

Comparison of specific IgE tests by Immulite 2000 and UniCAP 100

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Aim of the study

Recent availability of a new method for specific IgE (sIgE) in total automation on Immulite 2000 (DPC, Los Angeles, CA, USA) present from several years in our, as in a big number of laboratories, for traditional immunochemical assays induce us to verify, more than organizing aspects of which we discussed previously, the analytical quality of proposed test with a dedicated experimentation with a protocol articulated and complete. We compared new product to the methodology historically used in our laboratory for "in vitro" allergic tests and in general considered as gold standard for sIgE determination. (UniCAP 100; Pharmacia Diagnostics, Uppsala, SWEDEN).

Methods

Evaluated test, Immulite 2000 Allergen-Specific IgE, performs determination of IgE allergen specific antibodies with a solid-phase, two-steps chemiluminescent immunoassay (each step with incubation time of 30') that exploits liquid phase kinetics in a bead format. The allergens, in liquid phase, are covalently bound to a soluble polymer/co-polymer matrix, which in turn is labelled with a ligand. For washing and measuring steps are employed traditional beads technology of Immulite, in this case coupled with anti-ligand. The comparison method, UniCAP Specific IgE, is based on ImmunoCAP technology (allergens are covalently coupled into a high capacity flexible hydrophilic carrier polymer - cellulose derivative- encased in a capsule) with fluorescence tracer, applied to the fully integrated and automated system UniCAP 100.

Materials

We performed about 1000 assays from 153 patients for both systems, according to manufacturer recommendations. 50 to 136 samples were assayed for each of the allergens (important for our laboratory) in Table 1, with a proportion of at least 75% of positive samples, spread all along the calibration curve. We analysed the reproducibility, quantitative and qualitative correlations, evaluation of crosswise calibration systems, dilution tests, and interferences of added elevated amounts of total IgE. For all assay runs we performed a dedicated scheme of Internal Quality Control, using both a negative and positive control serum.

Conclusion

Beyond organizing aspects due to elevated automation of Immulite 2000, from an analytical point of view it's necessary highlight that imprecision seems excessive, at least on positive controls. We think that is opportune to verify this variability on different control material and allergens because is too high respect system's routine performances. It's necessary to not underestimate the trend to provide higher values of nominal when we test Pharmacia calibrators as samples on Immulite 2000 and also the evidence of interference due to total IgE on evaluated allergens. These aspects could probably explain (but not justify) higher values obtained on Immulite 2000 for biological samples.

For sIgE determination is necessary to take in consideration that every allergen should be evaluated, also within the same methodology, as an independent parameter. It's difficult and hazardous to extend conclusion from a single allergen to all. After this preliminary remarks, it's dutiful to emphasize significant discordance between the two systems and that regards more than 50% of employed samples. In particular we have observed that about 20% of the samples are classified by Immulite 2000 in a different status (sensitized or not sensitized to that particular allergen) rather than UniCAP 100 and this can bring clinicians to a different approach towards the patient.

On account of differences obtained with the two systems on analytical results and without the availability of the "true" value of sIgE on single biological sample, if a laboratory would decide to introduce a new system, with different performances in term of sensitivity and specificity, it should at least take in consideration the necessity of a reassessment for both cut-off values of negative and positive samples and levels of classification supplied to clinicians to avoid mistakes on the clinical classification of patients. This parameters are not easy and practicable for individual laboratories and should be verified, for each allergen, with cumulate data and suitable statistical technique.

