Comparison of specific IgE against allergen components measured on the ALEX²® Allergy Xplorer and the ImmunoCAP™ ISAC multiplex assays

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Background. Allergic disease, mediated by immunoglobulin E (IgE), is common worldwide and its incidence is on the rise.
Objectives. To compare common food, inhalant and cross-reactive molecular components detected by the ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC™) and the Allergy Xplorer (ALEX²®) multiplex IgE assays.
Methods. The study analysed serum samples from 100 patients with suspected allergies. Allergen-specific IgE (sIgE) molecular component measurements were performed using the ImmunoCAP™ ISAC E112i assay (ISAC™) (Thermo Fisher Scientific, Sweden). Subsequently, sIgE molecular component measurements were performed using the Allergy Xplorer 2® (ALEX²®) (Macro Array Diagnostics, Austria). The ISAC™ method tests 112 molecular allergen components. The ALEX²® method offers 295 reportable molecular allergen components and whole allergen extracts.
Results. The overall Kappa analysis showed very good agreement in 58.33% (n=28/48) of components, good agreement in 33.33% (n=16/48) of components, moderate agreement in 8.33% (n=4/48) of components and no fair or poor agreements seen among the analysed components. The four components with a moderate agreement were Gly m 4 (PR-10, soy), Ara h 8 (PR-10, peanut), Gly m 5 (storage protein, soy) and Tri aA/TI (alpha amylase/TI, wheat), with K values of 0.52, 0.51, 0.48 and 0.44, respectively. Tri-AA/TI exhibited the lowest agreement.
Conclusions. The study findings demonstrated a good correlation between the ALEX²® and ISAC™ assays for the detection of sIgE against molecular allergen components. ALEX²® offers the benefit of testing 295 molecular allergen components and extracts, as well as cross-reactive carbohydrate determinants (CCD) inhibition.
Keywords. Allergic disease; allergy IgE testing, molecular allergy testing.

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Allergic disease, mediated by IgE, is common worldwide and its incidence is on the rise.[1] Elevated IgE levels are not always indicative of clinical allergy.[2] The main aim of allergy testing is to distinguish primary sensitisation from cross-sensitisation, achieved through either whole extract or component IgE allergen testing.[2] Fig. 1 illustrates the difference between extract-based and component-based testing for allergen-specific IgE (sIgE) using milk allergen as an example. True sensitisation occurs when specific IgE antibodies are restricted to a given allergen component.

Laboratory-based tests form an integral part of diagnosing IgE-mediated allergic disease. Despite the availability of various laboratory tests to measure sIgE, identifying the offending allergen can be complex. There are multiple reasons for this: (*i*) manufacturers of sIgE tests use different sources and quality of allergen extracts; (*ii*) elevated measured sIgE indicates allergen sensitisation which can occur in the absence of clinical disease and (*iii*) sIgE tests that use whole allergen extracts can be elevated owing to the presence of cross-reactive components.[3] Therefore, all sIgE tests should be interpreted given the patient's clinical picture.

Allergen extracts contain both allergenic and non-allergenic protein molecules. Specific IgE tests that utilise allergen extracts alone cannot distinguish cross-sensitisation between protein molecules that share a similar structure. In contrast, assays measuring sIgE to molecular allergen components—termed precision allergy molecular diagnostic applications (PAMD@)—can distinguish allergic disease from true primary sensitisation, clinically relevant cross-reactivity and clinically irrelevant cross-sensitisation.[4] Allergenic molecules are named using their Latin genus and species and numbered in order of their discovery e.g. Phl p 1 (Timothy grass pollen, *Phleum pratense*).[4] The WHO/IUIS Allergen Nomenclature Subcommittee maintains a database accessible at http://www.allergen.org.

An immune response to cross-reacting components (owing to their sequence homology) such as pathogenesis-related protein 10 (PR-10), cross-reactive carbohydrate determinants (CCD), profilins and lipid

transfer proteins (LTP), is defined as a cross-reaction.[5,6] Allergen sIgE targeted towards whole allergen extracts can be elevated owing to these cross-reactive components and do not always indicate clinical allergic disease.

