

Measurement of IgE antibodies using liquid allergens – an inter-method and inter-laboratory quality assessment

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Die Bestimmung von IgE-Antikörpern für die Allergiediagnostik – ein Methoden- und Laborvergleich

Zusammenfassung. *Hintergrund:* Die Bestimmung von IgE-Antikörpern stellt einen wichtigen Bestandteil der *in vitro* Allergiediagnostik dar. Allerdings erfüllen nicht alle Systeme, die am Markt vertreten sind, wichtige Qualitätskriterien wie etwa hohe Sensitivität und Spezifität, und positiven Ergebnissen fehlt häufig die klinische Relevanz. Neuere Entwicklungen reduzieren die Arbeitsbelastung für den Ausführenden und helfen so Zeit und Geld einzusparen. Noch wichtiger ist aber, dass dadurch auch potentielle Fehlerquellen reduziert werden.

Methodik: Zwei Testsysteme, das etablierte Pharmacia CAP System, bei welchem die Allergene konventionell an die feste Phase gebunden sind, und das ALLERgen System, bei dem Flüssigallergene eingesetzt werden, wurden in Hinblick auf Qualitätsmerkmale und Praktikabilität verglichen. Definierte Serumpools wurden in zwei unabhängigen Laboratorien auf „within-run“ und „between-days“ Genauigkeit bezüglich IgE-Antikörper-Nachweis untersucht. Daneben wurde Serum von allergischen Patienten und Gesunden mit beiden Methoden auf Gesamt-IgE und antigenspezifische IgE-Antikörper in den zwei Laboratorien getestet. Zusätzlich beteiligte sich ein Labor, das ausschließlich mit dem ALLERgen System arbeitet, am Österreichischen Qualitätskontroll-Ringversuch für IgE-Antikörper.

Ergebnisse: Die beiden Systeme erbrachten sehr gute Ergebnisse, die Sensitivität und Spezifität betreffend und zeigten eine befriedigende Korrelation. Die „within-run“ Ergebnisse waren hervorragend für Gesamt-IgE und antigenspezifische IgE-Antikörper, die „between-days“ Genauigkeit erwies sich als akzeptabel. Die Variationskoeffizienten bewegten sich innerhalb einer zulässigen Bandbreite für die verschiedenen Allergengruppen. Im externen Qualitätskontrollprogramm zeigten die mit dem ALLERgen System ermittelten Werte gute Übereinstimmung mit den anderen Systemen; bezüglich der klinisch relevanten Sensibilisierungen waren 94% der Werte identisch. Die Nutzerfreundlichkeit erwies sich für beide Systeme als sehr gut. Das ALLERgen System überzeugte ganz besonders aufgrund der vollständigen Automatisierung, was in kürzeren Arbeitszeiten, sowohl

die fixen als auch die variablen Phasen betreffend, für das Personal resultierte.

Zusammenfassung: Beide Systeme, sowohl das Pharmacia CAP System als auch das ALLERgen System, sind Mitglieder einer modernen Generation von Allergietestsystemen. Beide sind Benutzer-freundlich, beide liefern gut reproduzierbare und vergleichbare Ergebnisse. Das ALLERgen System weist aufgrund der Verwendung von Flüssigantigenen und der Vollautomatisierung gewisse Vorzüge auf.

Schlüsselwörter: IgE, PRIST, RAST, ALLERgen System, CAP System, Allergie-Ringversuch.

Summary. *Background:* The determination of IgE antibodies is important for the *in vitro* diagnosis of allergic diseases. However, not all systems currently available in the market fulfil essential quality criteria, e.g. regarding characteristics such as sensitivity and specificity, and the data do not always reflect true clinical relevance in the required fashion. Recent innovations may reduce the workload for the technician, and thus help save time and money. More importantly, they might reduce potential sources of error.

Objective: Two allergy systems, the well established Pharmacia CAP system that uses the allergens conventionally in a solid phase and the ALLERgen system that employs liquid allergens, were compared with regard to quality criteria and practicability.

Methods: Defined serum pools were checked for within-run and between-days imprecision of IgE antibody detection in two independent laboratories. Serum specimens from allergic patients and controls were tested in parallel using both methods for total and antigen-specific IgE antibody detection under standardized conditions. In addition, one laboratory working exclusively with the ALLERgen system participated in the Austrian inter-laboratory quality assessment program.

Results: The two systems were comparable in terms of sensitivity and specificity, and also showed good correlation. Within-run evaluations were excellent for total IgE and antigen-specific IgE, and the between-days imprecision was satisfactory. Coefficients of variation were within an acceptable range for the different groups of allergens. In the external quality control program the data obtained

with the ALLERgen system showed good concordance with other systems in use; up to 94% of the results were identical when considering clinically relevant sensitizations. Regarding practicability, both systems were most satisfactory for the operator. The ALLERgen system offered a certain advantage in terms of automated operation, which resulted in shorter fixed and variable phases of personnel time.

Conclusion: Both the Pharmacia CAP system and the ALLERgen system belong to an advanced generation of allergy test systems and are easy to handle. The reproducibility of results is good with both methods, and the imprecision data fall within an acceptable range. Thus, the ALLERgen system is a reliable *in vitro* system for evaluating specific and total IgE in serum, providing data equivalent to those obtained with the CAP system.