Results

Table 1 - Evaluated Allergens (n)

w1	Common Ragweed - <i>Ambrosia elatior</i>	124
w19	Wall pellitory - <i>Parietaria officinalis</i>	116
d1	House dust mite - <i>Dermatophagoides pteron.</i>	124
t3	Birch - <i>Betulla verucosa</i>	136
i9	Olive tree - <i>Olea Europea</i>	100
g6	Timoty - <i>Phleum pratense</i>	113
m2	Hormodendrum Herbarum - <i>Cladosporium</i>	56
e1	Cat epithelium and dander	50
f1	Egg white	98
f4	Wheat	99
i3	Common wasp - <i>Vesputa Spp.</i>	104

Table 2 - Global results

(test performed in replicate for both of two different analytical runs: for each allergen n= 4)

UniCAP 100: n = 1114 Immulite2000: n = 1009

265 negative (24%) 375 negative (37%)

849 positive 634 positive

(33 with conc >100) (103 with conc >100)

UniCAP 100	assay run	Immulite 2000
42	n	7 (21 test)
83	x*	44
42.48	(min-max)	(71055-121980)
6.49	ds*	9549
9.75%	CV	10.38%

*: analytical signal
 @ mean of analytical signal for calibrator at 0.35 KU/L - 200 system blank = 20

Figure 1a - Internal Quality Control : negative = f1

UniCAP 100	assay run	Immulite 2000*
42	n	7 (20 test)
84	x (kU/L)	29.49
12.81	(min-max)	(15.4-45.5)
1.39	sd	7.06
10.91%	CV	23.94%

* mean CV% for immunochemical test: 4.09% for tPSA 13.10% for intact PTH

Figure 1b - Internal Quality Control : positive = g6

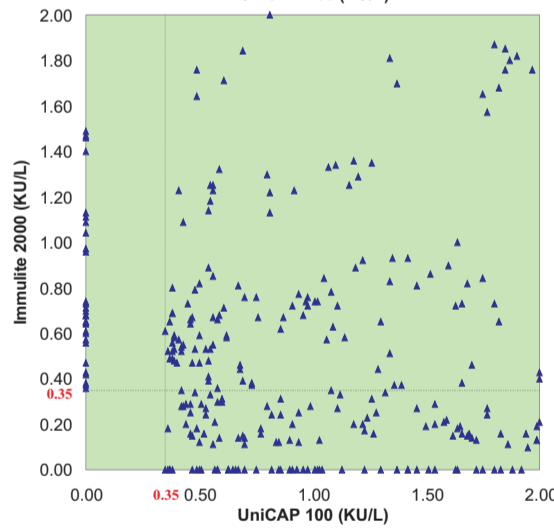
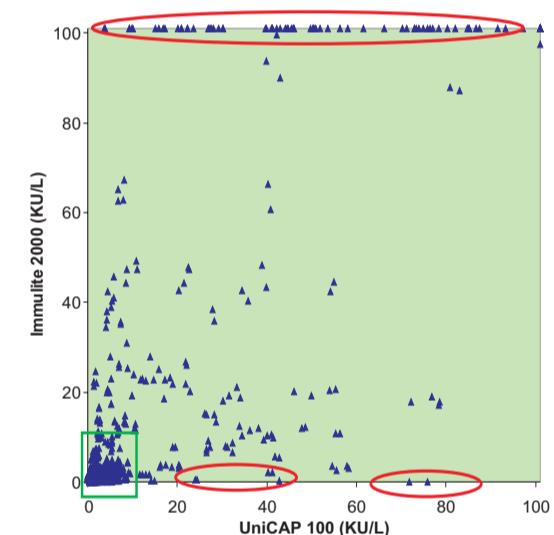


Figure 2 - Analytical results: global comparison (right part: detail of green square)

