

IN VITRO ALLERGY DIAGNOSIS: COMPARISON OF A NEW METHOD OF FULLY AUTOMATED DETERMINATION OF SPECIFIC IGE, USING IMMULITE 2000 COMPARED WITH UNICAP 100

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INTRODUCTION

The recent availability of a method for the fully automated determination of specific IgE (sIgE) based on a system which has normally been used for conventional determination by immunochemical methods (Immulite 2000; DPC Los Angeles, CA, USA; represented in Italy by Medical Systems, GE), and which has been used for this purpose for several years in our organisation, led us to verify the analytical quality of the proposed method, leaving aside the purely organisational aspects which we have discussed elsewhere (1), by carrying out appropriate testing in accordance with a sufficiently comprehensive and detailed protocol. We chose as our control method the procedure historically used in our laboratory and generally considered valid for the in-vitro determination of sIgE (UniCAP 100; Pharmacia Diagnostics, Uppsala, Sweden).

The problem faced in the evaluation of methods of determining sIgE relates to the large number of parameters affecting the outcome (exhaustive in-vitro allergy diagnosis requires the examination of approximately 150 allergens in the laboratory). Since each of the allergens considered has such distinctive characteristics, in terms of preparation, purification, and antibody behaviour towards the many different antigen determinants, that the investigator is effectively dealing with completely different analytes. Thus the conclusions drawn from analytical comparisons are frequently inconsistent or even contradictory, depending on the allergen in question. Moreover, a laboratory is obviously not free to choose different methods of determination for the different allergens according to objective evaluation parameters, as is commonly done, for example, in the conventional immunochemical deter-

mination of hormones and tumour markers. When choosing a method, it is therefore essential to have an exact knowledge of the analytical quality of the product in question, and especially of any problems which may arise with the chosen method.

In view of these considerations, we attempted to devise the tests according to a system which would provide the greatest possible amount of information in relation to any decisions that were made (2). We therefore considered a large number of samples relating to different patients (more than 150) in sufficient numbers for each allergen concerned in order to achieve a high level of statistical significance. We also selected the allergens from those which were generally most important in terms of requests to the laboratory. We considered that it was essential, for the purposes of comparison and evaluation, to prioritise the positive samples (at least 80% of the tests conducted) in our selection (with data distributed over the whole range of concentrations (0.35-100 kU/l) and good representation of the low concentrations. Regarding the quantitative analytical methodology, we considered it essential to use data expressed in International Units (WHO 75/502) (3).

MATERIALS AND METHODS

The Immulite 2000 Allergen-Specific IgE test which was to be evaluated provided for the determination of specific IgE allergens by immunoassay in two stages (each with an incubation time of 30 min.) using allergens in the liquid phase (covalently bound to a soluble polymer/copolymer matrix, which in turn is marked with a ligand). The washing and detection system used the conventional bead method of the Immulite system, bonded with an anti-ligand in this case (4). The control method, UniCAP Specific IgE, is based on the

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immunoCAP technology (allergens bonded on a solid high-capacity cellulose-based matrix) with fluorescence reading, using the UniCAP 100 automatic system (5, 6).

Table 1 shows the allergens used in our evaluation, selected according to their incidence in the requests made to the laboratory, with the aim of representing the various types of allergens and enabling a comparative study to be conducted on characterized biological samples relating to 153 different patients. We verified the reproducibility in respect of the selected samples, and the determinations for each allergen were replicated in both analytical systems in each of two different analysis sessions, giving a total of $n = 4$ replicates for each allergen. An important aspect of the evaluation of a method is the verification of its linearity at different concentrations, which is a prerequisite for ensuring the correctness of the results over the whole measurement range (7). For this purpose, we set up a dilution study for different allergens (w1, d1, e1, m6, t3), in which samples at a sufficiently high concentration, but not exceeding the limit of linearity of the calibrators (100 kU/l), were diluted by serial dilution (1/3, 1/9, 1/27, 1/81). For each analytical system we used the diluent recommended by the manufacturer and a serum free of IgE. To reduce any sources of imprecision in the dilution test to a minimum, we replicated each determination in each of two different analysis sessions ($n = 4$ replications). A comparison was also made between the calibrators used in each system (by cross-checking) and the possible effect of high total IgE levels on the determinations was evaluated. A dedicated IQC system, based on one negative and one positive control serum, was established for all the determinations.

	Allergen	n
w1	Ambrosia elatior	124
w19	Parietaria officinalis	116
d1	Dermatophagoides pteron.	124
t3	Birch	136
t9	Olive	100
g6	Phleum pratense	113
e1	Cat epithelium	50
m2	Hormodendrum-Cladosporium	56
f1	Albumen - Egg white	98
f4	Wheat	99
i3	Polistes species - Wasp	104

Table 1 : Allergens subjected to evaluation.

