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ANALYTICAL EVALUATION OF IMMUNOCAP ISAC AND RESULTS FOR A MULTI-SENSITISED PATIENT

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INTRODUCTION

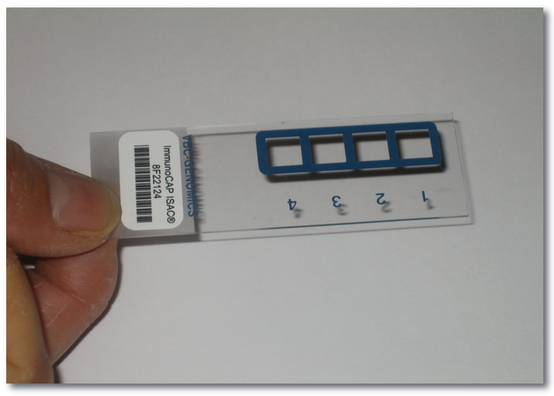
ImmunoCAP ISAC is a biochip that provides in one run semi-quantitative results for 103 specific IgEs of recombinant or native components corresponding to 40 allergenic sources, using only 20 µl of serum. The aim of the study is to evaluate ISAC in comparison with individual components performed on UniCAP and to show an ISAC profile for a patient with pollinosis and food symptoms.

METHOD

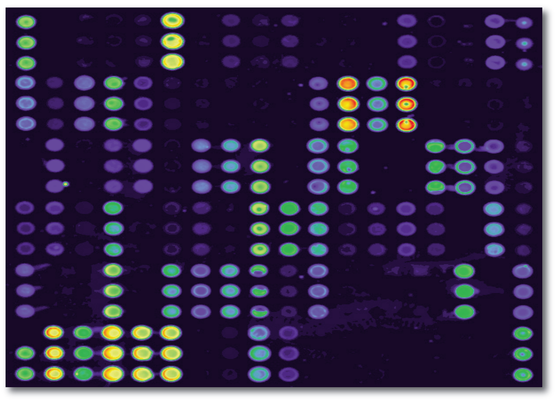
Sera of patients and controls were analysed by UniCAP and ISAC biochip according to manufacturer's guidelines. ISAC IgEs are quantitative results in ISU (ISAC Standardized Units) ranging from 0.3 to 100 ISU but they have to be changed into classes to interpret them.

A comparison study was performed between 110 allergen components results obtained by testing 14 samples on ISAC biochip and UniCAP for the unitary available components (timothy, birch, mites, latex, peanut, cow milk proteins, egg proteins).
UniCAP Specific IgEs were ranged into classes to allow comparison with ISAC results. The specific IgE Quality Control serum was tested in 20 different ISAC runs (with different batches of reagents) to calculate the inter-assay precision of 11 representative components at various levels.

The four reaction sites of the microarray slide, containing each 103 allergen components in triplicate



Fluorescence signal obtained with the specific IgE Quality Control serum ; IgE levels are evaluated according to the calibration serum



RESULTS

We obtained a concordance of 98 % with 1 class of difference and of 78 % at identical class for components tested with ISAC and UniCAP. The results were satisfying for the allergens that we could compare. We couldn't conclude that one method was more sensitive than the other one for the components compared.

Using the quantitative ISAC results released in ISU, inter-assay CVs were between 16 % (Bet v 2: mean 2.1 ISU) and 39 % (Fel d 1: mean 6.5 ISU).
The other CVs are shown in *table 1*. Other teams that also experienced high CV % pointed out the role of the spotting step and the variability of immunoreactivity of the components on the biochip.

Case study

We tested the serum of a teenager living in the South West of France, allergic to grass pollen and treated by specific immunotherapy for 3 years. Recently, oral symptoms to food have appeared. Skin prick tests were positive to many fruits and vegetables (banana, avodaco, kiwi , mango, cherry, raspberry, peach, apricot, apple, pear, melon, orange, tomato). We wanted to discriminate genuine food allergy from cross-reactivity.
The tested serum revealed a high reactivity of all timothy components confirming the grass pollinosis (*table 2*). Almost all PR10 were positive whereas LTP were negative, explaining the oral syndrome. Avoidance of fresh fruits or vegetables is advised but they should be tolerated if cooked. Species specific markers for peanut, hazelnut, brazil and cashew nut, soya, latex are negative. High level of IgE against profilins and polcalcins were in relation with the wide cutaneous reactivity. This reactivity profile did not reveal any sign of severity of allergy.

Table 1: Inter-assay CVS of composants obtained in 20 ISAC runs

	Level, mean (ISU)	Inter-assay CV (%)
Der p 1	3.3	19
Der p 2	2.7	22
Der p 10	1.3	24
Fel d 1	6.5	39
Alt a 1	8.1	38
Phl p 1	8.8	22
Phl p 5	11.2	28
Bet v 1	5.8	19
Bet v 2	2.1	16
Gly m beta conglycinin	10.1	16
Gly m glycinin	7.6	23

Table 2: Summary of ISAC specific IgE for the 14 years old patient

Species Specific components	PR-10	Cross reactive components	Other components
Phl p 1 = 27 Phl p 2 = 10 Phl p 4 = 15 Phl p 5 = 10 Phl p 6 = 7 Phl p 11 = 2	Bet v 1 = 53 Aln g 1 = 20 Cor a 1 = 20 Mal d 1 = 30 Pru p 1 = 42 Gly m 4 = 3 Ara h 8 = 5 Act d 8 = 13 Api g 1, Dau c 1 =0	**Profilins +++** Bet v 2 = 25 Ole d 2 = 25 Hev b 8 = 23 Mer a 1 = 21 Phl p 12 = 10	Der f 2 = 12 Der p 2 = 8 Eur m 2 = 2 Der p 1 = 0 Der f 1 = 0
Cyn d 1 = 6 Ole e 1 = 47 Pla a 2 = 3		**Ca Bp +++** Bet v 4 = 40 Phl p 7 = 18	Tropomyosins = 0 Albumins = 0
Ara h1/h2/h3 = 0 Cor a 9 = 0 Ber e 1 = 0 Ana o 1 = 0 Gly m b c = 0 Hev b 1/3/5/6 = 0		**LTP = 0** (Pru p 3, Cor a 8, Art v 3, Par j 2) CCD = 0	Egg, cow milk, cat, dog, mould, fish = 0

CONCLUSION

ISAC performances are satisfying if interpreted on a semi-quantitative basis. Its main interest is to provide a complete profile of sensitisation which were useful to investigate multi-sensitised patients or complex clinical cases and then to differenciate co-sensitisation and cross-sensitisation in the diagnosis of allergy. Of course all the results have to be interpreted according to the clinical history.

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