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Comparison of two multiplex arrays in the diagnostics of allergy

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Abstract

The objective of this analysis was to compare the multiplex ImmunoCAP ISAC (Thermo Fisher Scientific, Sweden) and the multiplex Alex Allergy Explorer (Macro Array Diagnostics GmbH, Austria) platform on specific IgE to grass pollen (Phl p 1, Phl p 5), tree pollen (Bet v 1), house dust mites (Der p 1, Der p 2) and cat (Fel d 1) allergens in allergic patients. Our findings demonstrate a good correlation of presently used methods to detect serum sIgE. Multiplex testing of allergen-specific IgE can be the method of choice for a prospective component-resolved diagnosis of type I allergy, and the basis for the design and monitoring of a patient-tailored specific therapy.

Keywords: Molecular allergology, Multiplex platform, ISAC, ALEX

To the Editor,

Molecular allergy diagnostics are increasingly entering routine care. This type of allergy diagnostics is used to map the allergen sensitizations of patients at the molecular level [1]. Both recombinant and purified natural allergen components are used in commercially available test systems. Allergenic molecules are classified into protein families, according to their structure and biological function. Knowledge of structures of allergens molecules can help physicians to improve the diagnostic workup of allergy [2].

The presence of IgE antibodies against allergenic molecules may be determined using a singleplex or multiplex measurement platform. Multiplex assays permit more than one allergen to be detected and quantified in a single assay analysis [3].

There are a few companies that offer multiplex assays for molecular allergy diagnostics. One of them is based on the Immuno Solid-phase Allergen Chip (ISAC) (Thermo Fisher Scientific, Phadia, Sweden), which has been available since 2001. The current version of this allergen chip enables the determination of specific IgE (sIgE) to 112 different single molecules from 51 different plant and animal allergen sources.

Recently a new multiplex platform is available, called ALEX (Macroarray Diagnostics, Vienna). This platform offers 156 allergen extracts and 126 molecular components.

The aim of this study was to perform a comparison between the results of ISAC and ALEX for the following allergen components: Bet v 1 (*Betula verrucosa)*, Phl p 1, Phl p 5 (*Phleum pratense*), Der p 1, Der p 2 (*Dermatophagoides pteronyssinus*) and Fel d 1 (*Felis domesticus*). We chose these allergens because they were the most common allergens in our patients. We wanted to have as many samples as possible to compare ISAC and ALEX multiplex assays. Heffler et al. [4] published a study where they compared these methods with the same allergens. We wanted to verify whether we achieved the same results in our population.

Serum samples from 198 patients of the Department of Immunology and Allergology at the University Hospital Pilsen, Czech Republic, were analyzed by two multiplex assay systems, ALEX, and ImmunoCAP ISAC. The study protocol was approved by the local ethics committee prior to the initiation of the study.

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ISAC

Briefly, allergen components are immobilized in triplicate on a glass slide. The sample (serum or plasma) is pipetted onto each reaction site (there are four reaction sites available per chip), and after incubation and washing, fluorescence-labeled anti-human IgE detection antibody is applied. After incubation, washing and drying the microarray is scanned by a confocal laser scanner. The image is processed using the Microarray analyzer (MIA), and test results are reported in semi-quantitative ISU units [5, 6]. Measurement values are reported semi-quantitatively, divided into four different categories: values < 0.3 ISU-E are defined as negative; values between 0.3 and 1 ISU-E as low-level positive; values between 1 and 15 ISU-E as moderately high and values > 15.0 ISU-E as very high positive [7].

ALEX

This array contains 282 reagents (156 allergen extracts and 126 molecular components). The different allergens and components are coupled onto polystyrene nanobeads, and then the allergen beads are deposited on a nitrocellulose membrane. The ALEX chip is then incubated with 0.5 mL of 1:5 diluted serum under agitation. Notably, the serum diluent contains a Cross-Reactive Carbohydrate Determinants (CCD) inhibitor. After incubation for 2 h, the chips are extensively washed, and an anti-human IgE detection antibody (labeled with alkaline phosphatase) is added and incubated for 30 min. Following another cycle of extensive washing, the enzyme–substrate is added, and after a few minutes, the reaction is complete. The membranes are dried, and the intensity of the color reaction for each allergen spot is measured by a Charge Coupled Device (CCD) camera. The Raptor software digitalizes the images and prepares a report that lists the allergens and components and their score in kUA/L. It is the same unit that is used for ImmunoCAP. Measured values are divided into four categories; values < 0.3 kUA/L are defined as negative; 0.3–1 kUA/L as low-level positive; values between 1 and 5 kUA/L as moderate-level positive; values between 5 and 15 kUA/L as high level positive and values > 15 kUA/L as very high positive [4].