An example of cross-reactivity is CCD, which can cause positive extract-based IgE results for plant-based foods such as peanut and wheat, where pollen is the true sensitiser (Fig. 2). Such individuals often do not have a primary food allergy and can tolerate these foods, a determination that must be confirmed by the clinical history and/or food challenges.

Singleplex assays, such as ImmunoCAP™ tests, use single reagents and have high analytical sensitivity, as they detect both high- and low-affinity antibodies.[3] Previous studies have shown a good correlation between the ISAC™ multiplex assay and the ImmunoCAP™ singleplex

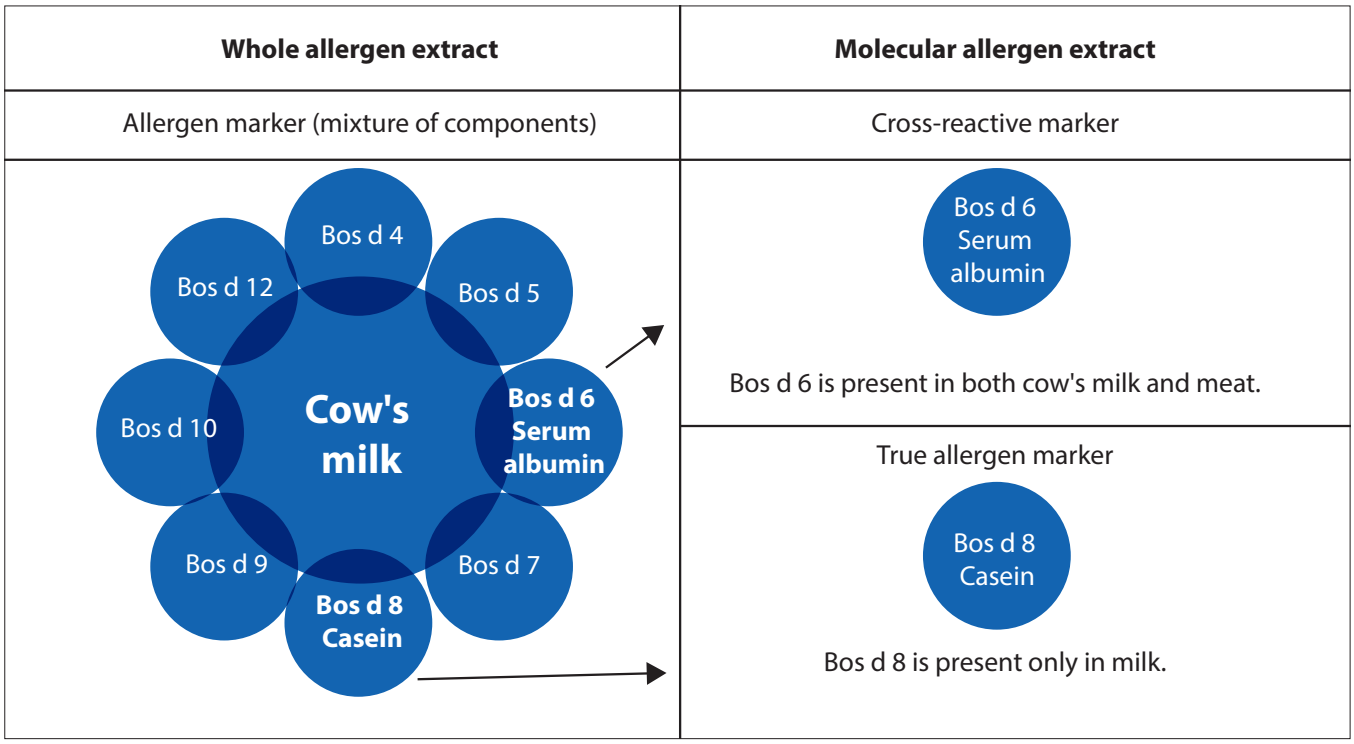

Fig. 1. Differences between extract- and component-based testing for allergen-specific IgE. (IgE = immunoglobulin E)