RESULTS

VARIABILITY OF ANALYSIS

Table 2 shows the results for the variability in different analysis sessions as yielded by control serums included systematically in the individual determinations. The positive control serum was determined by using the g6 allergen, and the negative serum was determined by means of the f1 allergen. For the negative control, since the concentrations were below the limit of sensitivity of the systems (namely 0.35 kU/l for UniCAP100 and 0.10 kU/l for Immulite 2000) and therefore no concentration value was available, the statistical evaluation was conducted on the analytical signal supplied by the instruments used. For UniCAP 100, the determinations were carried out, as for the routine samples, by using two of the three instruments present in the laboratory (instruments 1 and 2) alternately in the two analysis sessions scheduled for each allergen, and evaluating the overall variability in a cumulative way. The imprecision levels appear satisfactory for UniCAP 100, being within a CV of 10% (as found normally in everyday operations) both for the negative control and for the positive control (8), this level also being maintained with Immulite 2000, at least in respect of the negative control serum (for the sake of correctness it should be noted that, as specified above, for UniCAP 100 we were dealing with a variability relating to the cumulated data found with two instruments, and that the imprecision levels are reduced further for an individual instrument). The imprecision found with Immulite 2000 for the positive control serum was markedly higher, the CV being in the region of 25%, a level which appears excessive for this type of determination (9) and which, for unknown reasons, is much higher than the average found with the same instrument in our laboratory for routine immunochemical determinations (where the CV ranges from 4% for tPSA to 11% for intact PTH).

COMPARATIVE TESTING

Regarding the analytical comparison for the 153 patients concerned, we obtained 1114 results for UniCAP 100 and 1009 for Immulite 2000 (the difference was due to minor operational problems encountered during testing which prevented us from using some of the replications for individual samples). The totals of negative results were 265 (24%) and 375 (37%) respectively, while the totals of positive results were 849 (76%) for UniCAP 100 and 634 (63%) for Immulite 2000. The concentration of 0.35 kU/l was accepted as the negative/positive discrimination thresh-

shold for both systems. As regards the samples classified as positive by UniCAP 100, 33 of these (0.3% of the total and 3.9% of the positive total) had concentration levels beyond the limit of linearity of the calibration curve (100 kU/l); for Immulite 2000, the number of these samples was 108 (equivalent to 10.7% of the total and 17.0% of the positive results). The details of the statistical evaluations for the total population and for the individual allergens are shown in table 3. In these evaluations, for the purposes of statistical calcu-

lation, concentrations below the sensitivity of the two methods were arbitrarily set at 0.10 kU/l; similarly, the concentrations above the limit of linearity were set at 100 kU/l. A global analysis of these data suggests to us that, in purely analytical and numerical terms, the two systems appear to act in such a way as to provide values of concentration which cannot be superimposed and are not comparable, since the levels of sIgE found with Immulite 2000 appear, for many allergens at least, to be markedly different (in that they show a

	Allergen fl			Immucite 2000	Positive control - allergen g6			Immucite 2000
	UniCAP 100				UniCAP 100			
	instrument 1	instrument 2	instrument 1+2		instrument 1	instrument 2	instrument 1+2 2	
sessions	22	22	42	7 (21 determin.)	22	22	42	7 (20 determin.)
n	44	39	83	44	44	40	84	43
x (kU/l)	39.2 *	44.3 *	42.4 *	91988 *	13.90	11.58	12.81	29.49
min-max	33-46 *	36-57 *	33-53 *	71055-121980 *	11.1-15.5	10.3-12.6	10.3-15.5	15.4-45.5
sd	2.29 *	3.96 *	6.49 *	9549 *	0.93	0.54	1.39	7.06
cv%	5.83	8.93	9.75	10.38	6.66	4.64	10.91	23.94

Table 2 : Internal Quality Control

* = analytical signal

a = mean value of the analytical signal relative to the calibrator at 0.35 kU/l: approximately 200; relative to the blank state of the system: approximately 20

Allergen	Determination				Samples											
	UniCAP 100				Imm											
	Tested	concluded	pos.	neg.	Tested	concluded	pos.	neg.	Tested	concluded	pos.	neg.	Tested	concluded	pos.	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	-
fl	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
el	50	13	7	9	6	4	14.83	24.48	1.87	38.06	44.31	7.01	-	-	9	2
Global	1094	273	207	176	66	97	11.66	23.75	1.66	14.50	30.97	0.73	86	39	52	7

Table 3 : Comparative analytical data

M = mean, sd = standard deviation, m = median

For statistical evaluation: results < 0.35 kU/L = 0.10 kU/L and results > 100 kU/L = 100 kU/L

clear reduction of the values found or, in other cases, increased values) from those found for the same samples with UniCAP 100. Moving on from a purely statistical global comparison, which in some situations can amplify or mask data, leading to evaluations and conclusions which cannot be generalised (and this appears to be one of those situations in which each allergen seems to act differently from, and sometimes in the opposite way to, the rest), we found, for each sample and for each allergen, situations showing marked discrepancies both in terms of analytical data and in terms of classification of the patient as sensitive or non-sensitive to a particular allergen. For this purpose, we arbitrarily described as inconsistent the results obtained with Immulite 2000 which varied, in terms of concentration expressed in kU/l, by more than 50% from the reference data (in our case, the value found with UniCAP 100), and we described the samples found to be negative with one system and positive with the other, or vice versa, as incorrectly classified. The results of these evaluations are shown in table 3 under the heading "inconsistent".

