_A/L)
Food (fruits and nuts)	Hazelnut (Cor a 1.0401: 12.33 kU_A/L) Apple (Mal d 1: 0.58 kU_A/L)
Food (legumes)	Soy (Gly m 4: 1.86 kU_A/L) Peanut (Ara h 8: 1.28 kU_A/L)
Animal epithelia	Dog (Can f 1, 2, 3, 4, 6: negative)

Table 4: Excerpt from ALEX³ findings (9 positive parameters).

Looking strictly at the upper rows of this raw data, one might initially assume this patient has a complex, multi-layered allergy profile requiring strict avoidance of nuts, legumes, and apples, while leaving the question of the suspected dog allergy confusingly unaddressed. Instead of manually dissecting this web of positive results, the physician processes the data through RAVEN², adding the patient's reported clinical history. The clinical narrative shifts instantly, correcting patient misconceptions and preventing unnecessary interventions.

Symptom category	RAVEN² analysis output
Seasonal (spring)	The reported mild eye and moderate nasal symptoms are explained by the observed genuine sensitisation to birch (Bet v 1) and cross-sensitisation to alder. Birch is suspected to be the primary cause of cross-sensitisation to other trees in the *Fagales* order.
Seasonal (summer)	The patient suspected rye pollen as the trigger. While no genuine sensitisation to rye was detected, the reported summer symptoms are explained by a genuine sensitisation to timothy grass (Phl p 1). This acts as the primary source of clinical cross-sensitisation to other grasses, explaining the patient's symptomatic reaction to rye.
Perennial	The patient reported symptoms presumably caused by dog exposure. However, no genuine sensitisation to dog was found.
Food	The patient reported no food-related symptoms. The software detected a broad cross-reactive PR-10 pattern (Aln g 1, Ara h 8, Bet v 1, Cor a 1.0401, Gly m 4, Mal d 1). Because the food-derived PR-10 proteins are heat-labile and the patient does not react to these foods, these specific food positivities represent clinically irrelevant laboratory phenomena driven by the primary birch allergy.

Table 5: RAVEN² automated clinical interpretation.

First, the software analyses the spring respiratory symptoms. It highlights a strong, genuine sensitisation to Bet v 1, the major allergen in birch pollen. In molecular allergology, a genuine sensitisation means the immune system was directly triggered by this specific source. RAVEN² confirms that this is the primary immunological driver of his spring misery.

Next, the software refines the clinical picture. The patient firmly believed rye pollen was ruining his summers and dogs were causing his perennial issues. The RAVEN² output clearly shows that there is no genuine IgE reactivity to either rye or dog. Instead, it pinpoints timothy grass (Phl p 1) as the immunological root cause, explaining that the patient's real-world reaction to rye is a symptomatic cross-reaction driven by this primary timothy sensitisation.

Finally, the software tackles the alarming list of positive results for foods. RAVEN² instantly recognises the underlying molecular pattern: the specific food molecules that tested positive, such as Mal d 1 in apple, Ara h 8 in peanut, Cor a 1.0401 in hazelnut, and Gly m 4 in soy, all belong to the PR-10 protein family. PR-10 proteins are structural homologues, essentially molecular look-alikes, of the major birch pollen allergen Bet v 1. The software clearly maps out that because the patient is highly sensitised to birch pollen, his antibodies are simply cross-reacting with these similarly shaped proteins in the laboratory. Because PR-10 proteins are easily degraded by cooking and digestion, and crucially, because the patient reported no symptoms, RAVEN² confirms these are harmless cross-reactions.

ACHIEVING BETTER PATIENT OUTCOMES

Armed with this RAVEN² interpretation, the physician's approach to the consultation completely changes. They can confidently sit down with the patient to deliver a clear, actionable diagnostic and treatment plan. It becomes easy to explain that the primary issues are birch and timothy grass allergies, which can be effectively targeted with a causal treatment like specific allergen immunotherapy.
Furthermore, this insight helps alleviate the patient's anxiety

about dogs by confirming he does not have a true dog allergy. Perhaps most importantly, the practitioner can point to the large list of positive food results in the report and assure him that his diet does not need to change at all. The goal of molecular allergy diagnostics is not to generate more data but to facilitate better patient outcomes. A multiplex test provides an incredibly detailed map, but without a key that map can easily lead a busy clinician astray. RAVEN² serves as that vital key. By translating complex, multi-layered immunological responses into structured, risk-assessed, and easy-to-interpret reports, RAVEN² ensures that any physician can focus entirely on what matters most: providing clear, confident, and targeted care to the patient sitting across from them.