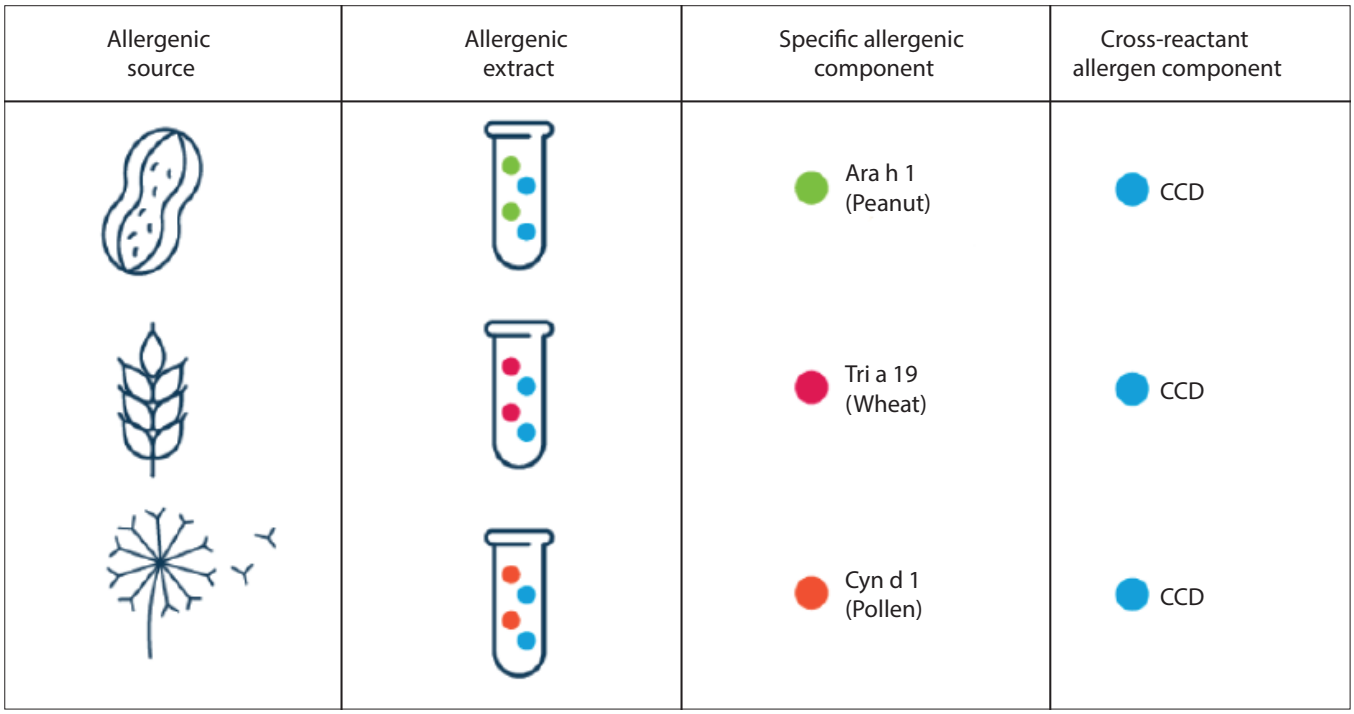

Fig. 2. Visual representation of specific and cross-reactive molecular allergen components. (CCD = cross-reactive carbohydrate determinants)

Table 1. Component analysis separated by food, inhalants and cross-reactive components