In the detailed study of the individual allergens, a first consideration related to the differences in the information yielded at times by the mean value of the data found and the corresponding median (for example, the d1, t3, m2 and t9 allergens); this finding is probably due to the presence of samples with concentrations which are very low (< 0.35 kU/l) or very high (> 100 kU/l) and which, although normalized, have different effects on the two parameters. However, there is evidence

of marked over-estimates for some allergens (t3, i3 and e1) and a similar number of major under-estimates for others (w1, f4 and especially m2 and w19, for which more than 50% of the positive values according to UniCAP 100 are evaluated as negative by Immulite 2000). The number of inconsistent samples was considerable: there were 138 out of 273, equivalent to 50.5% of the tested samples, of which 86 (31.5%) showed values according to Immulite 2000 which were over 50% smaller than the values according to UniCAP 100, while there were also 52 (19.0%) with Immulite 2000 values more than 50% greater than the values found with UniCAP 100. Practically most of the 46 differently classified samples (16.8% of the total) appeared to be non-sensitive results according to Immulite 2000 (to be precise, the number was 39, i.e. 14.3%) where UniCAP 100 provided evidence of sensitisation for the same allergen. The opposite situation was much less frequently encountered: only 7 samples out of 273 (2.6%) were assessed as positive by Immulite 2000 and negative by UniCAP 100, and these mainly concerned two allergens (i3 and e1) for which the presence of markedly higher values with Immulite 2000 was also evident in the analysis of the global statistical parameters.

Figure 1 shows an overall view of the comparison between all the analytical data found with the two systems. When compared with a "formally" acceptable global correlation ($r = 0.801$), areas representing results differing markedly between the two methods are clearly apparent, as was to be expected in view of the numeric data in Table 3. The details of these data,

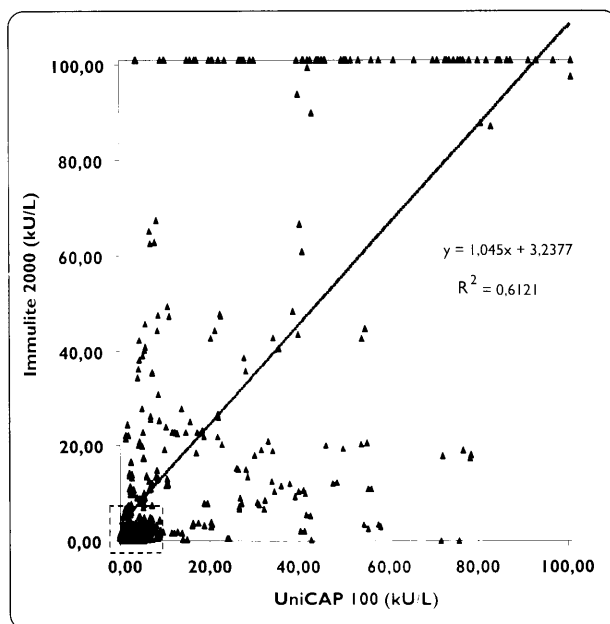


Figure 1 : Comparison of all results (using only data for samples with at least one positive value out of those provided by the two systems; those with negative values for both determinations were excluded). The frame includes values from 0 to 2.0 kU/l.

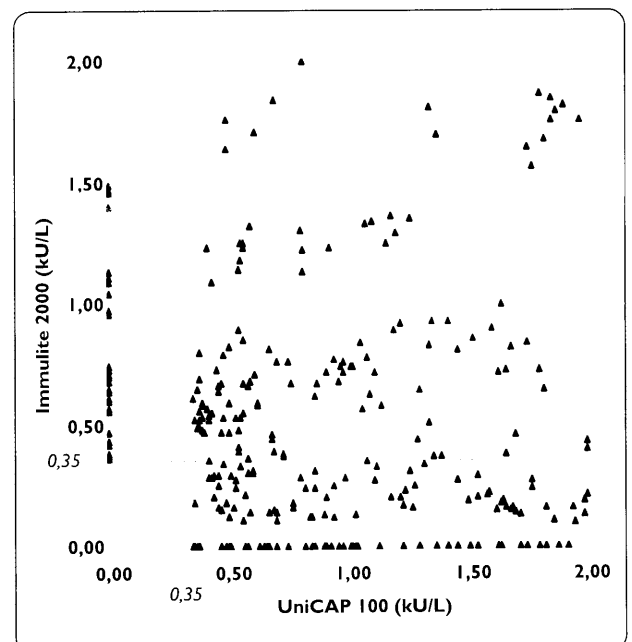


Figure 1b : Detail of figure 1. Samples with concentrations of sIgE from 0.35 to 2.0 kU/l are represented.

taking into account only the values included in the frame, are shown more clearly in Figure 1b. Here we can identify various samples which have a negative value (< 0.35 kU/l) with UniCAP 100 but show values above the limit of 0.35 kU/l with Immulite 2000, and vice versa. Figures 2 to 6 show correlation diagrams for certain allergens representative of this situation of particular non-uniformity in the comparison of the two systems.

EVALUATION OF CALIBRATORS

We considered it useful to verify how each analytical system measured the calibration samples (or recalibration samples, in the case of Immulite 2000 where

the calibration curve is supplied by the manufacturer as the value of the analytical signal) of the other system when they were used as biological samples. Given the results obtained for the samples, it was to be expected that the UniCAP 100 calibrators would show higher concentration levels when tested by Immulite 2000. As shown in table 4 and in graphic form in figure 7, this was indeed the case for practically all points of the UniCAP 100 calibration curve. This fact may also be attributable to the different matrix in which the calibrators are placed, and it would therefore be advisable to conduct further verification, preferably using the WHO 75/502 international standard to which both systems supposedly conform, before

