Evaluation of PR-10 allergen sensitization profiles in two different multiplex test systems

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Background

Diagnosis of type I hypersensitivities is based on anamnesis, skin- and blood testing and provocation testing. Serum testing for specific IgE (sIgE) can be performed in both single- and multiplex formats. While single plex testing gives a limited view over the patients potential cross-reactions, multiplex tests easily allow to scrutinize the sensitization pattern within a protein family.
The PR-10 like family, with its most prominent member Bet v 1, is one of the most common sensitizers in birch endemic countries like Austria.
The objective of this study was to evaluate the performance of the novel ALEX ® (Allergy Explorer) multiplex platform for the detection of sIgE against PR-10 like allergens and to compare it with the ImmunoCAP ISAC® test system.

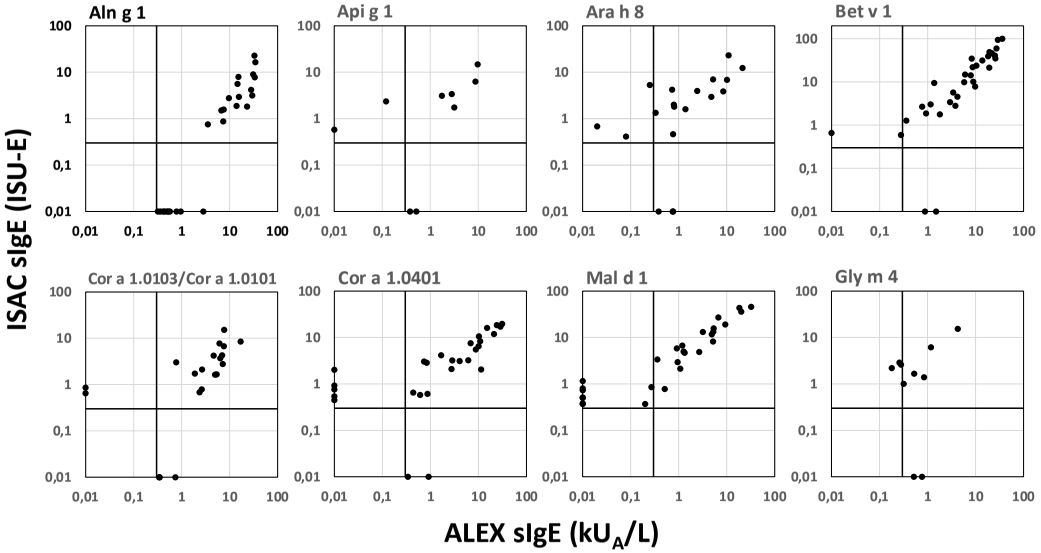

Figure 1. Scatter plot of sIgE values for relevant PR-10 like family allergens obtained by ALEX versus ISAC.

Methods

Serum samples of 32 PR-10 sensitized individuals were analyzed for the presence of specific IgE with the two multiplex test systems ALEX ® and ImmunoCAP ISAC ®.
Assuming a cutoff value of 0.3 (kU_A/L or ISU-E), results for the PR-10 like allergens Aln g 1, Api g 1, Ara h 8, Cor a 1.01, Cor a 1.04, Gly m 4 and Mal d 1 were subjected to statistical analysis using MedCalc. Correlation coefficients and rank order correlation coefficients were calculated according to the methods of Pearson and Spearman, respectively. sIgE data pairs which were above the cutoff in at least one of the two test systems were displayed in a scatter plot (values below 0.01 were plotted as 0.01 to be shown in the graph). A qualitative analysis of results was performed in a 2x2 table format by comparing the results obtained by ALEX ® with the ISAC ® data (as a non-reference standard) and by computing positive percent agreement (PPA) and negative percent agreement (NPA) values as indicated below:

Positive percent agreement (PPA) = 100% x a/(a+c)
Negative percent agreement (NPA)= 100% x d/(b+d)

	ISAC +	ISAC -
ALEX +	a	b
ALEX -	c	d
	a+c	b+d

Results

The statistical analysis of the sIgE test results is summarized in the table and positive results are plotted in the figure.
Bet v 1 was found to be the most prevalent sensitizing molecule by both tests systems and data pairs obtained by both methods were strongly associated (r and rho >0.9). The ALEX test showed good positive agreement with ISAC (>90% PPA) for the detection of this important allergen.

More positive samples for the food allergens Api g 1 and Gly m 4 are needed to come to a statistically meaningful conclusion as reflected by the influence of outliers on the calculation of their correlation and agreement values.
Both test systems display one PR-10 allergen with substantially more positive results near the LoD (Aln g 1 for ALEX, Mal d 1 for ISAC). Potential explanations for such varying results are the different methods utilized to immobilize the molecules to different solid-phases resulting in the potential misrepresentation of certain allergen epitopes.

Allergen	ALEX positive	%	ISAC positive	%	PPA	%	(95%CI)	NPA	%	(95% CI)	Correlation coefficient r		r²	Coefficient of rank correlation rho	
Aln g 1	26	81.3	16	50.0	16/16	100.0	(79.4 - 100)	6/16	37.5	(15.2 - 64.6)	0.77	P<0.0001	0.60	0.94	P<0.0001
Api g 1	7	21.9	7	21.9	5/7	71.4	(29.0 - 96.3)	23/25	92.0	(74.0 - 99.0)	0.89	P=0.0015	0.78	0.73	P=0.0262
Ara h 8	16	50.0	16	50.0	13/16	81.3	(54.4 - 96.0)	13/16	81.3	(54.4 - 96.0)	0.72	P=0.0005	0.52	0.66	P=0.0021
Bet v 1	30	93.8	30	93.8	28/30	93.3	(77.9 - 99.2)	0/2	0.0	(0.0 - 84.2)	0.92	P<0.0001	0.85	0.93	P<0.0001
Cor a 1.0103/ 1.0101	18	56.3	17	53.1	16/18	88.9	(65.3 - 98.6)	11/14	78.6	(49.2 - 95.3)	0.69	P=0.0008	0.47	0.83	P<0.0001
Cor a 1.0401	23	71.9	26	81.3	21/26	80.8	(60.7 - 93.5)	4/6	66.7	(22.3 - 95.7)	0.94	P<0.0001	0.88	0.86	P<0.0001
Gly m 4	7	21.9	8	25.0	5/8	62.5	(24.5 - 91.5)	22/24	91.7	(73.0 - 99.0)	0.93	P=0.0001	0.86	0.16	P=0.6505
Mal d 1	19	59.4	28	87.5	19/28	67.9	(47.6 - 84.1)	4/4	100.0	(39.8 - 100)	0.94	P<0.0001	0.89	0.94	P<0.0001

Table 1. Statistical analysis of the sIgE test results.

Conclusion

The ALEX multiplex IgE test holds promise to be a valuable tool for the multiplex molecular IgE diagnosis of PR-10 like allergen sensitizations.
Considering the methodological differences, both test systems generated comparable results.
In the absence of a reference standard for multiplex sIgE testing, care must be taken when comparing different test methods and interpreting their results.