Key words: IgE, PRIST, RAST, multicenter evaluation, ALLERgen system, CAP system.

Introduction

The measurement of total IgE and allergen-specific IgE in serum is important for nosologic discrimination of atopic diseases (bronchial asthma, rhinitis, urticaria, eczema) from pollen, food, insect venom, drug and occupational allergies, as well as from parasitic infections. Several assay systems for the detection of these antibodies are available in the market. The assay systems in clinical use today differ in terms of coupling methods (solid phase with cellulose, polystyrol and ferrum oxide, or liquid systems), detection techniques (fluorescence, chemiluminescence, color precipitation, or radioactivity), and technical configurations (manually performed, semiautomated, or fully automated).

Although the WHO standard 75/502 is prescribed for calibration, the data obtained with the various systems are considerably different in terms of sensitivity and specificity [1–4]. These variations determined in national and international multicenter evaluations may be explained by differing company-specific in-house standards, various antigenic sources and extraction methods, and by a lack of

public control mechanisms for quality control: several companies offer antigens such as metals, biocides, plastics, insecticides, and herbicides, for which IgE-mediated reactions are not proven to date [5]. Moreover, antigens are quite commonly offered when no positive human reference materials or literature reports are available [5].

The incidence of allergies in European populations has considerably increased over recent decades [6]. A fully automated quantitative technique of diagnosis would be helpful, as it might reduce the frequency of errors in handling samples [7] and also reduce costs. The present multicenter evaluation was undertaken to compare the performance criteria of an automated allergy system that functions with liquid antigens against a well established system that is semi-automated, works conventionally with a solid phase, and is currently used in many laboratories. In addition to comparative inter- and intra-assay controls and multicenter evaluation, the data were also investigated in a laboratory quality control program, where several different methods are regularly employed.

Materials and methods

The Pharmacia CAP system

For the measurement of total IgE and allergen-specific IgE, the Pharmacia CAP system (Pharmacia, Uppsala, Sweden) uses as solid phase a flexible hydrophilic carrier polymer encased in a capsule, the ImmunoCAP [7]. The allergen-carrier consists of a CNBR-activated cellulose derivative. The allergens are mostly purified extracts, but for some allergens recombinant antigens such as bet v1 and bet v2, the major allergens of the birch tree, are offered. Owing to the large surface area of this "sponge", the protein binding capacity of an ImmunoCAP is at least three-fold higher than that of the corresponding paper disk, and about fifty-fold higher than that of a coated tube. Polyclonal and monoclonal β -galactosidase conjugated antibodies generating fluorescence by splitting the fluorogenic substrate 4-methylumbelliferyl- β -D-galactoside are used. The results are expressed quantitatively in kilounits per liter for total IgE and antigen-specific IgE. For a single determination, 50 μ l of the patient's serum are required. The instrument specifications have been published elsewhere [7].

Table 1a. Evaluations of total IgE were carried out in different serum pools (A–D) using both methods in parallel. Samples in Graz and Salzburg were different. Total IgE (within-run imprecision)

CAP system												
Sample	A			B			C			D		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	53.5	1.4	10	198.1	4.7	10	473.3	3.9	10	929.6	4.4	10
Graz	38.3	6.6	10	138.4	5.2	10	351.6	4.3	10	794.1	3.6	10
ALLERgen system												
Sample	A			B			C			D		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	38.9	5.2	10	172.6	2.6	10	432.0	6	10	807.7	12.7	10
Graz	34.7	4.5	10	117.6	3.7	10	283.2	6.5	10	864.4	5.8	10

x mean values; CV% coefficient of variation; n number of determinations.

Table 1b. Evaluations of total IgE were carried out in different serum pools (A–D) using both methods in parallel. Samples in Graz and Salzburg were different. Total IgE (between-days imprecision)

CAP system												
Sample	A			B			C			D		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	52	7.4	10	201.7	3.0	10	482.5	3.9	10	954	5.1	10
Graz	35.2	9.0	10	119.6	8.5	10	314	6.8	10	676.8	8.3	10

ALLERgen system												
Sample	A			B			C			D		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	34.6	12.2	10	155.6	8.9	10	444.2	10.6	10	796.6	10.9	10
Graz	29.3	13.0	10	122.3	11.1	10	313.5	13.0	10	617.2	14.5	10

x mean values; *CV%* coefficient of variation; *n* number of determinations.

The ALLERgen system

The ALLERgen system (Adaltis Italia SpA, Bologna, Italy) is composed of test kits with biotinylated allergens (ALLERgen) and an automated instrument for processing and detection (ALLERTECH). The system is based on the EIA immunocapture principle and employs the same solid phase, i.e. wells coated with anti-human IgE, for total and specific IgE assays. After the first incubation of the samples in the coated wells and subsequent washing – to avoid negative interference from other immunoglobulin classes (e.g. antigen-specific IgG) – the IgE is selected and determined by reactions with liquid biotinylated anti-IgE for total IgE assay and the specific IgE calibration curve, or with liquid biotinylated allergens for the allergen-specific IgE of the samples. Peroxidase-streptavidin conjugate and ready-to-use liquid chromogen allow colorimetric detection of the bound IgE. For both total and allergen-specific IgE, the ALLERgen system – as well as the CAP system – uses standard curves expressed in terms of total IgE (calibration against WHO 2nd IRP 75/502). The calibration ranges are 0 to 1,000 kIU/l for total IgE, and 0.36 to 100 kIU/l for specific IgE.