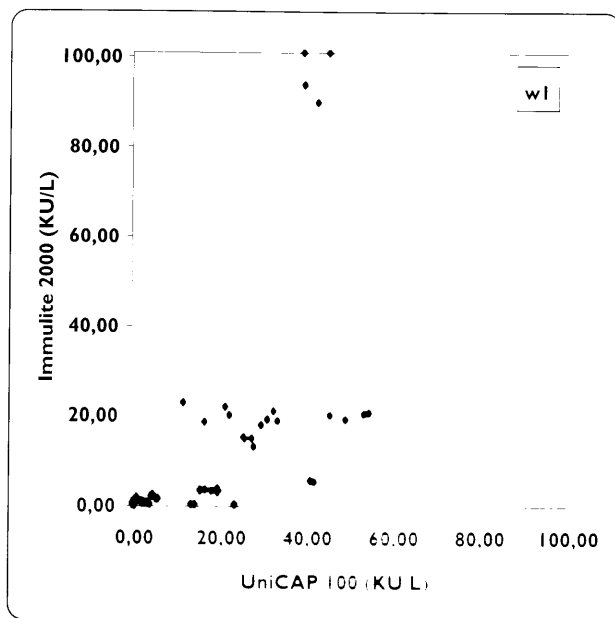


Figure 2 : Comparative test: allergen: amaranthus aleuter (w1).

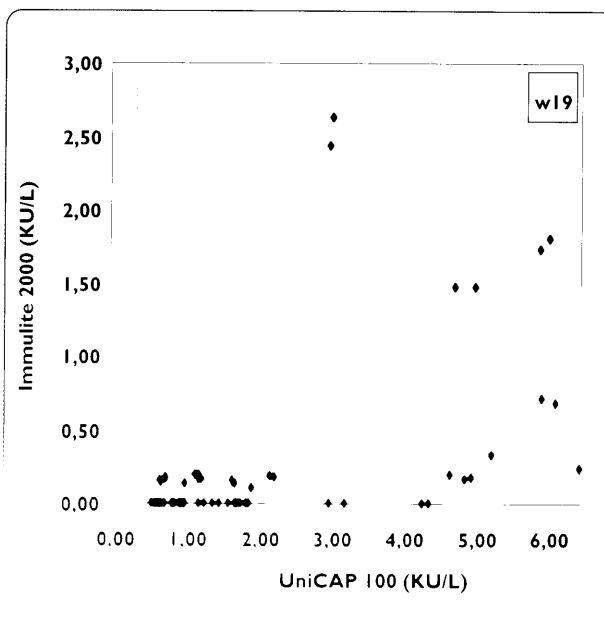


Figure 3 : Comparative test: allergen: parietaria officinalis (w19).

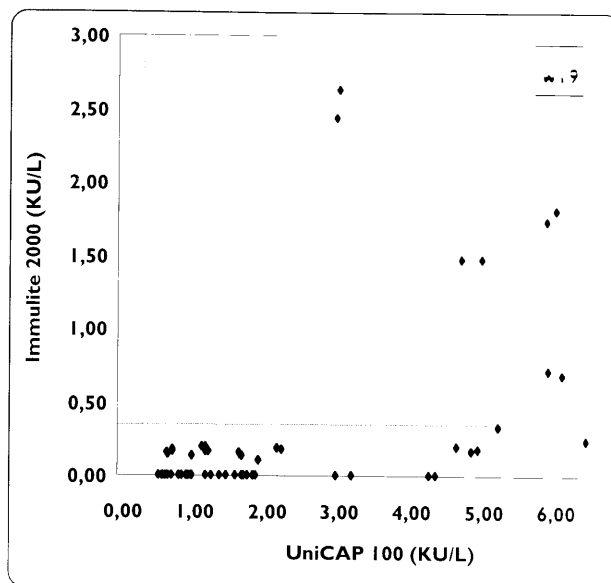


Figure 4 : Comparative test: allergen: wheat (f4).

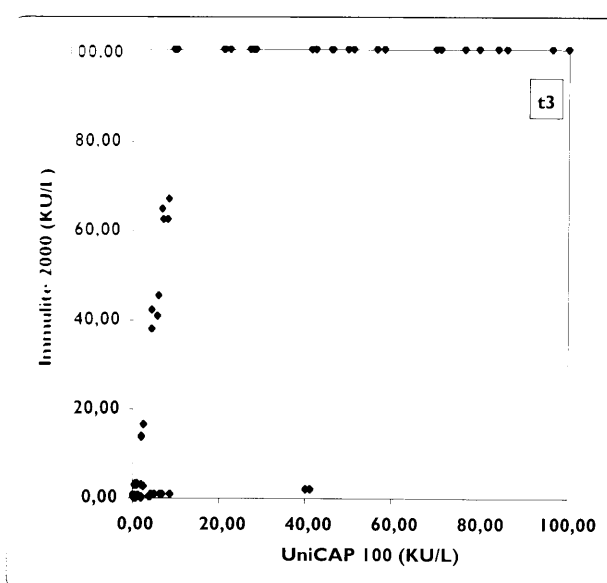


Figure 5 : Comparative test: allergen: birch (t3).

re giving this reason for the higher values found for the biological samples with Immulite 2000.

DILUTION TESTING

Using the concentration presented in table 5, figures 8 and 9 show the data obtained by plotting the concentration values as a function of the dilution used (Part a of each figure) or by normalizing the experimental value with respect to the corresponding expected theoretical value (Part b of each figure); in the second case, the ideal ratio at each point is considered to be 1 and the ideal test response corresponds to a horizontal straight line at the same value. The behaviour of the different allergens varied in the dilution test also: while we found some completely parallel variations (for both diluents and both systems), represented well on the ideal dilution line, as in the case of the w1 allergen (Figure 8a and 8b), we also found a significant response to dilution by UniCAP 100, with insufficient

Experimental values	Nominal values
0.43	0.35
0.94	0.70
4.79	3.50
25.53	17.50
88.76	50.00
> 100	100.00

Table 4 : Inversion of calibrators: UNICAP 100 on Immulite 2000.