GENUINE SENSITISATION VS CLINICAL CROSS-REACTIVITY

- **Genuine sensitisation:** In molecular allergology, 'genuine' refers to the original immunological source of sensitisation – the allergen that first triggered the patient's immune system to produce IgE antibodies. The term does not imply that other reactions are less important; it simply identifies where the allergy began.
- **Cross-reactivity:** A cross-reaction occurs when existing IgE antibodies bind to a structurally similar protein from another source. This is not a harmless laboratory artefact – it can lead to genuine clinical reactions in patients.
- **Tropomyosin example:** A patient may have a genuine sensitisation to house dust mite tropomyosin (Der p 10). Because shrimp tropomyosin (Pen m 1) is structurally very similar, the same antibodies can cross-react. These proteins are heat-stable, so eating shrimp can trigger severe reactions such as anaphylaxis.
- **Clinical implications:** The genuine sensitisation indicates where the allergy originated and helps guide causal treatments such as immunotherapy, while symptomatic cross-reactions identify the allergens a patient must avoid in daily life to stay safe.

Is There Competition between Extracts and Allergens on the Same Chip?

by Dr Christian Lupinek
and Dr Irene Mittermann

For some allergen sources, the ALEX^3 test contains both the extract and one or more molecular allergens. Examples include pecan (Car i extract, Car i 1, Car i 2, and Car i 4), lamb's quarters (Che a extract and Che a 1) and ragweed (Amb a extract, Amb a 1 and Amb a 4). This sometimes raises the question of whether the presence of two or more spots that attract the same specificity of IgE antibodies can lead to a lower signal level of those spots, or whether one or more spots can become negative because of this competition.

One frequently expressed concern is that the extract would 'hijack' IgE antibodies at the expense of the molecular allergens from the same allergen source (Figure 1A). However, if such competition were to occur at all, it would be the other way round, since the 'pure' allergen spot contains substantially more copies of that allergen than the spotted extract, which is a mixture of various allergens and non-allergen components. Another scenario that could theoretically lead to competition between different spots on the same array is the presence of several members of the same allergen family that exhibit a high degree of cross-reactivity because of shared epitopes. Examples of this phenomenon are profilins, polcalcins, tropomyosins, and PR-10 allergens.

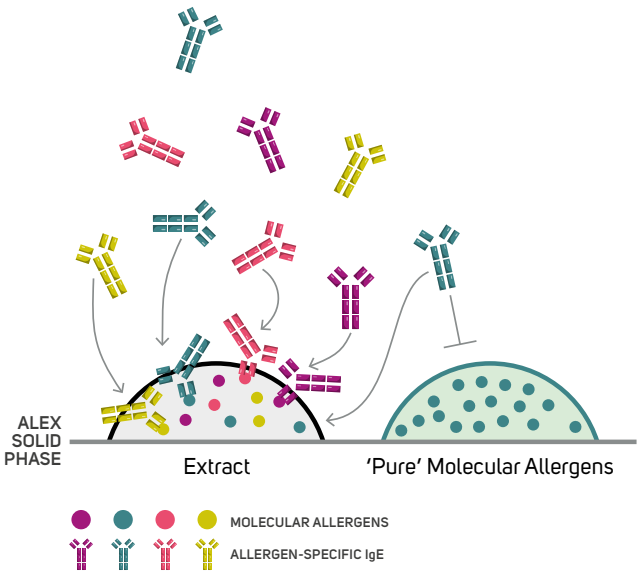


Figure 1A

Around 40 years ago, when microarrays first emerged, the biophysics of this unique assay type was meticulously investigated. One common feature of these tests is that only small quantities of the specific test reagent (e.g., allergens) that specifically combine with the respective analyte (e.g., IgE) are immobilised on the solid phase. Consequently, only a small fraction of the analyte in the biological specimen is bound,

whereas the vast majority remains in the liquid phase (Figure 1B). Since the immobilised binding partner is concentrated on a small spot, creating a high local signal density, such tests can achieve high sensitivity [38] [39].