All reagents, including the biotinylated allergens, are in liquid form and ready to use. For a single determination, 50 µl of the patient's serum are required.

ALLERTECH is an ELISA processor dedicated to the fully automated processing of ALLERgen kits, including processing and reporting of all data. In addition, the software allows the investigator to manage Quality Control sera and to perform statistic evaluations of the results. The total incubation time is no more than 135 minutes.

Participating laboratories

Two allergy laboratories (Graz and Salzburg, Austria) performed the studies. Both investigated patients' sera for total IgE and a wide range of specific allergens, using the two systems for the purpose of comparison. Within-run and between-days imprecision tests were performed in defined sera from allergic patients in the two laboratories, again with both systems.

The manufacturer of the ALLERgen system has participated in the quality control program of the Austrian Society of Allergy and Immunology (ÖGAI) since 1999. At three-month intervals, 5 sera from allergic patients are sent to 23 allergy laboratories in Austria, Germany and Italy for the determina-

tion of antigen-specific antibodies and total IgE [1, 4]. A wide variety of commercial assay systems are used by the participating laboratories, the most common ones being the Pharmacia CAP FEIA system and the Pharmacia UniCAP. Some laboratories work with the HYCOR, Allergopharma, or other systems.

Specimens to be evaluated

For the within-run imprecision, total IgE was determined (*n* = 10) in the two laboratories in different serum pools containing very low, low, medium or high levels of total IgE (between >30 and <1,000 kIU/l), in parallel using both methods. Each of the two laboratories carried out these determinations, yielding one coefficient of variation per analyte and per specimen (Table 1a). For the within-run imprecision of allergen-specific IgE (*n* = 10), the patient samples were initially determined as test-positive against a panel of common inhalant allergens (timothy grass – g6, common silver birch – t3, mugwort – w6, dermatophagoides pteronyssinus – d1, cat dander – e1, dog epithelia – e5) and insect venoms (honey bee – i1 and wasp – i3). Both laboratories carried out these determinations, yielding one coefficient of variation per allergen-specific IgE (Table 2a).

For the between-days imprecision, total IgE levels were determined daily in duplicate on 10 working days in both laboratories, using the panel of serum pools mentioned above (Table 1b). In order to calculate the between-days imprecision for allergen-specific IgE, the same panel of specimens was analysed daily in duplicate on 10 working days (Table 2b).

In order to compare the analytical procedures, 343 serum samples from allergic persons and 290 samples from non-allergic controls were investigated; RAST categories were determined by the two methods and the two sample pools were compared (Table 3, Fig. 1). A detailed evaluation was done for groups of "related" allergens (Table 3, Fig. 2a–h).

In the quality control program of the Austrian Society of Allergy and Immunology, 5 sera from allergic patients are sent at 3-month intervals to allergy laboratories for the determination of various antigen-specific antibodies and total IgE. The results obtained from testing the sera of allergic patients using the two methods (Pharmacia CAP or ALLERgen), were subjected to statistical evaluation (Fig. 3 for total IgE and Fig. 4 for antigen-specific IgE).

Table 2a. Evaluations of antigen-specific IgE were carried out in sera from patients allergic to a wide variety of antigens, using both methods in parallel. Samples in Graz and Salzburg were different. Specific IgE (within-run imprecision)

CAP system												
Allergen	d1			e1			t3			g6		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	15.9	11.8	10	3.9	5.2	10	9.6	6.2	10	23.6	7.0	10
Graz	2.6	3.4	10	6.5	7.2	10	10.9	4.4	10	10.9	4.5	10
Allergen	w6			e5			i1			i3		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	0.8	10.6	10	3.0	5.9	10	3.5	5.0	10	2.5	17.6	10
Graz	2.2	3.7	10	6.7	4.3	10	1.6	9.2	10	2.7	7.6	10
ALLERgen system												
Allergen	d1			e1			t3			g6		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	13.4	2.1	10	1.8	3.7	10	13.5	4.8	10	26.6	5.9	10
Graz	0.4	4.4	10	4.7	3.2	10	28.2	9.7	10	7.0	3.3	10
Allergen	w6			e5			i1			i3		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	0.5	3.9	10	1.9	3.1	10	0.5	5.2	10	2.3	3.5	10
Graz	1.9	2.9	10	5.6	3.4	10	1.2	1.8	10	4.0	2.6	10

x mean values; *CV%* coefficient of variation; *n* number of determinations; *d1* dermatophag. pteronyssinus; *e1* cat epithelium; *t3* birch, white; *g6* timothy grass; *w6* mugwort; *e5* dog dander; *i1* bee venom; *i3* common wasp venom.