The following allergen components: Bet v 1, Phl p 1, Phl p 5, Der p 1, Der p 2 and Fel d 1 were evaluated by Spearman's rank correlation coefficient and Bland–Altman plots for quantitative level of comparison and Cohen kappa analysis for semi-quantitative level of comparison between the results of ALEX and ISAC. The kappa analysis results were divided into four classes 0, 1, 2 and 3 (Table 1). Statistical analysis was performed by using the statistical software MedCalc version 18.6.

Table 1 Division into classes for kappa analysis

Class	Interval	Interval	
		ISAC	ALEX
0	< 0.3	< 0.3	< 0.3
1	≥ 0.3–1	0.3–0.9	0.3–1
2	1.1–14.9	1–14.9	1.1–5
			5–14.9
3	≥ 15	≥ 15	≥ 15

Comparison was made by plotting the results of components present in both ALEX and ISAC in the same sample. The results of correlation between two methods are shown (Fig. 1). The correlation coefficients were highly significant for every comparison (Bet v 1; r = 0.95; $p < 0.0001$); (Der p 1; r = 0.94; $p < 0.0001$), (Der p 2; r = 0.93; $p < 0.0001$), (Phl p 1; r = 0.94; $p < 0.0001$), (Phl p 5; r = 0.94; $p < 0.0001$), (Fel d 1; r = 0.91; $p < 0.0001$).

Bland–Altman plots were used to demonstrate the agreement between ISAC and ALEX multiplex platforms. The Bland–Altman plots showed that differences depend strongly on the magnitude of measurement (Fig. 2). There is a different dynamic range between ISAC and ALEX. The dynamic range of ALEX is 0.3–30 kUA/L and ISAC has the dynamic range 0.3–100 ISU-E.

The Cohen kappa statistic (κ) and its 95% CI was used to test the level of agreement between ISAC and ALEX platforms. The strength of agreement was considered poor for κ < 0.2; fair for κ = 0.21–0.40; moderate for κ = 0.41–0.60; good for κ = 0.61–0.80; and very good for κ > 0.81) [8].

The kappa analyses showed that there was a very good agreement between ISAC and ALEX method (Bet v 1; κ = 0.88); (Der p 1; κ = 0.89); (Der p 2; κ = 0.91); (Phl p 1; κ = 0.9); (Phl p 5; κ = 0.86); (Fel d 1; κ = 0.83) (Fig. 3).

We observed that ALEX and ISAC yielded different results in terms of positivity in one and negativity in the others test. We have 16 out of 198 cases as negative controls (patients were negative in all allergens in ISAC and ALEX). There were no discrepancies. We have 182 out of 198 cases as subjects with positive allergens. We found discrepancies in the following allergens Bet v 1 (9 out of 182 cases), Phl p 1 (5 out of 182 cases), Phl p 5 (7 out of 182 cases), Der p 1 (6 out of 182), Der p 2 (7 out of 182 cases), Fel d 1 (14 out of 182 cases). We observed no statistical significant differences between these discrepancies.

Molecular allergology (MA) is increasingly used in clinical routine worldwide, providing enhanced diagnostic depth in addition to conventionally extract-based sIgE-testing. Corresponding to the rising use of MA, there is a