These theoretical considerations can be directly translated to the ALEX test: assuming that a) one spot accommodates around 1 ng of allergen and b) the average affinity (KD) of the IgE antibodies in the specimen is in the range of 1 nM, then the reduction in the concentration of free IgE in the sample would be of the order of a few percent [40]. However, these theoretical values depend not only on the average affinity of IgE but also on the molecular weight of the immobilised allergen.

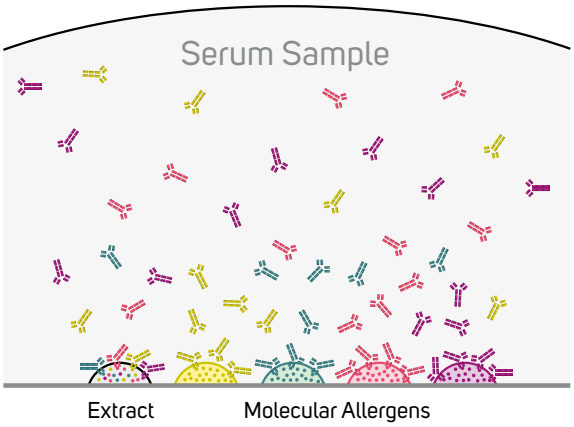


Figure 1B

Therefore, to provide experimental evidence that there is no relevant interference between different spots attracting the same IgE specificity on the ALEX^3 test, the following experiments were conducted (Figure 2): two special research arrays based on ALEX technology were produced, containing a) either only an extract of a defined allergen source or the extract alongside molecular allergens of the same allergen source (Pecan extract vs molecular allergens Car i 1, 2 and 4; Figure 2A), b) either one or 10 replicates of the same allergen (Bet v 1; Figure 2B), or c) one or six members of the same allergen family with a high level of cross-reactivity (profilin; Figure 2C). Different sera with reactivity to the respective allergens or extracts and sIgE levels in the range of 0.4 to 38 kU_A/L were tested on these experimental arrays. For all tested sera, average changes in sIgE when competing spots were contained on the same array were between –15% and –5%, i.e., clearly within the acceptable 20% range of interassay variation for microarrays. In no serum did a signal close to the cut-off (0.3 kU_A/L) turn negative.

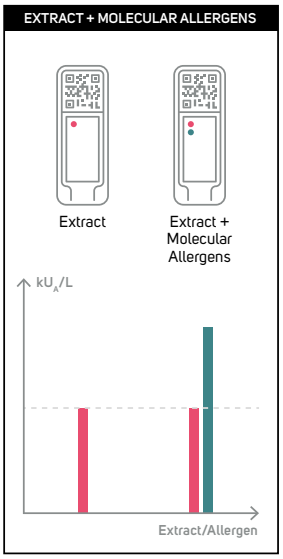


Figure 2A

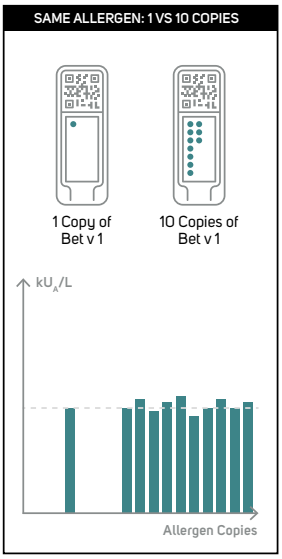


Figure 2B

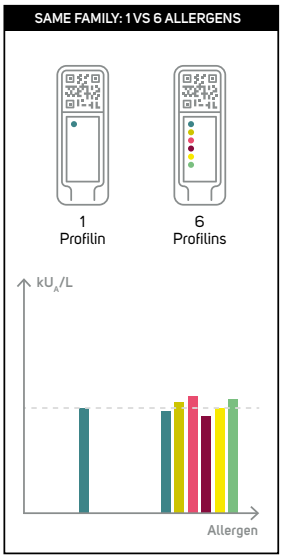


Figure 2C

Thus, due to the design of the ALEX^3 test and as shown by experimental evidence, competition between different spots containing cross-reactive molecular allergens neither produces false-negative results nor substantially reduces the specific IgE signal intensity.

Is ALEX Too European?

Expanding Allergen Representation with ALEX^3

by Dr Raffaela Campana

As allergy diagnostics have expanded to include multiplex testing platforms, clinicians increasingly expect tools that reflect the broad range of allergens patients encounter in everyday life. The ALEX IgE multiplex test has gained a strong reputation for its comprehensive coverage, enabling the simultaneous assessment of specific IgE sensitisation to numerous molecular allergens and allergen extracts, while also providing integrated total IgE measurement from a single patient sample. By combining extensive allergen coverage with practical usability, ALEX offers clinicians a detailed and clinically meaningful overview of a patient's sensitisation profile.