Table 2b. Evaluations of antigen-specific IgE were carried out in sera from patients allergic to a wide variety of antigens, using both methods in parallel. Samples in Graz and Salzburg were different. Specific IgE (between-days imprecision)

CAP system												
Allergen	d1			e1			t3			g6		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	16.9	8.1	11	4.0	4.6	11	10.6	10.0	11	24.4	9.3	11
Graz	3.0	8.9	10	8.8	13.5	10	16.9	16.2	10	17.0	15.9	10
Allergen	w6			e5			i1			i3		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	0.8	12.1	11	2.9	5.1	11	3.6	13.5	11	2.4	18.8	11
Graz	3.2	20.0	9	10.2	18.7	10	2.4	21.9	10	3.9	17.6	10
ALLERgen system												
Allergen	d1			e1			t3			g6		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	13.7	18.6	10	1.5	21.2	10	12.6	16.4	10	30.5	16.0	10
Graz	0.6	14.9	9	4.6	13.8	9	26.1	14.2	9	8.1	16.3	8
Allergen	w6			e5			i1			i3		
	x	CV%	n	x	CV%	n	x	CV%	n	x	CV%	n
Salzburg	0.5	19.7	10	1.7	22.0	10	0.5	23.9	10	2.0	24.2	10
Graz	2.2	13.9	8	5.3	15.5	9	1.5	19.0	9	4.4	12.4	9

x mean values; *CV%* coefficient of variation; *n* number of determinations. Allergens – see Table 2a.

Statistical evaluation

The data obtained from the comparison of methods were calculated and graphically evaluated according to standard procedures for statistical evaluation of laboratory data. Standard deviations and mean values were calculated as described elsewhere [8].

Results

Within-run imprecision

Table 1a shows the mean values ($\bar{x}$), the coefficients of variation (CV%) and the number of samples (n) for total IgE determination performed under standardized

conditions (= same operator, same reagent lot, same instrument and same calibrators) using the various samples at the different locations. The CVs ranged from 1.4 to 6.6% for the CAP and the ALLERgen system in the 3 lower total IgE pools, and were slightly higher for the high total IgE pools (5.8 and 12.7%). The overall mean was 4.4% for the CAP system and 5.9% for the ALLERgen system.

Table 2a shows the within-run imprecision for the determination of allergen-specific IgE. The coefficients of variation for the different antigens ranged from 3.4 to 17.6% (mean = 7.1%) for the CAP system and from 1.8 to 9.7 (mean = 4.0%) for the ALLERgen system.

Table 3. Comparison of specific IgE values obtained by the two evaluation systems in parallel runs for a wide variety of allergens. CAP – ALLERgen system comparison (specific IgE)

Allergen	Classified samples	CAP pos – ALLERgen pos	CAP pos – ALLERgen neg	CAP neg – ALLERgen pos	CAP neg – ALLERgen neg	Concord. on classified samples	Cap Cl. 1 ALLERgen Cl. 0	Cap Cl. 0 ALLERgen Cl. 1	Total n° of samples
Type*	n°	n°	n°	n°	n°	%	n°	n°	n°
All	633	296	42	5	290	92.6	39	17	689
Animal dander	87	46	9	3	29	82.6	6	4	97
Foods	101	28	10	0	63	90.1	7	2	110
Grass pollen	54	34	0	1	19	98.2	6	0	60
Drugs	27	1	3	0	23	88.9	8	6	41
Mites	103	59	3	1	40	96.1	4	0	107
Moulds	46	8	10	0	28	78.3	0	1	47
Occupational allergens	4	3	0	0	1	100	1	0	5
Tree pollen	61	35	2	0	24	96.7	2	1	64
Venoms	96	52	5	0	39	94.8	2	3	101
Weed pollen	54	30	0	0	24	100	3	0	57

* Allergens, grouped specific allergens, e.g. all data regarding egg white, cows' milk, cod, wheat, pea nut, soybean, carrot etc. are summarized under the term "foods".

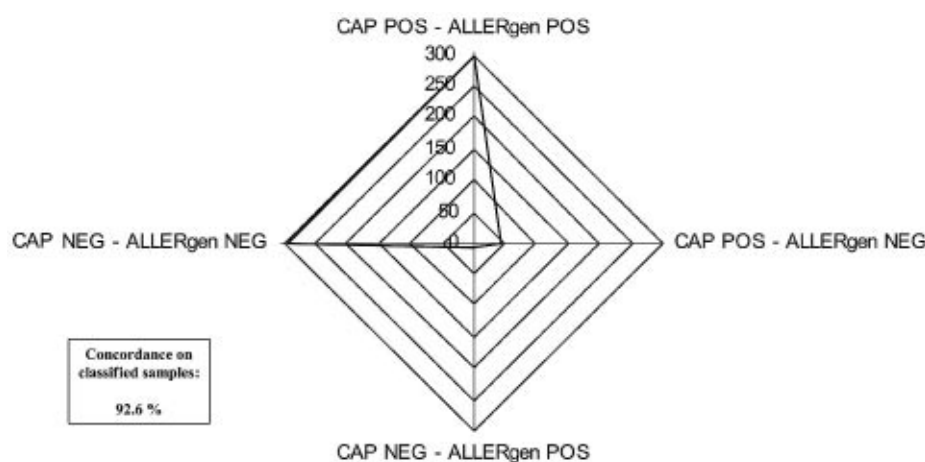


Fig. 1. Comparison of specific IgE determined by CAP and ALLERgen in 633 allergic patients or non-allergic control persons, depicted in the format of radial plot(s): the center represents zero and the outer frame the maximum value. The lines connect the data registered for the four respective categories. Nearly identical results were obtained with the two systems (data from Graz and Salzburg)