Allergen group	Component		Cohen's kappa (K)	PPA (%)	95% CI (%)	NPA (%)	95% CI (%)
Food	**Hen's egg**						
	Ovomucoid	Gal d 1	0.70	100.0	76.84 – 100	95.4	89.62 – 97.99
	Ovalbumin	Gal d 2	0.87	93.8	67.90 – 99.07	96.4	90.57 – 98.70
	Conalbumin	Gal d 3	0.90	85.7	60.38 – 95.94	100	95.80 – 100.0
	Cow's milk						
	α-lactalbumin	Bos d 4	0.77	100	54.07 – 100	95.8	91.33 – 97.96
	β-lactoglobulin	Bos d 5	0.89	100	66.37 – 100	98.9	93.34 – 99.83
	Casein	Bos d 8	0.76	85.7	45.06 – 97.77	97.9	93.20 – 99.34
	Seafood						
	Cod	Gad m 1/Gad c 1	0.74	75.0	41.84 – 92.60	97.8	93.13 – 99.34
	Shrimp	Pen m 2	0.93	100	69.15 – 100	98.9	93.21 – 99.83
		Pen m 4	0.66	100	15.81 – 100	100	96.31 – 100
	Legumes						
	Peanut (Storage proteins)	Ara h 1	0.89	86.4	67.60 – 95.06	100	95.38 – 100
		Ara h 2	0.98	95.0	73-07-99.26	100	95.48 – 100
		Ara h 3	0.84	84.2	63.71 – 94.19	100	95.55 – 100
		Ara h 6	0.92	90.5	70.74 – 97.39	100	95.44 – 100
	Soy (Storage proteins)	Gly m 5	0.48	100	29.24 – 100	93.8	90.53 – 96.01
		Gly m 6	0.72	73.1	56.65 – 84.93	94.6	87.75 – 97.72
	Wheat						
	Omega-5-Gliadin	Tri a 19	1.00	100	2.50 – 100	100	96.34 – 100
Inhalants	**Grass pollen**						
	Bermuda grass	Cyn d 1	0.84	100	92.29 – 100	85.2	75.21 – 91.60
	Timothy grass	Phl p 1	0.78	100	92.13 – 100	92.7	83.29 – 97.03
		Phl p 2	0.90	83.3	40.79 – 97.32	98.9	93.95 – 99.82
		Phl p 5	0.85	62.5	35.37 – 83.54	100	96.07 – 100
		Phl p 6	0.79	100	29.24 – 100	97.9	94.20 – 99.29
		Phl p 7	0.93	100	39.76 – 100	100	96.23 – 100
	Mould						
	Alternaria	Alt a 1	0.91	100	84.56 -100	97.44	90.98 – 99.31
		Alt a 6	0.66	100	66.37 – 100	97.8	92.79 – 9.36
	Aspergillus	Asp f 1	1.00	100	2.5 – 100	100	96.34 – 100
		Asp f 3	0.80	100	15.81 – 100	98.98	95.14 – 99.79
		Asp f 6	0.88	100	47.82 – 100	100.0	96.19 – 100
	House dust mite						
	Dermatophagoides pteronyssinus	Der p 1	0.86	95.5	74.87 – 99.33	96.15	89.66 – 98.63
		Der p 2	0.95	88.9	72.52 – 96.04	100	95.07 – 100
		Der p 23	0.99	100	83.89 – 100	100	95.44 – 100
	Dermatophagoides farinae	Der f 1	0.83	100	83.16 – 100	96.25	89.93 – 98.66
		Der f 2	0.98	96.2	78.11 – 99.43	100	95.14 – 100.0
	Animals						
	Dog	Can f 1	0.95	100	76.84 – 100	97.7	91.99 – 99.35
		Can f 2	0.69	66.7	32.16 – 89.41	98.9	94.16 – 99.81
		Can f 4	0.94	94.4	70.79 – 99.17	100	95.60 – 100
		Can f 6	0.86	80.0	49.53 – 94.22	97.8	92.72 – 99.35
	Cat	Fel d 1	0.95	100	90.51 – 100	98.4	89.96 – 99.77
		Fel d 4	0.66	100	54.07 – 100	92.6	88.25 – 95.36
	Cross reactive components						
	Lipid transfer protein (LTP)	Pru p 3	0.72	92.3	62.35 – 98.86	90.8	85.23 – 94.41
		Tri a 14	0.85	100	39.76 – 100	100	96.23 – 100
	PR-10	Ara h 8	0.51	100	29.24 – 100	95.9	92.45 – 97.79
		Bet v 1	0.83	100	59.04 – 100	96.8	92.09 – 98.72
		Gly m 4	0.52	100	29.24 – 100	96.9	93.37 – 98.59
	Profilin	Bet v 2	0.66	100	75.30 – 100	91.9	86.28 – 95.41
		Hev b 8	0.62	100	75.30 – 100	90.8	85.13 – 94.46
	Tropomyosin	Der p 10	0.72	100	47.82 – 100	94.7	90.64 – 97.10
	Serum albumin	Bos d 6	0.89	81.8	52.64 – 94.80	97.8	92.55 – 99.35

PPA = positive percent agreement; NPA = negative percent agreement; CI = confidence interval.