At the same time, a recurring perception in some clinical discussions is that multiplex allergy diagnostics such as ALEX may be 'too European', reflecting allergen exposures common in temperate climates while underrepresenting allergens encountered in other parts of the world. This perception highlights a broader challenge in allergy diagnostics, namely how to represent the diversity of global allergen exposures within a single predefined test panel. Data from regions such as South Africa illustrate how sensitisation patterns may differ from European datasets, demonstrating the limitations any fixed panel may face across different geographic settings [41]. With the introduction of ALEX^3, however, this perception deserves reconsideration.

WHERE DOES THE PERCEPTION OF EUROCENTRISM COME FROM?

Molecular allergy diagnostics were initially shaped by European allergen research, and early multiplex tests, including ALEX, focused on allergens extensively studied in Europe [37]. As a result, prominent allergen families such as PR-10 proteins, profilins, and seed storage proteins from well-characterised European sources became central components of these pioneering molecular diagnostics [35] [37]. For clinicians in Europe and similar climates, this represented a major advance in diagnostic precision.

However, patterns of allergic sensitisation are influenced by geographic region, genetic background, and environmental exposures. When patients have convincing clinical histories but limited IgE findings, even on a broad multiplex test, this can give the impression that certain 'local' allergens are missing. This gap has sometimes been interpreted as Eurocentric bias, rather than as a limitation of any predefined diagnostic panel.

Importantly, this is not a limitation specific to ALEX. No multiplex assay can capture the full complexity of global allergen exposures. The challenge lies in balancing broad allergen coverage with practical clinical interpretation across different populations.

REDESIGNING AND EXPANDING ALEX FOR GLOBAL ALLERGY DIAGNOSTICS

With ALEX³, multiplex allergy diagnostics progress from historically shaped panels towards a data-driven approach informed by real-world results. The analysis of nearly 400,000 ALEX² test results from over 90 countries [1] allowed molecular allergy experts at MADx to identify which allergens were most informative, which were redundant, and where additional coverage was needed. The redesigned panel adds new molecular allergens, including underrecognised protein families (e.g., glutenins, haemocyanins) and clinically requested components such as α-gal, a carbohydrate linked to tick bites that can trigger delayed allergic reactions to red meat, as well as an expanded range of storage proteins. ALEX³ now contains 23 nut allergens (+4 extracts), which can cause life- threatening reactions, and 17 legume allergens (+2 extracts), enhancing detection of complex and cross-reactive sensitisation patterns across diverse allergen sources [42].

These new molecular allergens include animal- and plant-derived foods (including fruits such as banana) as well as respiratory allergens and venoms, reflecting non-European dietary habits, environmental exposures, and animal contact. For instance, seafood allergens relevant to coastal and Asian populations, expanded animal allergen coverage from household and occupational exposures, and plant allergens beyond traditionally studied European pollen sources have been incorporated.

Overall, the redesign and expansion of ALEX³ illustrate how a multiplex allergy test can move from a Eurocentric focus to a globally relevant diagnostic tool, providing clinicians with a comprehensive, clinically meaningful view of patients' sensitisation profiles worldwide.

IMPLICATIONS FOR CLINICAL PRACTICE

For clinicians, the practical value of ALEX³ lies in its ability to bridge epidemiological insight and individual patient history. A more globally representative allergen panel increases the likelihood of identifying clinically relevant sensitisations, particularly in patients with complex exposure histories across different regions. The expanded use of molecular allergens supports more precise interpretation of sensitisation patterns, helping guide decisions around avoidance strategies, risk assessment, and allergen immunotherapy selection. Integrated total IgE measurement, now reported quantitatively between 2 and 1000 kU_A/L and semi-quantitatively at higher levels, together with improved assay standardisation, also contributes to consistent performance of the test.

Nevertheless, even the most comprehensive multiplex test remains a diagnostic tool, not a substitute for clinical judgement. A positive result indicates IgE sensitisation, but the diagnosis of allergy must always be interpreted in the context of clinical symptoms and patient history. Likewise, negative results do not exclude allergy, and unexplained symptoms should still prompt consideration of regionally relevant or emerging allergens that may not yet be represented. Ongoing panel updates and real-world validation remain essential to maintaining clinical relevance.