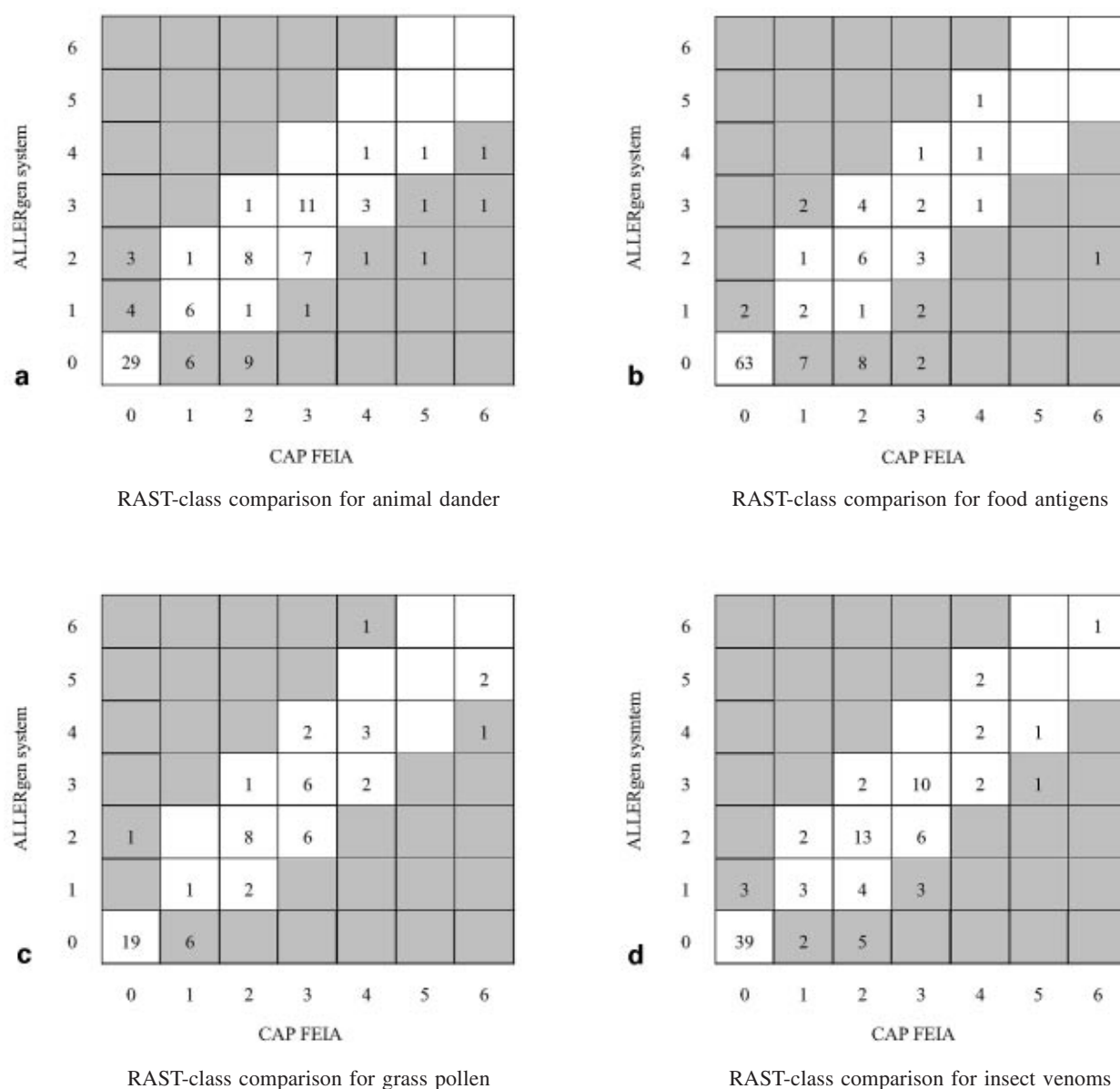


Fig. 2a–h. RAST-class comparison for 8 different groups of antigens tested in parallel in 633 sera from allergic patients and non-allergic control persons

Between-days imprecision

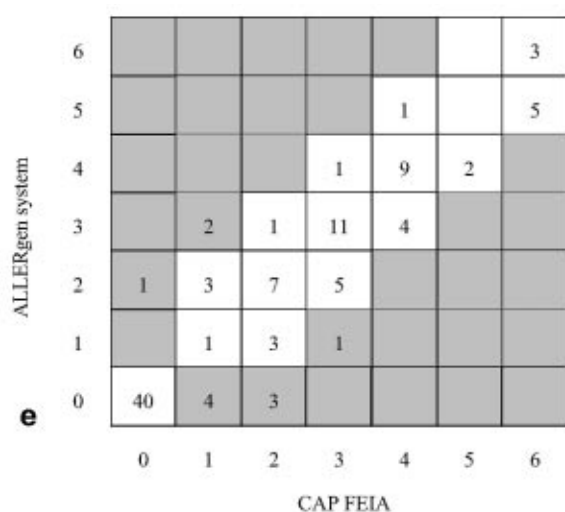
Table 1b shows the total IgE data using the CAP system (values ranging from 3.0 to 9.0%; mean = 6.5%) and the ALLERgen system (values ranging from 8.9 to 14.5%; mean = 11.8%).