assays for detecting molecular allergen components.[7] Both ISAC™ and ALEX²® are multiplex testing methods that use microarray formats to simultaneously test multiple allergenic proteins with a small sample volume. This offers a cost-effective way to examine sIgE sensitisation patterns, especially in polysensitised patients. Multiplex assays that detect a preselected panel of molecules mainly detect high-affinity antibodies.[3] These assays can therefore be less sensitive, especially in patients with low sIgE concentrations. However, multiplex assays improve analytical specificity by detecting antibodies to stable sIgE allergens.[3] Given their comprehensive nature, multiplex assays are often preferred for patients with complex symptoms, polysensitisation, high total IgE and idiopathic anaphylaxis, especially when the pretest probability of allergic disease is high.

Molecular allergy diagnostics aim to identify sIgE components associated with severe allergic reactions (e.g. LTP or storage proteins) or those typically linked to milder clinical symptoms (e.g. profilins and PR-10).[5,6] A stepwise approach is essential, beginning with clinical history, physical examination and skin prick test (SPT) as the first-level diagnostic tools (a top-down approach).[5,6] Extracted-based (whole allergen) sIgE testing serves as a second-level test, followed by PAMD@ diagnostics.[5,6] Some have suggested that a bottom-up diagnostic approach could also be advantageous.[5,6]

This study aimed to compare common food, inhalant and cross-reactive molecular components between the ISAC™ and the ALEX²® multiplex IgE assays. Previous studies have shown strong correlations between the ALEX²® and ISAC™ results, [5,8-11] confirming the utility of both tests in allergy diagnostics.

Methods

Data and sample size

The study analysed serum samples from 100 patients with suspected allergies, all tested using the ISAC™ assay. Of these, 80 samples tested positive for sIgE molecular components, while 20 tested negative. All 100 samples were subsequently tested on the ALEX²®. Testing was conducted at the Ampath National Reference Laboratory (NRL) in Centurion between February 2022 and December 2022.

Allergen sIgE molecular component measurements were performed using the ImmunoCAP™ $ISAC_{E112i}$ assay (ISAC™) (Thermo Fisher Scientific, Sweden). Allergen sIgE molecular component measurements were subsequently performed on the Allergy Xplorer 2® (ALEX²®) (Macro Array Diagnostics, Austria). The ISAC™ method tests 112 molecular allergen components, including food, inhalants and cross-reactive components. In comparison, the ALEX²® method analyses 295 reportable molecular allergen components and whole allergen extracts, including food, inhalants, venom, latex and cross-reactive components. ALEX²® also includes CCD inhibition, which reduces clinically irrelevant cross-sensitisation and improves test specificity. For this study, 48 common food and inhalant allergen components that are available on both platforms were assessed.

Data analysis

Allergen sIgE food and inhalant molecular components were compared between the ISAC™ and the ALEX²®. Data were captured in Microsoft Office Excel (Microsoft, USA) and analysed using MedCalc® version 19.4 (MedCalc® Software, Belgium).

Kappa analysis and percentage agreement per component were used to compare the two methods. Kappa analysis assessed the strength of agreement between the two methods, with the following interpretation: poor (K<0.20), fair (K=0.21 - 0.40), moderate (K=0.410.60), good (K=0.610.80) and very good (K=0.811.00). Allergen sIgE molecular component concentrations from both methods were categorised into the following diagnosis ranges: negative (<0.3 kUA/L), low (0.3-1 kUA/L), moderate/high (1-15 kUA/L) and very high (>15 kUA/L). These ranges are as per the manufacturers' instructions for use.

The negative percent agreements (NPA) and positive percent agreements (PPA) were used to assess method agreement. The NPA and PPA calculations were based on qualitative (negative or positive) interpretations of allergen sIgE concentrations.

Allergy questionnaire

A questionnaire on allergic symptoms was sent to all patients testing positive in the study, and feedback was received from 60 of the 80 participants. The questionnaire included questions about symptoms of atopic dermatitis, oedema/urticaria, anaphylaxis, adverse food reactions and oral allergy syndrome.

Ethical considerations

This study was approved by the Faculty of Health Sciences Research Ethics Committee at the University of Pretoria (ref. no. 312/2022). Informed consent was obtained from all patients who participated by completing the allergy questionnaire.