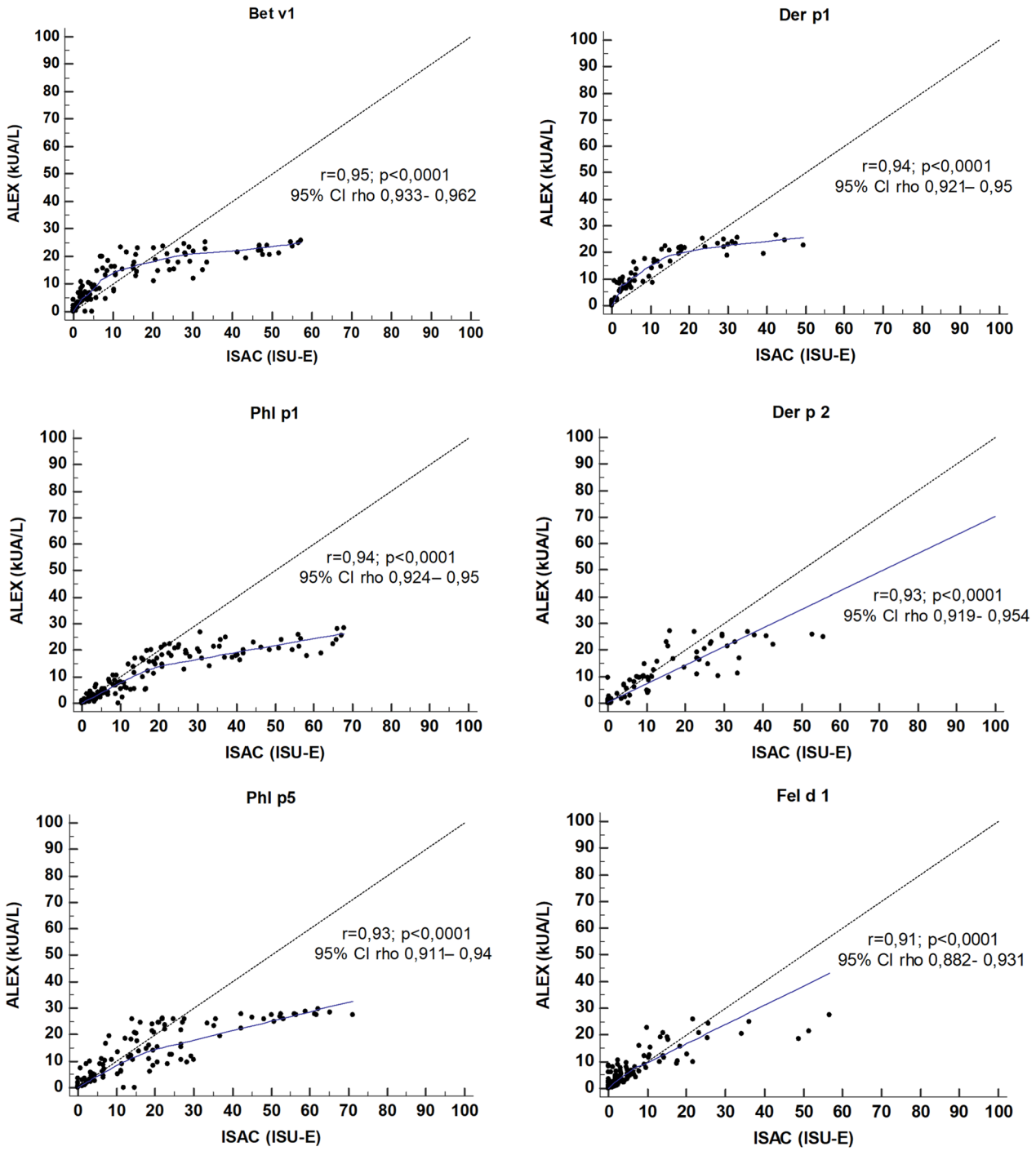

Fig. 1 Correlation between ISAC and ALEX

growing need for information on the optimal methodology and how to correctly interpret the results [9].

Regarding the sequence of the diagnostic steps, the authors of the WAO—ARIA—GA [2] LEN consensus document consider MA in general as a third-line approach to be used in the case of inconclusive first- and second-line investigations, which usually provide sufficient information in the majority of patients [6]. The use of recombinant allergens for molecular allergy diagnosis currently revolutionizes the diagnosis of IgE-associated