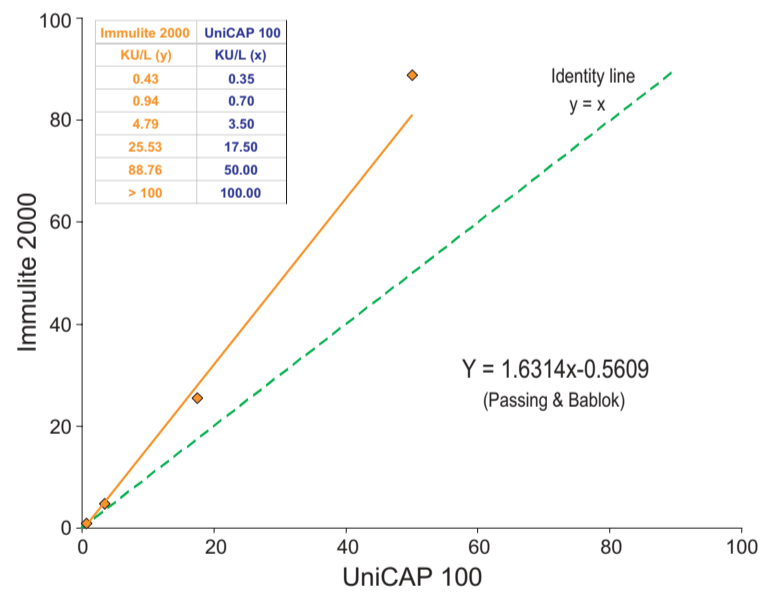


Figure 3 - Calibrators crosswise: UniCAP 100 tested on Immulite 2000

Table 3 - Analytical results comparison (Immulite 2000 vs UniCAP 100)

Allergen	Performed analysis		POSITIVE sample		NEGATIVE sample		UniCAP 100			Immulite 2000						
	tests (n)	samples (n)	Uni CAP 100	Imm 2000	Uni CAP 100	Imm 2000	KU/L			KU/L			sample classification			
							M	sd	m	M	sd	m	conc. (KU/L) <50%	neg vs pos	conc (KU/L) >50%	pos vs neg
w1	124	31	25	22	6	9	10.05	14.77	1.76	7.11	18.68	0.68	12	3	1	-
d1	119	29	22	19	7	10	15.06	26.15	2.00	18.81	33.55	0.86	4	3	6	-
t3	120	30	23	21	7	9	20.23	32.60	2.10	35.02	35.31	2.34	9	3	8	-
w19	116	29	23	10	6	19	11.84	20.81	1.90	3.51	8.97	0.14	20	13	-	-
g6	111	28	24	20	4	8	20.20	35.13	3.63	26.71	41.46	3.28	5	4	4	-
m2	56	12	6	2	6	10	1.38	2.14	0.48	1.39	0.85	0.10	4	4	1	-
t9	100	25	20	18	5	7	6.36	13.07	2.14	10.53	25.52	1.84	7	2	6	-
f1	98	25	18	16	7	9	5.82	18.95	0.89	5.24	19.82	0.56	9	4	3	2
f4	96	25	19	17	6	8	4.13	6.38	1.99	1.29	1.67	0.65	13	2	-	-
i3	104	26	20	22	6	4	11.38	25.62	1.96	15.96	27.11	3.64	3	1	14	3
e1	50	13	7	9	6	4	14.83	24.48	1.87	38.06	44.31	7.01	-	-	9	2
All	1094	273	207	176	66	97	11.66	23.75	1.66	14.50	30.97	0.73	86	39	52	7

M = mean; sd = standard deviation; m= median
 For statistical evaluation: results < 0.35 KU/L = 0.10 KU/L; results > 100 KU/L = 100 KU/L

