

A Comparison of ISAC_{E112i} and ALEX² Immunoassay Test Results

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Introduction

Both the ISAC_{E112i} (Immuno Solid Phase Allergy Chip) and the ALEX² (Allergy Explorer 2) assays allow for testing for sIgE to a large number of allergen components from a small volume of patients sample (30µl for the ISAC_{E112i} and 100µl for the ALEX²). The ISAC_{E112i} tests for up to 112 allergen components and the ALEX² tests for up to 300 components (including total IgE). There are 96 allergen components that are present on both the ALEX² and the ISAC_{E112i} (i.e. have identical nomenclature). The ALEX² assay can be run as an automated assay on the MAX45K processor, whereas the ISAC_{E112i} assay is a more manual assay. This study aims to compare results for Peanut Ara h9 LTP, Peanut Ara h2 2S albumin, Hazelnut Cor a8 LTP, Birch Bet v1 PR-10 and Mal d1 PR-10 components between the 2 methodologies.

Methodology

33 patient samples were tested using both the ISAC_{E112i} and the ALEX² assay. The ISAC_{E112i} and ALEX² use different units (ISU-E and KUA/L respectively) so values cannot be directly compared in a quantitative manner. Both the ISAC_{E112i} and ALEX² come with ranges to give an indication of the level of sIgE present to an allergen. For the purpose of this qualitative analysis of data each grade classification has been assigned a number of 1 to 4 and then this classification number compared in a qualitative analysis spreadsheet. The ALEX² had an additional grade of 1- 5 KUA/L (moderate) which has been classified as a 3 in line with the ISAC_{E112i} range of 1 – 14.9 which is classified as moderate/high. Classification of ALEX² and ISAC_{E112i} for data analysis purposes is shown in the table below:

ALEX ² Range KUA/L	ALEX ² Classification	Classification number assigned for data analysis	ISAC _{E112i} Range ISU-E	ISAC _{E112i} Classification	Classification number assigned for data analysis
<0.3	Negative of uncertain	1	<0.3	Undetectable	1
0.3 - 1	Low	2	0.3 – 0.9	Low	2
1 - 5	Moderate	3	1 – 14.9	Moderate / high	3
5 - 15	High	3			
>15	Very high	4	>= 15	Very high	4

Results

Qualitative comparison summaries of the ISAC and ALEX² are shown below:

Peanut Ara h9 LTP

Assay 1 - ISAC Results		Assay 2 - ALEX Results				
	1	2	3	4	Total	Percentage
1	30	0	0	0	30	90.9%
2	0	1	0	0	1	3.0%
3	0	0	1	0	1	3.0%
4	0	0	0	0	0	0.0%
Total	30	1	1	0	33	
Percentage	90.9%	3.0%	3.0%	0.0%		

Agreement	30.00	1.00	1.00	0.00	32.00
By chance	27.27	0.06	0.06	0.00	27.39

Observations in agreement (Pa)	0.970
Observations expected by chance (Pe)	0.830
Cohen's Kappa (k)	0.822
Standard Error of Kappa (SEk)	0.145
95% confidence interval from 0.537 to 1.106	
The strength of the agreement is considered to be very good.	

Peanut Ara h2 2S albumin

Assay 1 - ISAC Results		Assay 2 - ALEX Results				
	1	2	3	4	Total	Percentage
1	25	0	0	0	25	75.8%
2	0	0	0	0	0	0.0%
3	0	0	2	0	2	6.1%
4	0	0	0	1	1	3.0%
Total	25	0	2	1	33	
Percentage	75.8%	0.0%	6.1%	3.0%		

Agreement	25.00	0.00	2.00	1.00	32.00
By chance	18.94	0.00	0.18	0.91	20.03

Observations in agreement (Pa)	0.970
Observations expected by chance (Pe)	0.607
Cohen's Kappa (k)	0.923
Standard Error of Kappa (SEk)	0.072
95% confidence interval from 0.782 to 1.064	
The strength of the agreement is considered to be very good.	

Hazelnut Cor a8 LTP

Assay 1 - ISAC Results		Assay 2 - ALEX Results				
	1	2	3	4	Total	Percentage
1	30	0	0	0	30	90.9%
2	0	0	0	0	0	0.0%
3	0	0	0	0	0	0.0%
4	0	0	0	0	0	0.0%
Total	30	0	0	0	30	
Percentage	90.9%	0.0%	0.0%	0.0%		

Agreement	30.00	2.00	0.00	0.00	32.00
By chance	28.18	0.18	0.00	0.00	28.36

Observations in agreement (Pa)	0.970
Observations expected by chance (Pe)	0.860
Cohen's Kappa (k)	0.784
Standard Error of Kappa (SEk)	0.208
95% confidence interval from 0.377 to 1.192	
The strength of the agreement is considered to be good.	

Birch Bet v1 PR-10

Assay 1 - ISAC Results		Assay 2 - ALEX Results				
	1	2	3	4	Total	Percentage
1	15	0	0	0	15	45.5%
2	0	0	0	0	0	0.0%
3	0	0	4	0	4	12.1%
4	0	0	0	0	0	0.0%
Total	15	0	4	0	19	
Percentage	45.5%	0.0%	12.1%	0.0%		

Agreement	15.00	0.00	4.00	0.00	19.00
By chance	7.75	0.06	0.91	0.03	11.73

Observations in agreement (Pa)	0.848
Observations expected by chance (Pe)	0.355
Cohen's Kappa (k)	0.765
Standard Error of Kappa (SEk)	0.090
95% confidence interval from 0.588 to 0.942	
The strength of the agreement is considered to be good.	