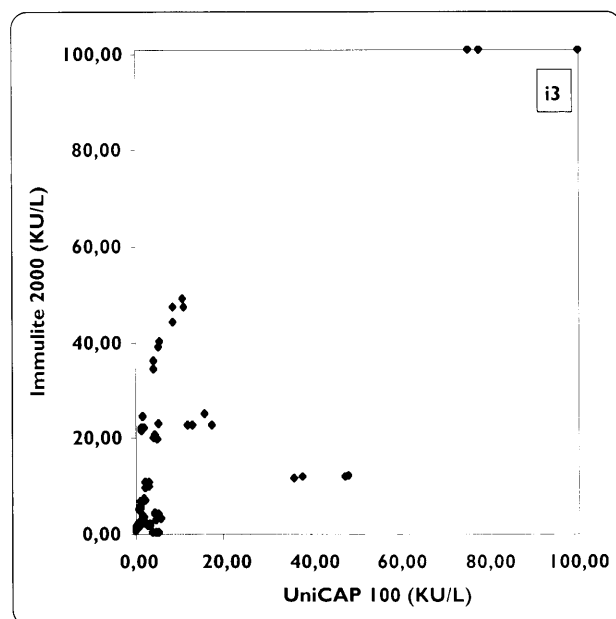


Figure 6 : Comparative test: allergen: polistes species, wasp (i3).

linearity for determination with Immulite 2000, for some other allergens. This was particularly true of allergens, such as i3, which had already been subject to major overestimates with Immulite 2000 as compared with UniCAP 100 (figures 9a and 9b). This result implied that there might be non-specific interference effects in respect of these allergens, or a difference in the selection of the epitopes in the preparation of the allergens by the different manufacturers (absence or different proportions of some major epitopes?).

EVALUATION OF INTERFERENCE

To verify the presence of possible interference by IgE other than those being specifically sought, we enriched some biological samples with a serum taken from human myeloma, containing 10000 kU/l of total IgE. This interference test was conducted on representative samples of all the tested allergens, with replicated determinations on OS and enriched serum (in a 1:1 ratio) with solutions containing 10,000, 5,000 and 2,500 kU/l IgE respectively. In order to align the initial values with those present in the sample after enrichment, a normalization was also carried out by diluting the sample with negative serum in the same ratio.

Table 6 shows the experimental data obtained for the various allergens in the two systems (the same preparations were used for both, to avoid effects due to the preparation of the samples). Figure 10 shows the diagram used to interpret the results of the interference test, and Figure 11 shows the results for certain significant allergens, which show non-uniform behaviour, as for the other tests described above: the interference of UniCAP 100 with all the allergens evaluated was

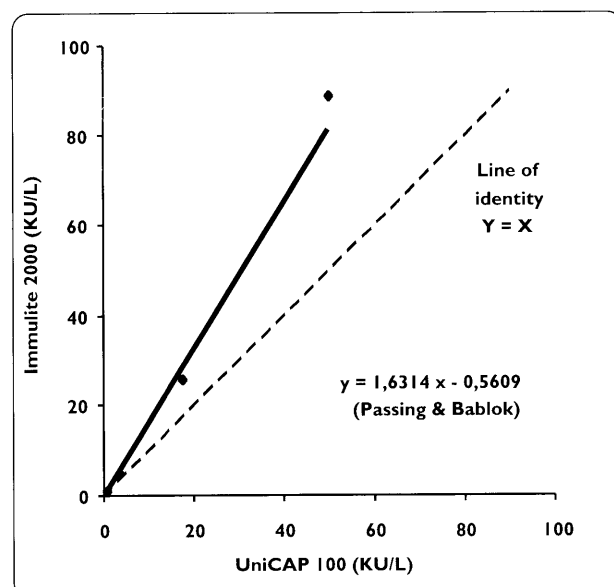


Figure 7 : UniCAP 100 calibrators (x) tested as samples with Immulite 2000 (y).

practically negligible, whereas with Immulite 2000 we found a match for some allergens (w1) but anomalous behaviour for many other allergens (d1, e1, t3).

CONCLUSIONS

Leaving aside the organisational considerations related to the high degree of automation of the Immulite 2000 system, we must point out, in relation to the purely analytical aspects, the analytical variability which appears excessive, at least in the positive control, and we would recommend the verification of this variability, which appears too high for the normal

performance of the system, in respect of control materials and various allergens. Attention should also be paid to the tendency for higher than nominal values to occur when the Pharmacia calibrators are measured with Immulite 2000, and the evidence of a certain degree of interference of the total IgE with the allergens concerned, which might perhaps partly explain (but does not justify) the higher values returned by Immulite 2000 for the various biological samples.