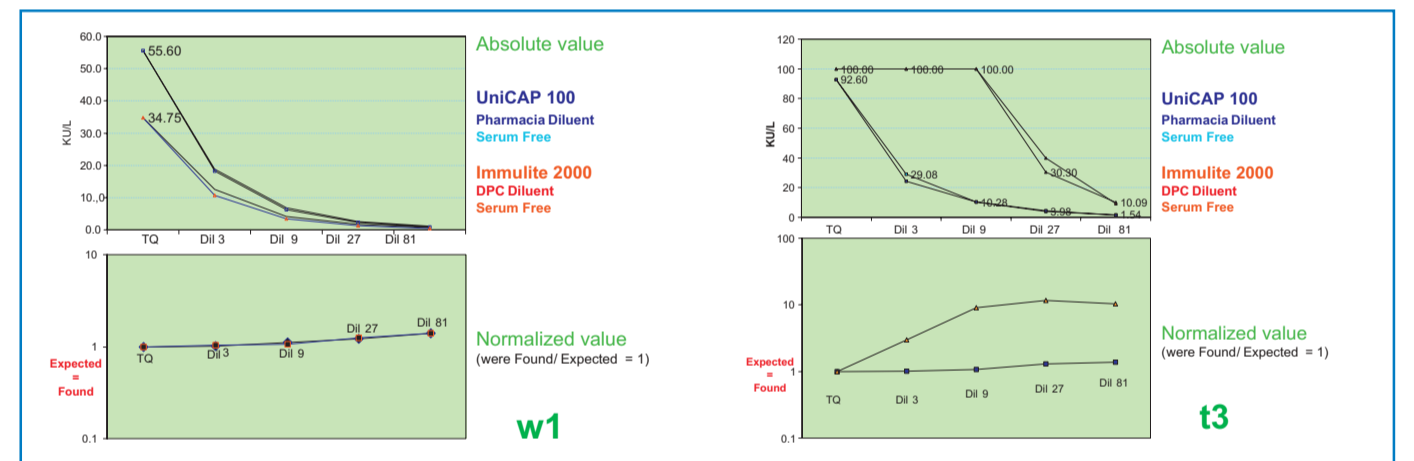


Figure 4 - Dilution test

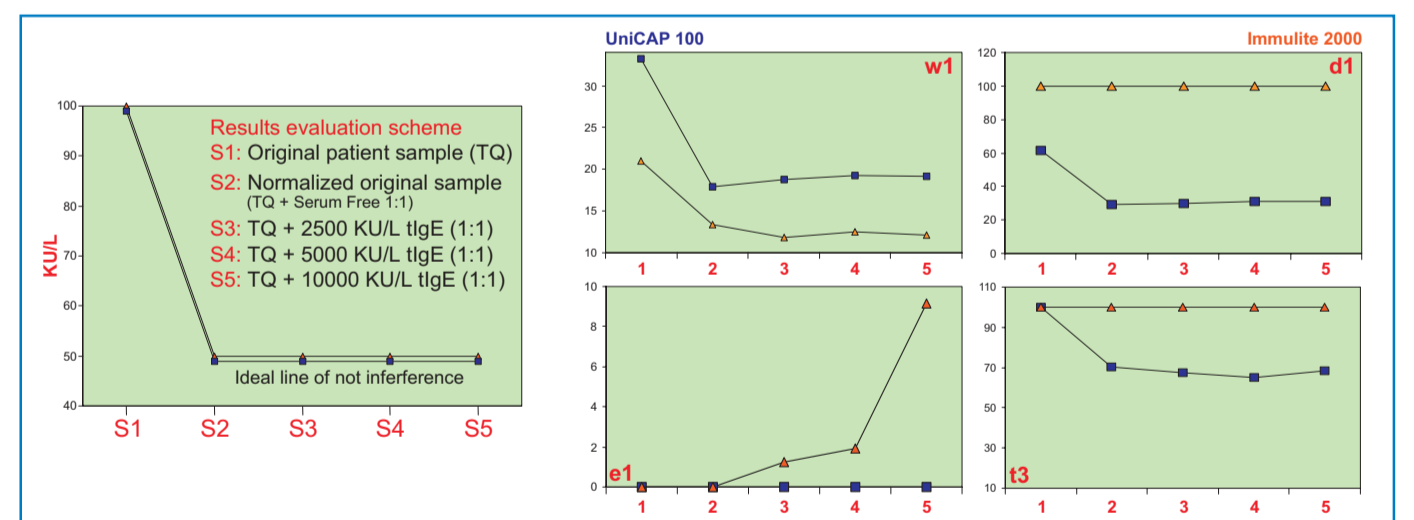


Figure 5 - Total IgE interferences

Quality Control

In Figure 1 we report results of commercial control sera used on all the assay runs for both systems. For negative control we used allergen f1 (Fig. 1a) and for positive control g6 (Fig. 1b). Regarding UniCAP 100, as normally in our daily routine work, we used for the replicate assay runs, two of the three instruments in our laboratory. Results of Internal Quality Control are reported as cumulate data from the two different instruments. Imprecision is good for UniCAP 100, with a CV less than 10% both for negative and positive control; same level for Immulite 2000 on negative control, but higher for positive control with a CV approximately of 25%. This variability seem too high (without explanation) for this test and for this system. In our laboratory on the same instrument we have imprecision from 4% for tPSA to a maximum of 13% for intact PTH. It is probably necessary to verify this bad performance on different control material and with other allergens.

Sample Comparison (Immulite 2000 vs. UniCAP 100)

In table 2 we report a global analysis of results. We can observe that, from the analytical point of view, the two systems give values that are not comparable. sIgE levels from Immulite 2000 are, for most of the allergens, different (both with higher and lower concentration) from sIgE obtained on the same sample with UniCAP 100. Table 3 shows that for some allergens (d1, t3, m2, t9) mean and median value give opposite information, probably for different incidence of samples with very low (<0.35 KU/L) or very high (>100 KU/L) concentration on two statistical parameters. We can note for Immulite 2000 an important over-estimation for t3, i3 and e1 and an important under-estimation for other allergens (w1, f4, and in particular m2 and w19; for this two allergens more than 50% of positive values on UniCAP 100 are negative on Immulite 2000). In the table, we have considered for sample classification (with arbitrary decision) like different the results given by Immulite 2000 if their concentration was more than 50% higher or lower when compared to the UniCAP 100 value; neg vs. pos means that sample has been classified negative with UniCAP 100 and positive with Immulite 2000 and pos vs. neg the opposite. With this classification we have 138 of 273 samples (50,5%) different with two systems with 86 (31,5%) under-estimated and 52 (19,0%) over-estimated by Immulite 2000; out of this 138, 46 samples (16,8%) give a different status of patient: 39 (14,3%) were allergic with UniCAP and not allergic (in particular to w19, g6, m2) with Immulite and 7 (2,6%) not allergic with