Table 2b shows the between-days imprecision for allergen-specific IgE. The coefficients of variation for allergens were below 21.9% for the CAP system (range: 4.6 to 21.9%; mean = 13.4%) and below 24.2% for the ALLERgen system (range: 12.4 to 24.2%; mean = 17.6%).

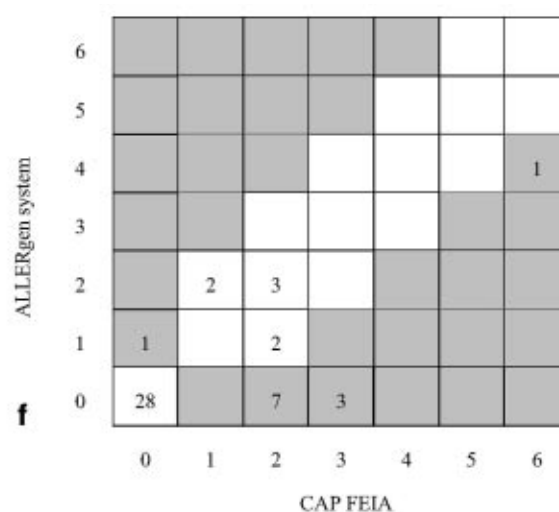
Comparison of the CAP and the ALLERgen systems for specific IgE

The overall concordance in classified samples was 92.6% (Table 3), ranging from 78.3% for moulds to 98.2%

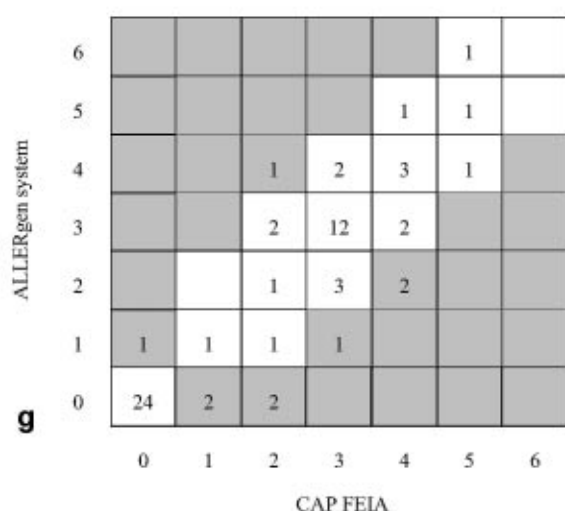
for grass pollen and 100% for the (limited number tested) occupational allergens (mostly latex). Data where one method revealed a class 1 (very low) and the other method a negative result were omitted in this presentation (Table 3, columns 7 and 8). The concurrence of data is shown in Fig. 1: nearly all CAP-negative sera were also ALLERgen-negative; nearly all CAP-positive ones were also ALLERgen-positive; almost no samples were CAP-negative/ALLERgen-positive, and only a limited number of CAP-positive values were ALLERgen-negative. These data may be explained by the very high – in some cases probably too high – sensitivity of the CAP system compared with any other system on the market [3]. Most determinations revealed identical RAST classes; variations in 1 or even 2 classes were rare for nearly all groups of antigens (Fig. 2). Striking differences were only regis-



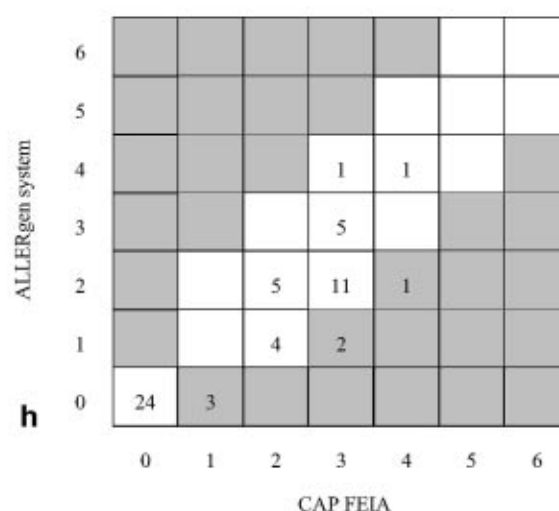
RAST-class comparison for dust mites



RAST-class comparison for moulds



RAST-class comparison for tree pollen



RAST-class comparison for weed pollen

tered for moulds (Fig. 2f), where 10 out of 18 CAP-positive sera were negative with the ALLERgen system. The clinical relevance of these discrepant findings remains obscure in most instances.

External quality control

The manufacturer of the ALLERgen system has participated in the ÖGAI external quality control program for the past 3 years. Total IgE-values correlated well when the data from the laboratory using the ALLERgen method were compared with the summarized data from those laboratories that employed the CAP system (Fig. 3). Regarding antigen-specific IgE values, the concordance was 87.4% in 95 determinations covering all groups of relevant allergens, as documented in Fig. 4. Almost no negative (control) specimens were included, as demonstrated by a tiny wedge-shaped area in the upper left quadrant.