In methodological comparisons, there is always a tendency to look for absolute identity between the system under evaluation and the reference system, and the end

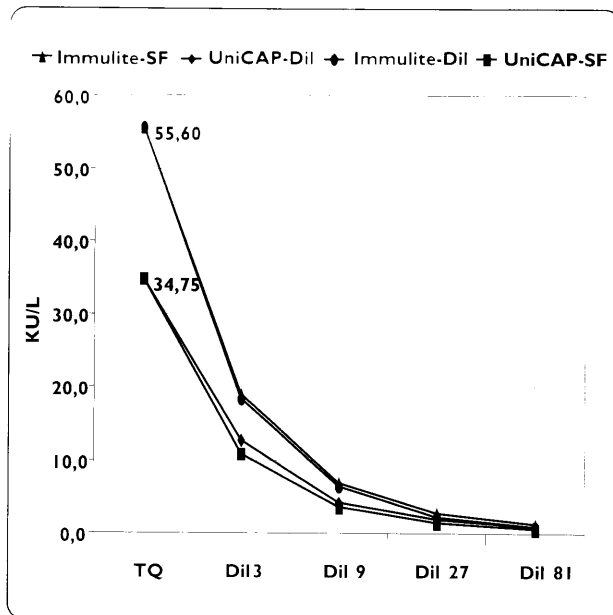


Figure 8a : Dilution test: allergen w1.

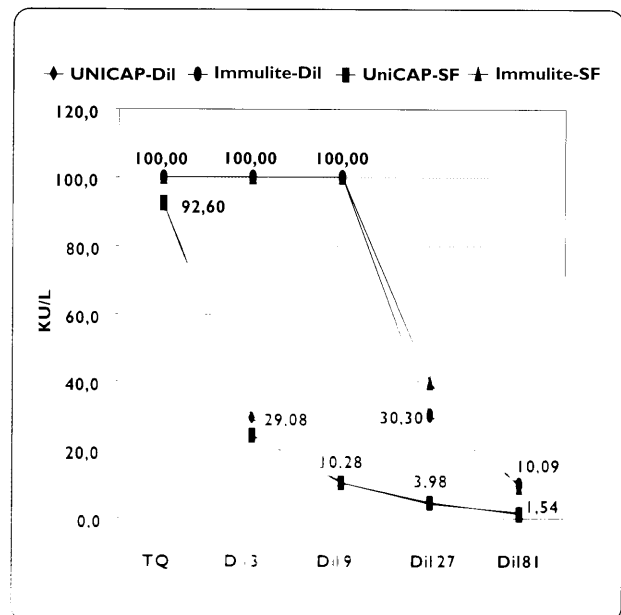


Figure 9a : Dilution test: allergen t3.

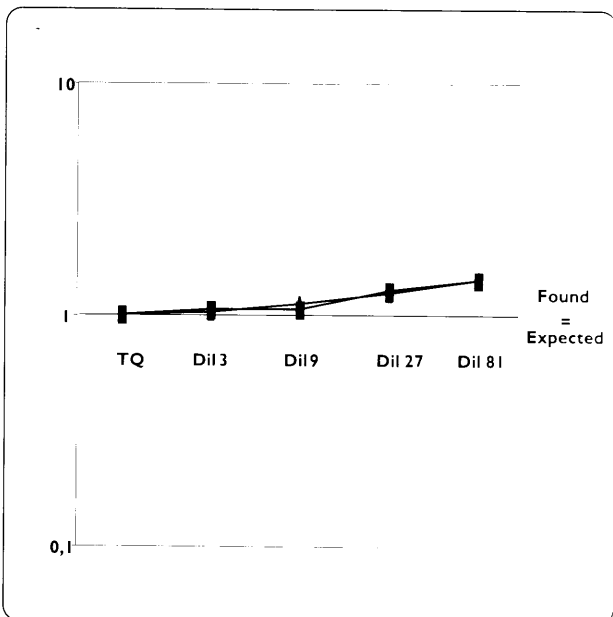


Figure 8b : Data from figure 8a expressed in normalized form.

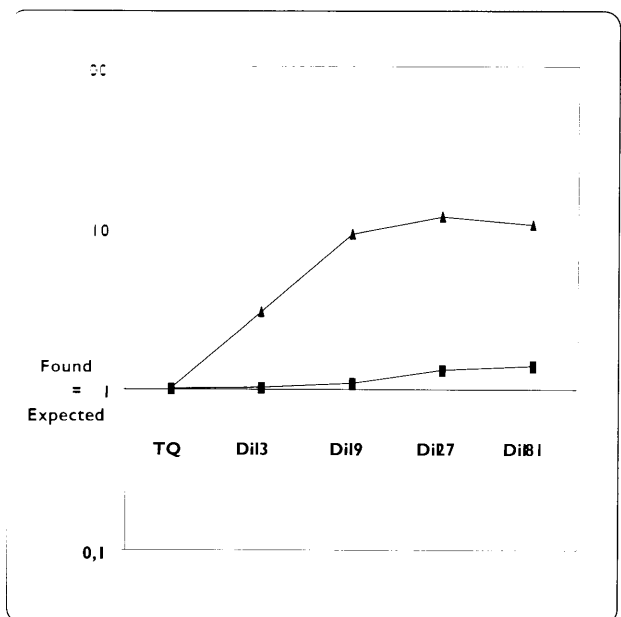


Figure 9b : Data from figure 9a expressed in normalized form.