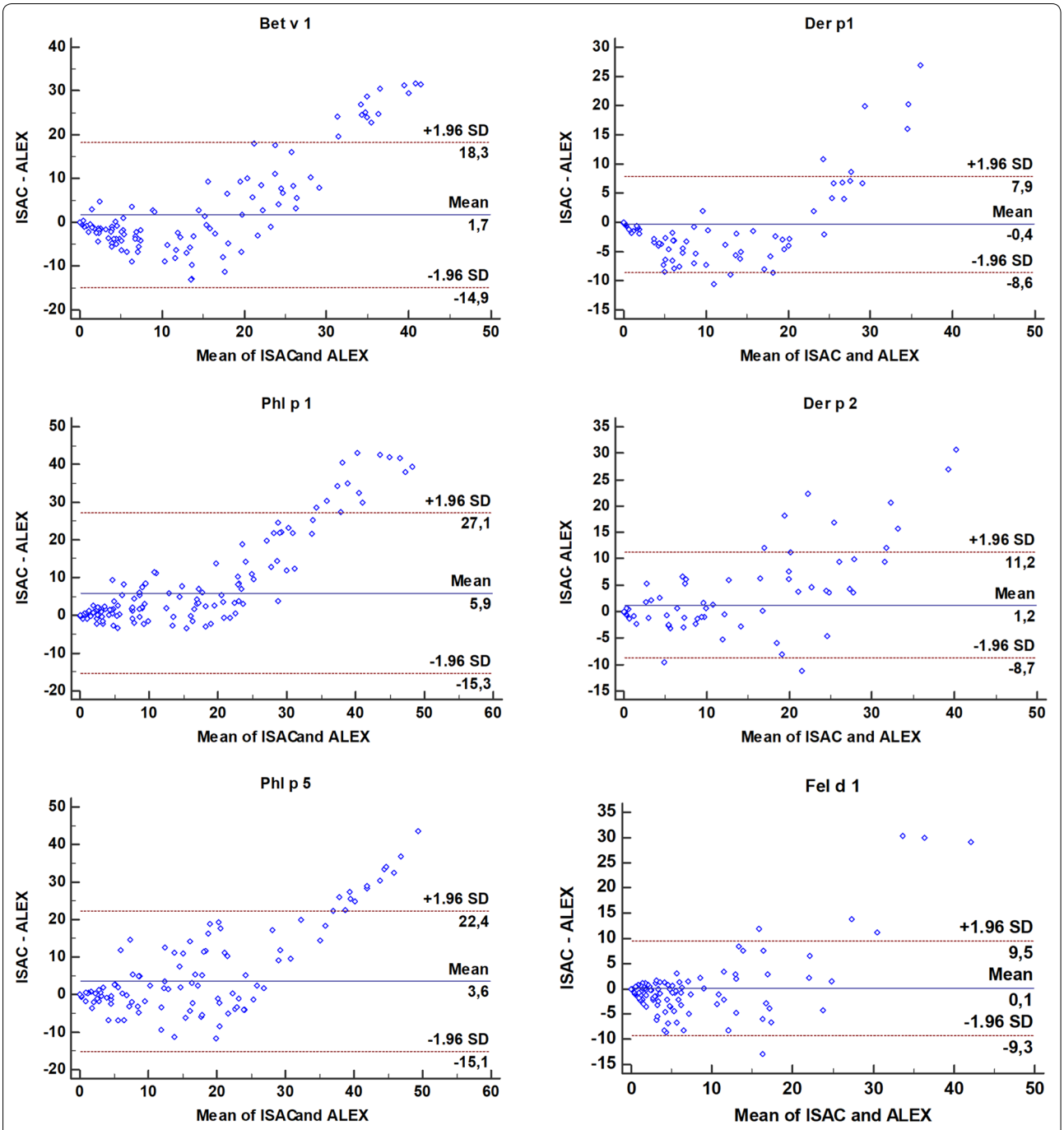

Fig. 2 Bland Altman plots for ISAC and ALEX

allergy. A recently published guide to molecular allergy diagnosis highlights the many advantages of molecular diagnosis. One interesting aspect revealed recently by molecular diagnosis is that sensitization to certain allergen molecules and a combination of allergens is more common for certain allergic manifestations than for others [10].

Every immunoassay is based on specific concentrations of antigens, test sera, enzyme-labeled detection antibodies and enzyme substrates suitable to offer the best dynamic range under the analytical conditions used. In allergy diagnostics, different platforms and substrates are currently used, and it is frequently observed that different serological assays generate