The slightly larger area in the right upper quadrant represents almost exclusively (false or unequivocal) CAP-positive results in patients with suspected penicillin allergy, where positive results (class 2 in most instances) proved to be without clinical relevance when the patients were exposed to penicillin. When the eleven "penicillin-sera" were excluded, the concordance rose to 94.1%. These figures prove both the comparability (if not identical nature) of allergy *in vitro* test methods and the excellent execution of the testing procedure by the investigators.

Assessment of practicability

Both systems are easy to install in terms of power and water supply; waste and the noise level are also low. Regarding the organization of work, the start-up/shut-down procedures, the processing of samples, the handling of reagents, and the work flow are satisfactory for the

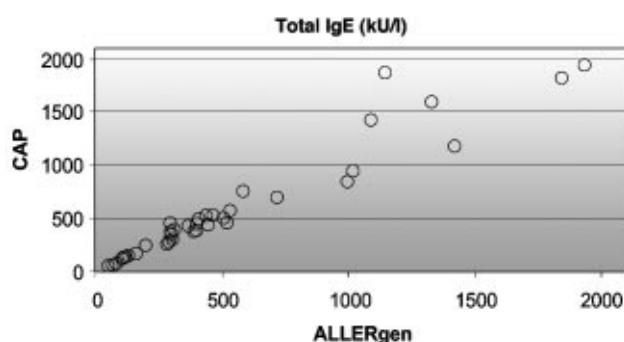


Fig. 3. Scatter plot visualizing the correlation between total IgE measured by the ALLERgen method versus the mean value given by the other CAP-participants of the external quality control program ($y = -25,685 + 0.952 \cdot x$; $R^2 = 0.97$; $n = 40$). Values higher than 2,000 kU/l were excluded from the figure

operators of both systems. With regard to work flow, the tests can be performed by single as well as profile definition. Requests may be continuously entered into the personal computer, resulting in a multitasking operating system. The monitoring of sample and reagent processing on the tray, the control of maintenance functions, the presentation of results for each patient on the screen/printer, and the storage of data are easy to control. During the period of evaluation, neither the Pharmacia CAP system nor the ALLERTECH system suffered any major breakdown that necessitated a service call. Host communication for data import/export is available for both systems.

In contrast to the Pharmacia CAP system however, ALLERTECH is a fully automated microplate system where the carousel can accommodate both samples and allergen tubes. Disposable plastic tips are used for the pipetting of samples and allergens, thereby avoiding any risk of carryover. The liquid form of the allergens is innovative and advantageous, as is the fact that the calibration curves are dispensed on each single plate. All data processing and reporting is also completely automated; the user only has to fill in the work list and load the

samples and reagents in the appropriate carousel and rack. The software allows the user to manage Quality Control sera and to perform statistical evaluations of the results.

Discussion

Historically, the diagnosis of allergic diseases has been mainly based on two types of *in vivo* testing: provocation (challenge testing) and skin testing. In the last decade, *in vitro* testing for IgE antibodies has gained importance for several reasons [9], the major one being that it eliminates the risk of potential systemic reactions [10]. It can also be performed or interpreted in patients with generalized dermatitis, or in those who must continue to take antiallergic medication. It is essential that allergy test results reflect true clinical sensitivity and specificity in order to prevent inappropriate management of patients [9].

However, in the data received from various investigators, the FDA found that the different allergens vary in terms of concordance with skin tests, ranging from greater than 80% for some allergens to less than 20% for others [9]. Therefore, the FDA requests manufacturers to provide allergy-specific clinical data for each new allergen they submit for the RAST-type test. Some of these problems might meanwhile be solved by the introduction of single antigens instead of allergen extracts; the use of recombinant technology has allowed the production of almost all defined type-1 allergens in a pure form [11]. In addition, the innovative arrayed investigation of IgE-reactivity profiles using natural and recombinant allergens may represent a further step forward in the reduction of current problems [12].

However, the sources of potential error in IgE detection are currently numerous: they arise from the collection and handling of the sample itself, the test performance, the equipment, and the laboratory personnel and routines. The Pharmacia CAP System belongs to an advanced generation and is easy to handle. It probably is the most widely distributed system for *in vitro* allergy testing world-wide. The majority of laboratories participating in the Austrian inter-laboratory quality assessment program used the CAP system. However, a few competitors' methods were also in use. The data collected in the laboratories were com-

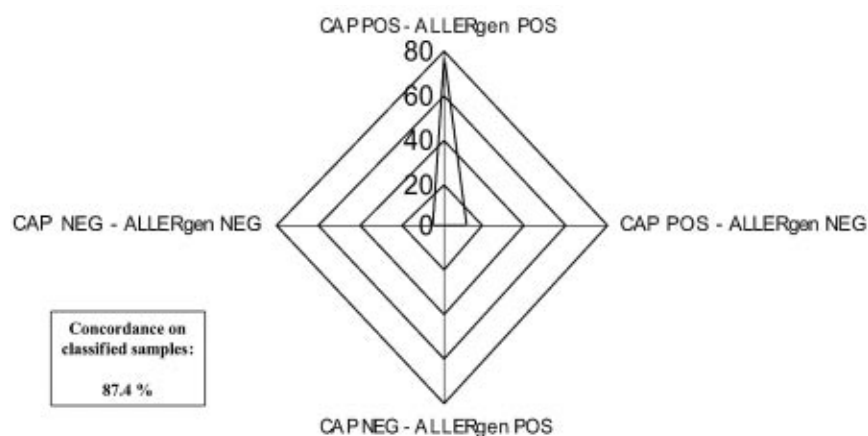


Fig. 4. Comparison of specific IgE determined by CAP and ALLERgen in the external quality control program, depicted in the format of radial plot(s): the center represents zero and the outer frame the maximum value. The lines connect the data registered for the four respective categories