Allergen	Instrument	Diluent	Sample OS	Dil. 3	Dil. 9	Dil. 27	Dil. 81
			kU/l	kU/l	kU/l	kU/l	kU/l
wl-sample 1	UniCAP	Pharmacia	27.40	9.82	3.81	1.50	0.58
		SF ^a		9.38	3.36	1.16	0.39
	Immulite	DPC	14.07	4.87	1.90	0.78	0.29
		SF ^a		4.69	1.56	0.52	0.17
wl-sample 2	UniCAP	Pharmacia	55.60	18.88	6.85	2.62	1.09
		SF ^a		18.25	6.33	2.23	0.81
	Immulite	DPC	34.75	12.58	4.18	1.76	0.76
		SF ^a		10.75	3.45	1.24	0.46
t3	UniCAP	Pharmacia	92.60	29.08	10.28	3.98	1.54
		SF ^a		22.85	8.78	4.43	1.32
	Immulite	DPC	> 100	> 100	> 100	30.30	10.09
		SF ^a		> 100	> 100	39.80	9.30

Table 5 : Dilution test: experimental data for some allergens. *a* = serum free of IgE.

Allergen	Instrument	Sample TQ	Sample TQ	Sample TQ	Sample TQ	Sample TQ
		kU/l	kU/l	kU/l	kU/l	kU/l
d1	UniCAP	60.30	29.10	30.90	31.20	30.10
	Immulite	> 100	> 100	> 100	> 100	> 100
e1	UniCAP	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35
	Immulite	< 0.10	< 0.10	3.80	1.93	1.22
f4	UniCAP	13.30	9.89	11.10	10.80	10.70
	Immulite	1.66	1.21	1.36	1.27	1.50
f13	UniCAP	3.57	1.55	1.20	1.83	1.69
	Immulite	0.22	< 0.10	0.15	0.18	0.35
g6	UniCAP	44.70	23.70	25.80	22.90	24.70
	Immulite	11.25	5.83	6.01	6.46	3.61
i3	UniCAP	> 100	73.80	71.80	77.50	77.80
	Immulite	> 100	> 100	> 100	> 100	> 100
m6	UniCAP	3.47	1.76	2.03	2.11	2.10
	Immulite	1.43	0.95	1.06	1.10	1.13
t3	UniCAP	> 100	70.20	68.20	65.00	67.20
	Immulite	> 100	> 100	> 100	> 100	> 100
t9	UniCAP	8.07	4.09	4.22	4.27	4.14
	Immulite	1.86	1.43	1.61	1.72	1.75
wl	UniCAP	33.70	17.90	19.20	19.30	18.80
	Immulite	19.60	13.35	12.15	12.50	11.85

Table 6 : Evaluation of interference by total IgE: experimental data. *a* = serum free of IgE.

use of the results in terms of their significance and clinical value is frequently overlooked. In the case of sIgE determination it is necessary to consider in the first place, as stated above, that each allergen has to be evaluated as an independent parameter, albeit in the context of the

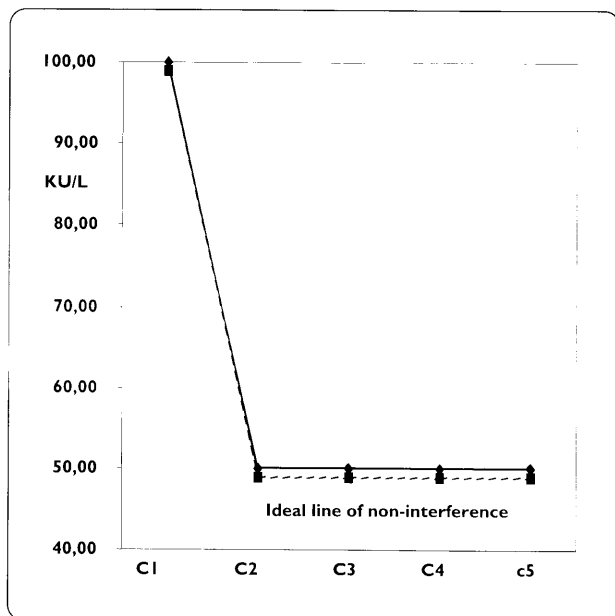


Figure 10 : Total IgE interference test: interpretation

Key:

- C1 : SIgE concentration in Original patient Sample (OS)
- C2 : SIgE concentration in normalized Original Sample resulting from a one to one (volume) mix of OS and SIgE serum free sample
- C3 : SIgE concentration resulting from a one to one (volume) mix of OS and a 2500 KU/L total IgE concentration sample
- C4 : SIgE concentration resulting from a one to one (volume) mix of OS and a 5000 KU/L total IgE concentration sample
- C5 : SIgE concentration resulting from a one to one (volume) mix of OS and a 10000 KU/L total IgE concentration sample

same method; it is therefore both difficult and dangerous to generalise the conclusions for one allergen to all other allergens (10). While taking the above factors into account, we consider that we should underline the significant discrepancy (relating to more than 50% of the samples used) found in the analytical data, and especially the fact that the classifications of about 20% of the samples used in the tests differ between Immulite 2000 and UniCAP 100 in respect of the description of patients as sensitive or non-sensitive to a given allergen, thus potentially causing the clinical specialist to take different approaches to the treatment of patients. On the other hand, these findings might lead laboratory researchers to question the sensitivity (for samples where the Immulite 2000 value is lower than the UniCAP 100 value) and specificity (for samples showing the opposite result) of the system for determining sIgE with Immulite 2000, these being parameters in respect of which no objective conclusions can yet be drawn on the basis of our data.