Apple Mal d1 PR-10

Assay 1 - ISAC Results		Assay 2 - ALEX Results				
	1	2	3	4	Total	Percentage
1	18	0	0	0	18	54.5%
2	0	0	0	0	0	0.0%
3	0	0	2	0	2	6.1%
4	0	0	0	1	1	3.0%
Total	18	0	2	1	21	
Percentage	54.5%	0.0%	6.1%	3.0%		

Agreement	18.00	1.00	2.00	1.00	22.00
By chance	12.55	0.09	1.64	0.15	14.42

Observations in agreement (Pa)	0.667
Observations expected by chance (Pe)	0.437
Cohen's Kappa (k)	0.408
Standard Error of Kappa (SEk)	0.122
95% confidence interval from 0.168 to 0.647	
The strength of the agreement is considered to be poor.	

There was good agreement in results for sIgE to Ara h9 (97%), Ara h2 (97%), Cor a8 (97%) and Bet v1 (85%). However, for Mal d1 just 67% of samples were in agreement. For Mal d1, 5 out of 33 samples (15%) differed by 2 classifications. All 5 samples were

graded as 1 on the ALEX² and grade 3 on the ISAC_{E112i}. It should be considered if these 5 patients are sensitised to cross reactive carbohydrate determinants (CCD's). CCD's are rarely associated with allergic reactions but may produce in-vitro positive test results to CCD containing allergens. MUXF3 (Bromelain) is a CCD in the ISAC_{E112i} panel (but not present in the ALEX²) which may help to determine if the ISAC_{E112i} Mal d1 sIgE results could be due to cross-reactive CCD's. The ALEX² assay includes a step during sample incubation to inhibit CCD reactions. Details for these 5 discrepant samples are shown in the following table:

Sample ID	sIgE to Mal d1 ISAC _{E112i} ISU-E	sIgE to Mal d1 ALEX ² KUA/L	MUXF3 on ISAC _{E112i} ISU-E	Patient sensitised to other PR-10 cross reactive allergens on ISAC _{E112i}
1	3.2 moderate/high	0.16 Negative/uncertain	0.6 Low	Bet v1 (12 ISU-E) Aln g1 (3.1 ISU-E) Cor a 1.0101 (6 ISU-E) Cor a 1.0401 (5.4 ISU-E) Pru p1 (4.7 ISU-E) Ara h8 (1.9 ISU-E)
2	1.2 moderate/high	<0.1 Negative/uncertain	0.4 Low	Bet v1 (5.8 ISU-E) Cor a 1.0401 (1.3 ISU-E) Pru p1 (1.1 ISU-E)
3	1.2 moderate/high	<0.1 Negative/uncertain	<0.3 Undetectable	Bet v1 (5.2 ISU-E) Aln g1 (0.4 ISU-E) Cor a 1.0101 (0.5 ISU-E) Cor a 1.0401 (3.2 ISU-E) Pru p1 (1.1 ISU-E) Gly m4 (0.5 ISU-E)
4	3.2 moderate/high	0.17 Negative/uncertain	<0.3 Undetectable	Bet v1 (22 ISU-E) Aln g1 (3.5 ISU-E) Cor a 1.0101 (0.5 ISU-E) Cor a 1.0401 (13 ISU-E) Pru p1 (0.9 ISU-E) Gly m4 (0.3 ISU-E) Ara h8 (0.5 ISU-E) Act d8 (0.9 ISU-E) Api g1 (1.3 ISU-E)
5	1.2 moderate/high	<0.1 Negative/uncertain	0.9 Low	Bet v1 (2.2 ISU-E) Cor a 1.0401 (0.4 ISU-E) Pru p1 (0.4 ISU-E)

Although the Bet v1 results were classed as having a good agreement (85%) between the two assays, there was a lower correlation than obtained for the Ara h9, Ara h2, and Cor a8 sIgE reactions. Bet v1 is also a PR-10 protein however from the data it is uncertain if the disagreements are due to cross reactive carbohydrate determinants as:

- One of these samples had sIgE to Bet v1 higher in the ALEX² assay than the ISAC_{E112i} assay.
- Three samples had undetectable sIgE to MUXF3 on the ISAC_{E112i} assay.
- The fifth sample showing a disagreement for Bet v1 between the ALEX² and the ISAC_{E112i} had low levels of MUXF3 on the ISAC_{E112i} assay and also had sIgE to PR-10 cross reactive components, these are shown in the table below:

sIgE to Bet v1 ALEX ² KUA/L	sIgE to Bet v1 ALEX ² KUA/L	MUXF3 on ISAC _{E112i} ISU-E	Patient sensitised to other PR-10 cross reactive allergens on ISAC _{E112i} ?
2.2 moderate/high	0.64 Low	0.9 Low	Cor a 1.0401 (0.4 ISU-E) Mal d1 (1.2 ISU-E) Pru p1 (0.4 ISU-E)

Conclusion

This work aims to give an idea of the performance of the ALEX² in comparison to the ISAC_{E112i} assay. It highlights that some differences may be observed between results which may potentially be due to the presence of a CCD inhibition step in the ALEX² assay.

References and Acknowledgements

Bradshaw, N. (2019). Go Molecular! A clinical reference guide to molecular allergy, Part 2: The allergen components (2nd ed.). ThermoFisher Scientific.

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