pared with those obtained with the ALLERgen method. With regard to the detection of total IgE and antigen-specific IgE antibodies, the grade of agreement between the two methods was excellent. Within-run and between-days imprecision results were within acceptable ranges, considering the state of the art for heterogeneous immunoassay systems. Regarding the clinical use of the analyzed data, the results are, of course, not comparable with the proposed quality specifications for analytical imprecision and inaccuracy for commonly analysed quantities, such as the immunoglobulins G, A and M [7].

Regarding sensitivity, at first glance the CAP system appears to be more sensitive for certain antigens. However, this results in at least partially reduced specificity – also a well established fact in the literature [3, 13]. This became obvious in the quality control program for specimens from multi-sensitized food and drug allergic patients, for which only laboratories working with Pharmacia systems reported positive results, whereas the competitor companies did not. The clinical relevance of these antibodies was lacking in several specimens. Various explanations have been suggested for such discrepancies in the detection of IgE antibodies: there is evidence that clinical cross reactivities between allergens are based on different immunologic mechanisms. However, patients with elevated IgE levels often have multiple weakly positive RASTs against non-cross-reactive allergens, pointing to a possible nonspecific IgE-allergen interaction [14]. *In vitro* data show that such nonspecific binding to IgE does not bear any clinical relevance [14].

In conclusion, the innovative ALLERgen system using liquid allergens for the detection of total IgE and antigen-specific IgE antibodies in a fully automated form yields reliable results in comparison with other allergy test systems (according to the external quality control program). It compares favorably with the Pharmacia CAP system in that costly and error-prone human work is reduced, while the major quality criteria for every lab method, i.e. sensitivity and specificity, are at least equal.

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References

1. Aberer W, Ebner H, Horak F, et al (1992) Qualitätskontrolle im Allergielabor – 4 Jahre „Ringversuch Allergie“ in Österreich. *Wien Klin Wochenschr* 104: 195–199
2. Fifield R, Hamilton RG (1993) Interlaboratory “external” quality assessment programs for the diagnostic allergy laboratory. *J Clin Immunoassay* 16: 14–52
3. Aberer W, Kränke B, Hager A, Wick G (1995) In vitro allergy testing needs better standardization – test results from different laboratories lack comparability mostly due to missing effective standards. *Int Arch Allergy Immunol* 108: 82–88
4. Schlenvoigt G, Jäger L (1996) Erfahrungen und Ergebnisse in der laborinternen Qualitätssicherung für spezifische IgE-Antikörper-Bestimmungen. *Allergo J* 5: 279–285
5. Kersten W, von Wahl PG, Lange CE, Wenning J (2000) Empfehlungen zur in-vitro-Diagnostik allergischer Erkrankungen. *Allergo J* 9: 21–24
6. Wüthrich B (1989) Epidemiology of the allergic diseases: are they really on the increase? *Int Arch Allergy Appl Immunol* 90: 3–10
7. Fink PCh, Aberer W, Hausen BM (1995) Measurement of total IgE and allergen specific IgE with the Pharmacia CAP system: a multicentre evaluation. *Klin Lab* 41: 119–133
8. Haeckel R (1975) Qualitätssicherung im medizinischen Labor. Deutscher Ärzte Verlag, Köln, S 149–187
9. Anonymous – www.fda.gov/cdrh/ode/guidance/800.pdf – (2001) Radioallergosorbent test (RAST) methods for allergen-specific immunoglobulin E (IgE) 510 (k)s; final guidance for industry and FDA, pp 1–16
10. AAAI – Position statement (1992) The use of in vitro tests of IgE antibody in the specific diagnosis of IgE mediated disorders and in the formulation of allergen immunotherapy. *J Allergy Clin Immunology* 90: 263–267
11. Kraft D, Ferreira F, Vrtala S, Breiteneder H, Ebner C, Valenta R, Susani M, Breitenbach M, Scheiner O (1999) The importance of recombinant allergens for diagnosis and therapy of IgE-mediated allergies. *Int Arch Allergy Immunol* 118: 171–176
12. Suck R, Nandy A, Weber B, Stock M, Fiebig H, Cromwell O (2002) Rapid method for arrayed investigation of IgE-reactivity profiles using natural and recombinant allergens. *Allergy* 57: 821–824
13. Moneret-Vautrin DA, Halpern GM, Brignon JJ, Nicolas JP, Kanny G (1993) Food specific IgE antibodies: a comparative study of AlaSTAT and Pharmacia RAST Phadebas CAP systems in 49 patients with food allergies. *Ann Allergy* 71: 107–114
14. Jensen-Jarolim E, Vogel M, Zavazal V, Stadler BM (1997) Nonspecific binding of IgE to allergens. *Allergy* 52: 844–852

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