Results

The component analysis investigated the performance of the ALEX²® compared with the ISAC™ assay. Forty-eight components were selected from the data, analysed and categorised into three groups: food, inhalants and cross-reactive components. The results from the comparison are presented in Table 1.

Food components

Of the 17 food components, 52.9% (*n*=9/17) demonstrated very good Kappa agreement, 35.3% (*n*=6/17) had a good agreement and 11.8% (*n*=2/17) had moderate agreement. Gly m 5 (storage protein, soy) and Tri aA/TI (alpha amylase/TI, wheat) had the lowest Kappa agreement overall, with K=0.48 and K=0.44, respectively.

Most food components (86.7%; *n*=14/17) had a PPA above 84%. The three exceptions were Gly m 6 (soya), Gad m 1 (cod fish) and Tri aA/TI (alpha amylase/TI, wheat), which had PPA values of 73.1%, 75% and 66.7%, respectively. All the food components had an NPA above 93%.

Inhalant components

Of the 22 inhalant components, 72.7% (*n*=16/22) demonstrated very good Kappa agreement, while 27.3% (*n*=6/22) had good agreement.

A majority of the inhalant components (90.9%; *n*=20/22) had a PPA above 80%, with the two exceptions being Can f 2 (dog) and Phl p 5 (Timothy grass), which had PPA values of 66.7% and 62.5%, respectively. All the inhalant components had an NPA above 85%.

Cross-reactive components

Of the nine cross-reactive components, 33.3% (*n*=3/9) demonstrated very good Kappa agreement, 50% (*n*=4/9) had a good agreement and the remaining two components, Ara h 8 (PR-10, Peanut) and Gly m 4 (PR-10, Soy), demonstrated moderate agreement. These two components were among the four with the lowest Kappa agreements in the study.

A total of 88.9% (*n*=8/9) cross-reactive components had a PPA above 92%, with the exception of Bos d 6 (milk, serum albumin), which had a PPA of 81.8%. All cross-reactive components had an NPA above 90%.

All components

The overall Kappa analysis showed very good agreement in 58.3% (*n*=28/48) of components, good agreement in 33.3% (*n*=16/48) of components, a moderate agreement in 8.3% (*n*=4/48) of components and no fair or poor agreements were observed among the analysed components. The four components with moderate agreement were Gly m 4 (PR-10, soy), Ara h 8 (PR-10, peanut), Gly m 5 (Storage protein, soy) and Tri aA/TI (alpha amylase/TI, wheat), with Kappa values of 0.52, 0.51, 0.48 and 0.44, respectively. Tri aA/TI demonstrated the lowest agreement.

Asp f 1 *(Aspergillus fumigatus)*, Tri a 19 (omega-5-Gliadin, wheat), Der p 23 *(Dermatophagoides pteronyssinus,* House dust mite*)*, Der f 2 (*Dermatophagoides farinae*, House dust mite) were the four components with the highest agreements, with Kappa values of 1.00, 1.00, 0.99 and 0.98, respectively. Overall, 91.67% (44/48) of components had Kappa agreements between 0.62 – 1.00 across both assays.

The PPA and NPA analysis showed a good correlation between the ALEX^{2*} and ISAC™ assays. The overall PPA was above 80% in 89.6% (*n*=43/48) of the components, and the NPA was above 90% in 97.9% (*n*=47/48) of the components evaluated. The lowest PPAs in the analyses included Gad m 1 (cod fish), Gly m 6 (soy), Can f 2 (dog), Tri aA/TI (alpha amylase/TI, wheat) and Phl p 5 (Timothy grass), with PPA percentages of 75%, 73.03%, 66.7%, 66.7% and 62.0%, respectively. The lowest NPA is Cyn d 1 (Bermuda grass), with an NPA of 85.2%. No discrepancies were observed in the negative group when ALEX^{2*} was compared with ISAC™.

Allergy questionnaire

Approximately 45% (*n*=27/60) of the participants who responded to the allergy questionnaire reported adverse food reactions, which included gastrointestinal tract (GIT) symptoms, abdominal cramps or discomfort and oral allergy syndrome (OAS) such as itchiness or swelling of the mouth, face, lips, tongue and throat. The majority of these participants tested positive for storage proteins (56%) and seafood (33%).