KEY MESSAGES

- Allergy sensitisation patterns may vary across geographic regions and environmental exposures.
- The perception that multiplex diagnostics are 'eurocentric' may reflect both their historical development in European allergen research and the challenge of capturing global allergen diversity within a single assay.
- ALEX³ was redesigned using real-world data from nearly 400,000 tests across more than 90 countries to improve global allergen representation.
- The expanded panel includes additional molecular allergens reflecting diverse dietary, environmental, and occupational exposures.
- As with all allergy diagnostics, laboratory findings should always be interpreted in the context of clinical history and exposure history.

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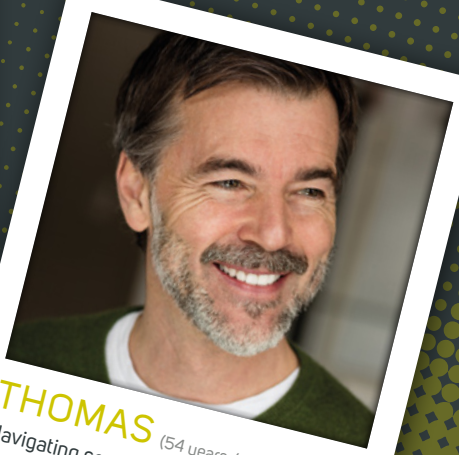
SCAN
FOR INSIGHTS

EVA (19 years / north-western Europe)
No more false-positive results:
Understanding the impact of
CCD blocking

ALEXANDER (35 years / Czechia)
A puzzling phenomenon:
Exploring the connection between
tick bites and meat allergy



THOMAS (54 years / Austria)
Navigating cross-reactivity:
Dealing with questions of
polysensitisation

Multiplex Testing in Modern Clinical Practice

Interview with Professor Schmid-Grendelmeier, MD

You were among the first to introduce multiplex testing into clinical practice. What arguments convinced you at the time?

Professor Dr Schmid-Grendelmeier: What makes multiplex testing so compelling is that it allows you to assess multiple groups of molecular allergens at once. This provides valuable insights into cross-reactivity and allergen relationships and helps to estimate the risk of anaphylaxis. Otherwise, you would need to perform many individual sIgE tests, which is far more time-consuming and ultimately more costly. In contrast, multiplex testing enables me to obtain a wide range of results in a single step. This is also beneficial for patients who want to be sure that as many allergens as possible are covered. I can reassure them that more than 200 allergens can be analysed simultaneously.

In everyday clinical practice, multiplex testing is also a valuable complement to the skin prick test (SPT), which typically includes only 12 to 14 allergens. While

the SPT provides immediate results, multiplex testing offers a much broader and deeper level of insight. It often identifies triggers that neither the physician nor the patient initially considered.

What role does multiplex testing play in your current clinical practice? Has scepticism among allergologists declined?

Professor Dr Schmid-Grendelmeier: In Switzerland, we are in a very privileged position, as multiplex tests are reimbursed by health insurance – so they have become almost routine. That said, expertise in interpreting the results is crucial.

For example, if the question is whether a patient is allergic to cats, the multiplex test can simultaneously reveal – via lipocalins – whether the patient is also sensitised to dogs or horses. It is important for physicians to be familiar with both singleplex and multiplex approaches.

Multiplex testing is certainly a valuable addition to diagnostics and is often the first – and sometimes the only – step I take. The ALEX test also measures total IgE, so in many cases no further testing is required.

So, for you, multiplex testing is not just an add-on but a central component of serological diagnostics. For which patient groups is this especially true?

Professor Dr Schmid-Grendelmeier: For example, in patients with anaphylaxis – especially when the trigger is unknown. Multiplex testing is also very useful in cases involving potential triggers such as latex or various foods, as well as in polysensitised patients, for instance those with both seasonal and perennial inhalant allergies. It can also be helpful when there is a suspicion that the patient's symptoms may not be caused by a food intolerance or a gastrointestinal disorder. In such cases, the test can provide valuable guidance on what further investigations may be needed.

Are there patient groups for whom you would not consider multiplex testing?

Professor Dr Schmid-Grendelmeier: In patients with a clearly defined, isolated Hymenoptera venom allergy, I would generally not use multiplex testing. The same applies to patients with strictly seasonal symptoms – for example, from May to July only – where the clinical picture is already quite clear.