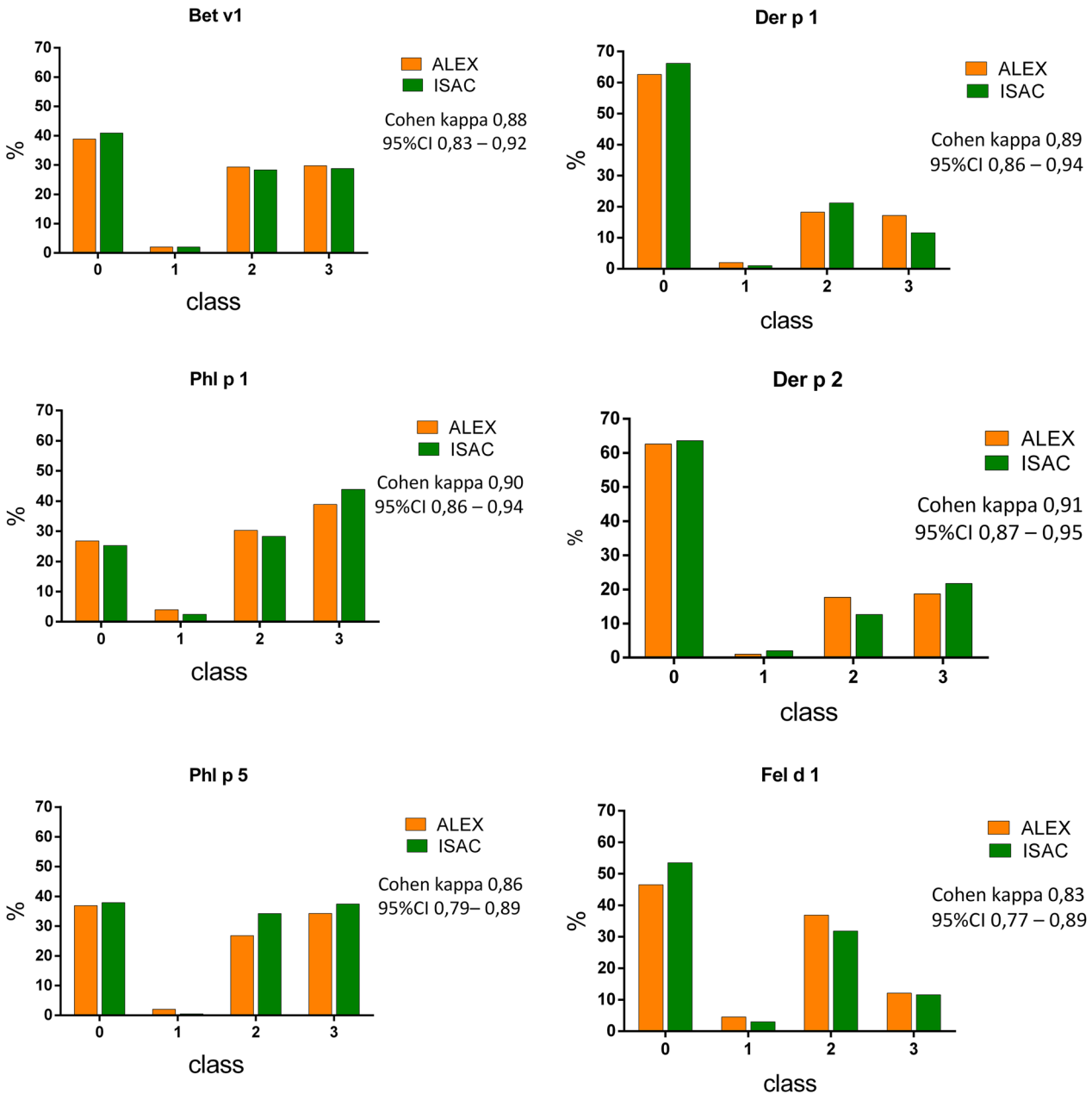

Fig. 3 Kappa analysis between ISAC and ALEX

different results, even if a correlation is observed under certain operative conditions. In addition, the more sophisticated the assay is, the greater the heterogenity of the results.

Having in mind these concepts, in the present study, we compared the ALEX multiplex platform, a novel tool that could be properly used in the bottom-up strategy of allergy diagnostics, and the ISAC multiplex platform.

When comparing the same molecular components on ALEX and ISAC, it should be considered that the solid phases were different, as well as the serum dilutions, the detection antibody, and the enzyme substrates. Additionally, ALEX uses a CCD inhibitor while ISAC does not. Nevertheless, laboratory methods are procedures that attempt to mimic in vitro what is suspected to occur in vivo and, more importantly, the results from in vitro tests are used to support the allergist's

diagnosis and therapy. However, despite technical differences, the results showed a substantial agreement between the two methods, similar results were showed at the study by Francesca et al. [11].

In conclusion, the results of ALEX correlated well with ISAC assay. Nevertheless, there is an overestimation of the ALEX methodology when comparing with ISAC at the high levels of concentration. However, as each diagnostic method has unique characteristics, the results are not interchangeable.

In the allergy workup, the ALEX assay is another additional diagnostic tool for the assessment of complex cases and the comparison to other tests has to be performed with care and based on profound knowledge of molecular allergy diagnostics.

Abbreviations
ALEX: allergy explorer; ISAC: immuno-solid-phase allergen chip; MA: molecular allergology; sIgE: specific IgE; CCD: charge coupled device; CCD: cross-reactive carbohydrate determinants.

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None.

Authors' contributions
JB, TV, PF, and PP each contributed to the design, acquisition, analysis, and interpretation of the data, and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval
This was a laboratory study where two methods commercially available were used to describe the IgE profile in a group of sera collected for diagnostic reasons in allergic patients. Sera were identified by a unique laboratory code and were processed in an anonymous procedure. The study protocol was approved by the local ethics committee prior to the initiation of the study.

Consent for publication
Not applicable.

Competing interest
P. Forstenlechner is an employee of Macro Array Diagnostics GmbH, Vienna (Austria).

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