UniCAP and allergic (in particular to i3 and e1) with Immulite. Figure 2 is a graphical evidence of comparison data within the two systems with a detail for concentrations between 0 and 2 KU/L. For this distributions we have used only data with at least one positive results in one of the two systems.

Crosswise calibrators

In Figure 3 table and correlation within nominal values of UniCAP 100 calibrators (x values) and experimental results when calibrators are processed as samples on Immulite 2000 (y values). Although both systems use calibrators from the same preparation (WHO 75/502) there is evidence of over-estimation of nominal values by Immulite 2000 (the possibility of a matrix effect should be evaluated).

Dilution test

For this study we employed samples with high level (but not higher than calibrators linearity) diluted on a serial scheme (1/3, 1/9, 1/27, 1/81) with a dedicated diluent and for both systems with a serum IgE free. Each point was tested as patient samples, in replicate for both of two different analytical runs (n = 4).

We report in Figure 4, results for two allergens as example of different performance of two systems. In general we had a good linearity for all tested allergens (w1, d1, e1, m6, t3) for UniCAP 100 and unsatisfactory for Immulite 2000 with some allergens (in particular for t3 and for allergens with which we note over-estimation on comparison test). For this behaviour we can suspect a non-specific interference or a different choice of epitopes in the allergen preparation.

Total IgE interferences

For this evaluation we added several patient samples (for all the compared allergens) with a serum containing high amount (2500, 5000 and 10000 KU/L) of total IgE. To avoid influences from added serum, a volume normalisation with serum IgE free was made and the same preparation was used for both systems. In Figure 5 results are shown for different allergens in which evidence of insignificant interference from tIgE on UniCAP 100 and critical behaviour (in particular for e1, t3, d1) for Immulite 2000 can be seen.

References

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