On the other hand, I find multiplex testing extremely useful when assessing allergies to edible insects. Testing for these individually would be very labour-intensive, whereas they are already included in the ALEX³ allergen panel – this is a major advantage. Although such cases are not very common, I treated two patients last year with unexplained anaphylaxis in whom edible insects turned out to be the actual trigger.

What is your experience with CCD-free diagnostics?

Professor Dr Schmid-Grendelmeier: The automatic CCD blocking used in the ALEX test increases the specificity of the results, which I greatly appreciate. The clinical relevance of CCDs is still debated, and there are only a few cases where they seem to have a real impact. Overall, however, it is clearly advantageous to obtain CCD-free results.

A common criticism of multiplex testing is that not all detected sensitisations are clinically relevant, and that the results can be difficult to explain. How do you handle this in practice?

Professor Dr Schmid-Grendelmeier: I explain to my patients in advance that the test is very sensitive and may detect sensitisations that indicate a predisposition but do not cause any symptoms. These findings do not necessarily need to be taken into account. This can usually be explained quite well, although it does require experience on the part of the physician.

Personally, I prefer having more information and discussing it in detail with the patient rather than missing something important. The more difficult situation is when a patient presents with an anaphylactic reaction and all test results remain inconclusive.

Finally: do you have a favourite allergen family?

Professor Dr Schmid-Grendelmeier: I find tropomyosins and arginine kinases particularly fascinating. They are found in seafood and insects and play a major role in tropical regions. I am also very interested in fungal allergens, especially Malassezia.

"I prefer having more information than missing something important."

ABOUT

PROFESSOR PETER SCHMID-GRENDELMEIER, MD is a professor of dermatology and allergology and former head of the Allergy Unit in the Department of Dermatology at the University Hospital Zurich. His research focuses on allergic skin diseases, and he has authored hundreds of scientific publications in dermatology and immunology.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Bringing Next-Gen Allergy Testing into Daily Lab Practice

Interview with Dr Caroline Parker

From a laboratory perspective, how does a test panel with 80% molecular allergens change the diagnostic confidence you can provide to clinicians?

Dr Parker: The transition from whole-allergen extracts to predominantly molecular allergens fundamentally changed the level of diagnostic precision we can offer clinicians. With traditional extract-based testing, a positive result often reflects sensitisation to a mixture of proteins – some clinically relevant and others cross-reactive – creating uncertainty about whether a patient truly has a clinically relevant allergy. By contrast, molecular allergy testing identifies IgE responses to individual allergenic proteins. This improves diagnostic confidence by allowing laboratories to distinguish genuine primary sensitisation from cross-reactivity, stratify risk regarding symptom severity, and achieve a clearer correlation with clinical history, as molecular profiles often align more closely with patient symptoms and exposure patterns. Having access to a clinically meaningful sensitisation profile at the first consultation enables doctors to prescribe targeted therapeutic measures without multiple rounds of testing. Another important advantage of expanding the number of molecular allergens in the ALEX^3 array is improved consistency of results. Crude extracts can vary because allergen content differs depending on the source material, manufacturing processes, and the degradation of unstable proteins. Molecular allergens, in contrast, offer more consistent composition, better assay standardisation, and greater inter-laboratory reliability, helping reduce conflicting results and providing clinicians with more dependable diagnostic information.

CCDs (cross-reactive carbohydrate determinants) have long been a thorn in the side of allergy diagnostics. How transformative is the integration of automatic CCD inhibition for reducing false positives in a high-throughput setting?

Dr Parker: It is highly transformative, particularly in high-throughput laboratory settings, because we can be confident that there is a significant reduction of false positives. We are providing patients and clinicians with cleaner sensitisation profiles and improved clinical interpretability, which reduces follow-up testing. From our perspective as a diagnostic lab, CCD inhibition effectively removes background 'noise' from the dataset, allowing the true molecular sensitisation pattern to emerge. For clinicians using our service, this means reports that are more reliable, clinically meaningful, and easier to interpret alongside the patient's history.

Molecular multiplexing often produces 100+ data points per run. How do the RAPTOR Server Analysis Software and RAVEN Interpretation Software assist in translating all this information into a clear, actionable report that a general practitioner can use?