In view of the differences found between the two systems in analytical terms, and since it is practically impossible to establish in quantitative terms the real value of sIgE present in individual biological samples, a laboratory deciding to use procedures having different characteristics in terms of sensitivity and/or specificity would at least have to consider the necessity of reviewing, in relation to the different analytical method used, as in the case of many simpler biological parameters, both the positive and negative discrimination cut-off points and the classification levels used by clinics (11-12). These classification levels should be verified since these are not simple routine procedures for individual laboratories using cumulative data and suitable statistical analysis techniques for each individual allergen.

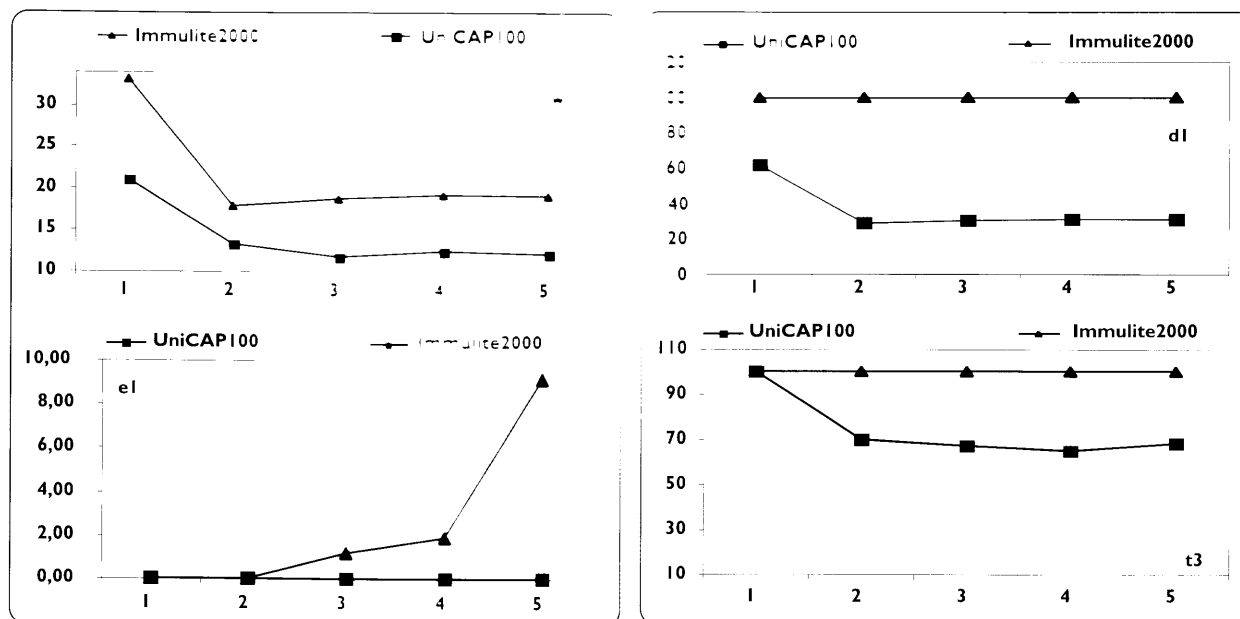


Figure 11.

REFERENCES

1. Vignati G, Griffanti D., Maronati A. *et al.* - Le determinazione delle IgE specifiche in automazione totale: nuove opportunità organizzative per il laboratorio. *Ligandassay* 2002;7(3):267.
2. Costongs G.-M., Janson P.-C., Hermans W.-J. *et al.* - Evaluation of performance characteristics of automated measurement systems for allergy testing. *Eur J Clin Chem Clin Biochem* 1995;33:295-305.
3. Bennich H., Ishizaka K., Johansson SGO. *et al.* - *WHO Bull* 1968;38:151-2.
4. Babson A.-L. - The cirrus IMMULITE Automated Immunoassay System. *J. Clin. Immunoassay* 1991;14:83-8.
5. Leimgruber A., Peitrequin R., Mosimann B. *et al.* - The Pharmacia CAP System: a new assay for specific IgE. *J Allergy Clin Immunol* 1989;83:176.
6. Leimgruber A., Masimann B., Claeys M. *et al.* - Clinical evaluation of a new "in vitro" assay for specific IgE, the immunoCAP System. *C Exp Allergy* 1991;21:127-131.
7. Ahlsted S. - Understanding the usefulness of Specific IgE blood tests in allergy. *Clin Exp All* 2002;32:11-6.
8. Liappis N., Starke A., Lantto O. *et al.* - Investigation of Pharmacia UniCAP 100 for *in vitro* allergy diagnosis. *Clin Laboratori* 1999;45:229-35.
9. Williams P.-B., Barnes J.-H., Szeinbach S.-L. *et al.* - Analytic precision and accuracy of commercial immunoassay for specific IgE: establishing a standard. *J Allergy Clin Immunol* 2000;105:1221-30.
10. Yunginger J.-W., Ahlstedt S., Eggleston P.-A. *et al.* - Quantitative IgE antibody assay in allergic diseases. *J Allergy Clin Immunol* 2000;105:1077-84.
11. Pastorello E.-A., Incorvaia C., Pravettoni V. *et al.* - A multicentric study on sensitivity and specificity of a new "in vitro" test for measurement of IgE antibodies. *Ann Allergy* 1991; 67:365-70.
12. Pastorello E.-A., Incorvaia C., Ortolani C. *et al.* - Studies on the relationship between the level of specific IgE antibodies and the clinical expression of allergy. I. Definition of level distinguish patients with symptomatic from patients with asymptomatic allergy to common aeroallergens. 1995;96:580-7.

