HEALTH SERVICES LABORATORIES

A Comparison of ISAC$_{E112i}$ and ALEX^2 Immunoassay Test Results

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Introduction

Both the ISAC$_{E112i}$ (Immuno Solid Phase Allergy Chip) and the ALEX^2 (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 ALEX^2). The ISAC$_{E112i}$ tests for up to 112 allergen components and the ALEX^2 tests for up to 300 components (including total IgE). There are 96 allergen components that are present on both the ALEX^2 and the ISAC$_{E112i}$ (i.e. have identical nomenclature). The ALEX^2 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.
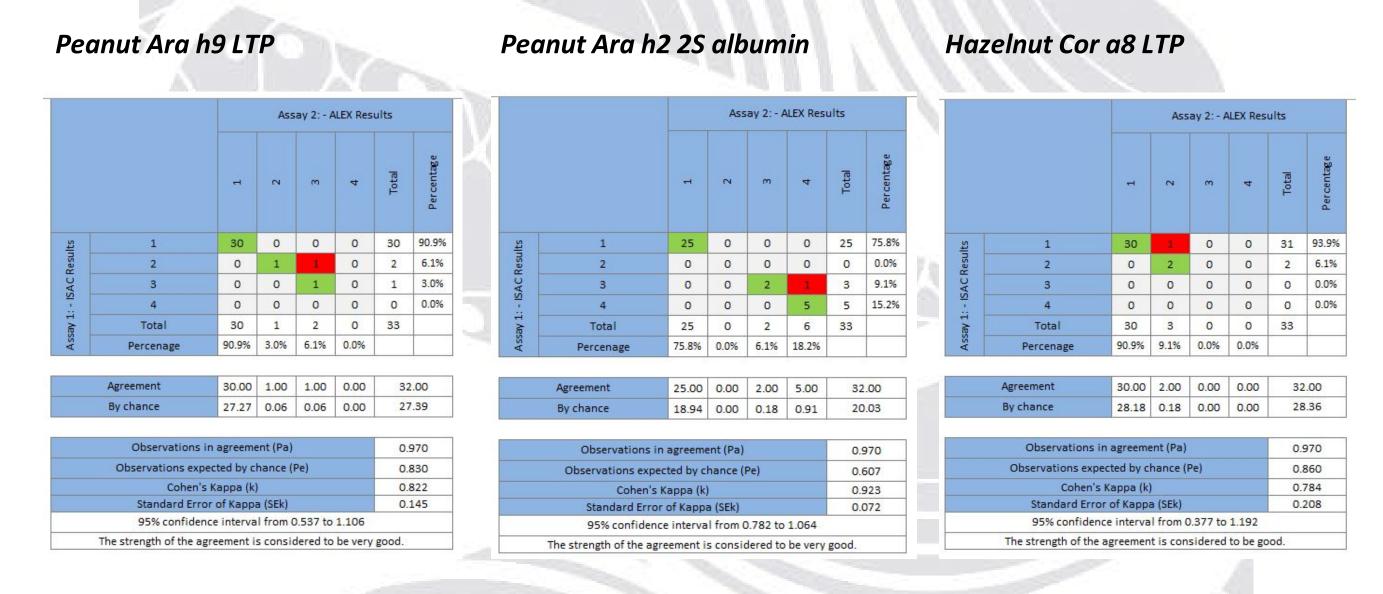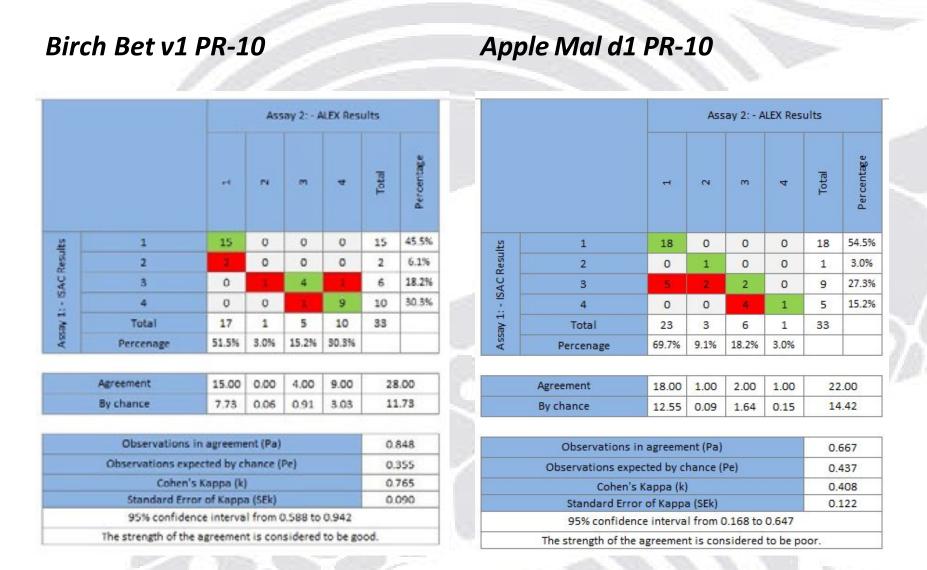
Methodology

33 patient samples were tested using both the ISAC$_{E112i}$ and the ALEX^2 assay. The ISAC$_{E112i}$ and ALEX^2 use different units (ISU-E and KUA/L respectively) so values cannot be directly compared in a quantitative manner. Both the ISAC$_{E112i}$ and ALEX^2 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 ALEX^2 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 ALEX^2 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 E$_{112i}$ Range ISU-E	ISACE$_{112i}$ 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 ALEX^2 are shown below:

Peanut Ara h9 LTP

Peanut Ara h2 2S albumin

Hazelnut Cor a8 LTP

Birch Bet v1 PR-10

Apple Mal d1 PR-10

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 ALEX^2 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 ALEX^2) which may help to determine if the ISAC$_{E112i}$ Mal d1 sIgE results could be due to cross-reactive CCD's. The ALEX^2 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 has 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 ALEX^2 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 ALEX^2 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 ALEX^2 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 ALEX^2 assay.