Dr Parker: Our goal as a laboratory is not simply to deliver raw IgE data, but to translate that information into a clear, clinically useful sensitisation profile. The RAPTOR Server Analysis Software and the integrated interpretation tool, RAVEN Interpretation Software, play a crucial role here. Both solutions support clinicians by identifying clinically relevant sensitisation clusters and distinguishing

primary allergens from cross-reactive molecular allergens. For example, the software can link molecules to known clinical syndromes and highlight potential food-pollen cross-reactivity patterns, and provide all this information in a single report. Our reports can also alert the clinician to potential risk indicators by flagging molecular allergens associated with systemic reactions or severe allergy. Essentially, the reporting process is designed to translate complex multiplex data into a clear narrative report, and we can provide service users with structured summaries suitable for clinical decision-making. Instead of receiving hundreds of isolated numbers, clinicians receive a clinically contextualised sensitisation map that can be directly integrated into the consultation with the patient.

Some see multiplexing as a 'luxury'. How do you make the financial case to hospital administrators that a comprehensive molecular panel is more cost-effective than a sequential testing strategy that often requires multiple patient visits?

Dr Parker: The first impression is that multiplex molecular testing is more expensive than individual allergen tests. However, when evaluated across the entire patient pathway, the economic picture is often the opposite. Traditional sequential testing typically involves an initial consultation followed by a limited screening panel and follow-up visits to

review results, with the need for additional targeted tests and possibly further consultations. This approach can result in multiple patient visits, repeated blood sampling, incremental laboratory costs, and delayed diagnosis. By contrast, a comprehensive molecular panel at the first consultation can provide a full sensitisation profile immediately, reduce the need for step-up testing, minimise repeat appointments, and accelerate clinical decision-making. From a healthcare-system perspective, the cost savings often arise from fewer consultations, fewer tests overall, and faster management decisions. For private patients, this approach can mean lower total costs and faster answers.

What happens to your lab's costs, complexity, and sustainability profile when you replace dozens of singleplex tests with one molecular multiplex assay covering 80% of diagnostic needs?

Dr Parker: From a laboratory perspective, complexity is reduced: instead of managing large inventories of individual allergen reagents, there is a single standardised assay platform. Using our MAX 9K lowers operational costs because automation reduces hands-on time for technicians and manual pipetting steps, reduces the error risk and increases confidence in result precision. Laboratory space is always at a premium, so the smaller inventory and storage requirements of multiplex panels drastically reduce cold-storage requirements and expiry-related waste. The MAX 9K instrument is also easy to use – and the closed nature of the system works well in our molecular facility. From a sustainability perspective, the MAX 9K has lower energy consumption, reduced plastic waste and small reagent volumes compared with other technologies. For a private laboratory, this means lower operational costs, simplified logistics, and a smaller environmental footprint while maintaining high diagnostic output.

What is your favourite molecule and why?

Dr Parker: It would have to be Ara h 2, one of the major peanut storage proteins. Ara h 2 is one of the clearest examples of how molecular diagnostics changes clinical practice. In extract-based testing, peanut allergy can be difficult to interpret due to cross-reactivity with other proteins. However, sensitisation to Ara h 2 is strongly associated with true peanut allergy and a higher risk of systemic reactions. For clinicians, detecting Ara h 2 helps answer the critical question: 'Is this a true peanut allergy, or just cross-reactivity?'. That level of clarity can avoid unnecessary dietary restrictions, identify patients who require strict avoidance and emergency medication, and reduce the need for oral food challenges in some cases. Another molecule that has become increasingly important in our laboratory practice is α-gal, which is now included in ALEX^3. α-gal is associated with α-gal syndrome, a delayed allergic reaction to mammalian meat that can occur hours after consumption. Because of the unusual timing and varied symptoms, patients often spend years seeking a diagnosis. Since using ALEX^3, we have identified previously unrecognised α-gal sensitisation in several patients with long histories of unexplained allergic symptoms. They finally received appropriate care, which highlights the real-world value of including emerging and clinically relevant allergens in molecular panels.

"Molecular allergens improve the diagnostic confidence we can provide."

ABOUT

DR CAROLINE PARKER, BMEDSC, PHD
is the founder and CSO of Forensic Genomics Innovation Hub (FGHI) in Southampton, UK. She has spent over 20 years working in private forensics, family DNA testing, clinical genetics and infectious diseases across Europe and South Africa. Her aim is to help individuals protect their health by providing scientifically and clinically validated tests that can remove uncertainty about both their emotional and physical wellbeing.

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