Fifty-eight percent (35/60) of the participants reported atopic dermatitis. Of the patients with atopic dermatitis, 57% (20/35) also reported adverse food reactions. Participants with atopic dermatitis mostly tested positive for storage proteins (54%) and seafood (29%).

Forty-three percent (26/60) of participants reported anaphylaxis. Of those, the majority tested positive for storage proteins (56%) and seafood (33%).

Discussion

Our study findings demonstrated a good correlation between the ALEX^{2*} and ISAC™ methods for detecting sIgE against molecular allergen components. The agreement between the two methods is in keeping with previous studies that found a strong correlation between the ALEX^{2*} and ISAC™ assays.[5,8-11] Heffler *et al.*[5] reported technical differences between the ISAC™ and ALEX^{2*} tests, including differential epitope recognition, different solid phases and different enzyme substrates. These technical differences could explain the moderate agreement of some components, although these findings could also have been compounded by the small sample size in this study.

The components with the lowest agreement in our analysis were the food allergens Gly m 4 (soy), Ara h 8 (peanut), Gly m 5 (soy) and Tri aA/TI (wheat). Similarly, Scala *et al.*[12] reported greater heterogeneity of results among food allergens. They also concluded that the ALEX^{2*} assay, which includes a larger number of allergens such as latex, insect venom, hemp and spices, offers a more comprehensive allergic profile detection. The lower agreement could be attributed to the lack of CCD inhibition in the ISAC™ assay, as noted by Platteel *et al.*[8] Nösslinger *et al.*[13] showed that CCD inhibition in the ALEX^{2*} reduced the number of positive CCD signals by 88.5% compared with the ISAC™. The allergen profiles differ between the two assays and the relevance of allergens may be patient-specific. A key advantage of the ISAC™ assay is its inclusion of the galactose-alpha-1,3-galactose (alpha-gal) allergen component, which is important for detecting delayed allergic reactions to red meat.

Čelakovská *et al.*[2] showed how component-resolved allergology diagnostics can be used in patients with atopic dermatitis. Their study highlighted the extensive profile of allergen extracts and molecular components in this subgroup of patients, making a molecular approach ideal for assessing the potential allergens, including rare ones, which can be detected through multiplex assays.

Multiplex testing to whole extract and molecular components is thought to be an alternative bottom-up approach in the diagnosis of allergies, as reported by Heffler *et al.*[5] They suggested that this approach is cost- and time-efficient, especially when allergy immunotherapy for polysensitised patients is required. These assays may be ordered to complement the history, examination and skin prick tests to understand complexes sensitisation profiles.

Limitations

This study included a smaller sample size, which resulted in a wide variation in the confidence intervals for some components. Additionally, the allergy questionnaire symptoms were self-reported, which may have led to misinterpretation of questions. We recommend further studies with larger sample sizes, more detailed clinical notes and a wider range of components to address these limitations and better evaluate the clinical utility of the ALEX^{2*} in assessing IgE sensitisation profiles.

Conclusion

The availability of molecular allergen components has ushered in a new phase of precision medicine. Molecular-based allergy diagnostics can improve the personalised management of IgE-mediated allergic disease through accurate diagnosis, risk assessment and treatment guidance. The study findings demonstrated a good correlation between the ALEX^{2*} and ISAC™ assays for detecting sIgE against molecular allergen components. ALEX^{2*} offers the benefit of simultaneously testing 295 molecular allergen components and extracts, along with CCD inhibition.

The scope of this article did not allow a more in-depth analysis of participating patients' self-reported symptoms and molecular allergen component results. The allergy questionnaire provides a reference for future larger cohorts and clinical correlation from clinicians guiding them to request appropriate allergy testing.

Declaration. None.

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Author contributions: CvR, SvdB and LM conceived the study. LM and SvdB compiled the protocol. LM and AvN obtained ethical approval. CdB performed the laboratory testing. CvH and HR performed statistical conversions. MJT, CvH, PS and LM drafted the manuscript. All authors have accepted responsibility for the content of the manuscript and approved submission.

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Data availability statement. The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest: None.

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