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Research Paper

Comparison between two commercial immunoassays:Dr. Fooke ALLERG-O-LIQ versus Phadia ImmunoCAP® System in detecting allergen-specific IgE and total IgE values☆

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Abstract

Objective: Beside patient's history and skin prick testing(SPT) the detection of specific IgE(sIgE) represents an important tool of allergy diagnostics. In recent years different technologies for the detection of sIgE have been developed. The objective of this study is the comparison of the ALLERG-O-LIQ with the ImmunoCAP® System using seven inhalant and four food allergens. **Methods:** Sera from patients were collected and tested for sIgE to inhalant(d1, d2, d5, i6, e1, e5 and m3) and food allergens(f1, f2, f24, f24) by ALLERG-O-LIQ(Dr. Fooke Laboratorien GmbH) and by ImmunoCAP® System(Phadia). Further, samples were also tested for total IgE in both systems. **Results:** Prevalence of positive test results varied between 0/20(f24) and 11/20(e5) for ALLERG-O-LIQ and between 3/18(f23) and 11/20(d1/d5) for ImmunoCAP®. The qualitative agreement between both methods was found between 75%(f24) and 100%(d2) depending on the allergen. Overall qualitative agreement for inhalant(n = 140), food(n = 78) and all allergens(n = 218) tested was 92.1%(kappa = 0.84), 83.3%(kappa = 0.58), 89.0%(kappa = 0.77), respectively. Sensitivity, specificity, positive predictive value (PPV), negative predictive value(NPV) and diagnostic efficiency(DE) were found at 88.2%, 95.8%, 95.2%, 89.6%, 92.1%(inhalant allergens), 62.5%, 92.6%, 78.9%, 84.7%, 83.3%(food allergens) and 81.5%, 94.4%, 91.5%, 87.5%, 89.0%(all allergens). **Conclusion:** Good to excellent qualitative agreement between ALLERG-O-LIQ and ImmunoCAP® for the detection of specific and total IgE could be observed. The degree of agreement depended on the allergen and was higher in the group of inhalant allergens. The ALLERG-O-LIQ System represents a reliable test for the detection of specific and total IgE.

Key words: specific IgE; allergy diagnostics; allergen

INTRODUCTION

World wide frequency of allergies has increased significantly over the past decades[1-3]. The term allergy is often used for type I hypersensitivity reactions (immediate type reactions)[4,5], whose symptoms generally occur within 30~60 minutes after contact with the allergen. Among the most frequent symptoms are: hay fever(rhinitis), conjunctivitis, hives(urticaria), allergic asthma and as the most dangerous manifestation anaphylaxis(the anaphylactic shock). Allergens causing type I hypersensitivity reactions are mostly proteins derived from the natural environment e.g. plant pollen, animal hair, food, mites, and insect venoms. A characteristic feature of type I allergies is the involvement of allergen specific immunoglobulins (antibodies) of class E(sIgE), thus the detection of sIgE is an important tool of modern allergy diagnostics[6-8].

Historically, sIgE to various allergens was determined by radio allergo-sorbent test(RAST) using allergen-coupled cellulose paper discs first described by Ishizaka

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K *et al.* in 1967[9]. Later on, the enzyme allergo-sorbent test(EAST) and more recently the reversed allegro-sorbent test(REAST) were developed and used for the detection of sIgE[6-8]. With the introduction of those second generation methods significant improvements such as greater speed, higher accuracy, use of nonisotopic labels, as well as reporting of IgE concentrations in a continuous range(U/ml) standardized according to the WHO reference preparation for IgE (WHO 75/702).

Today a high number of commercial test systems are available for the detection of sIgE and total IgE following different protocols. The vast majority of test systems make use of allergens immobilized on a solid support such as cellulose discs, cellulose membranes or so called carrier polymer(CAP). The ALLERG-O-LIQ System(Dr. Fooke Laboratorien GmbH, Neuss, Germany) follows the REAST protocol using anti-IgE coated mircotiterplates and biotinylated allergens combined with streptavidin HRP conjugate.

State of the art allergy diagnosis includes detailed patient's history, physical examination, SPT and *in-vitro* tests for the detection of sIgE based on EAST or REAST protocol. Furthermore, provocation challenges and / or cellular tests such as the basophile degranulation test are needed in case of food allergies[6-8].

The objective of this study was to compare the results of the ALLERG-O-LIQ System with the results of the ImmunoCAP® for sIgE to seven inhalant, Dermatophagoides pteronyssinus(d1), Dermatophagoides farinae (d2), Blomia(d5), German cockroach(i6), Cat epithelia (e1), Dog epithelia(e5) and Aspergillus fumigatus (m3) and four food allergens egg(f1), cow's milk(f2), crab (f23) and shrimp(f24) as well as for total IgE.

MATERIALS AND METHODS

Test persons and serum samples

Sera from patients(bronchus asthma, allergic rhinitis and chronic cough) were collected from October 2004 to May 2007 at outpatient department of Guangzhou Institute of Respiratory Disease and stored in aliquots at -20℃ until use. Samples were treated according to the local ethical regulations.

Diagnostic tests *In-vitro*

Sera were tested for sIgE to seven inhalant(d1, d2, d5, i6, e1, e5 and m3) and four food allergens(f1, f2, f23 and f24) by ALLERG-O-LIQ System(Dr. Fooke Laboratorien GmbH, Neuss, Germany) and by ImmunoCAP®(Phadia, Upsalla, Sweden) according to the instructions for use. Further, all samples were also tested for total IgE in both systems. The ALLERG-O-LIQ System is based on the REAST protocol and is performed in microtiter plates. During the first incuba-tion step, IgE is selectively purified from the patient's sample and immobilized on the anti-IgE coated surface of microtiter plates. All interfering substances such as allergen specific IgG, which can compete with sIgE for allergen binding, are removed from the system by a subsequent washing step. Subsequently, immobilized sIgE binds to its corresponding biotinylated allergen. Non IgE binding molecules in the allergen solution are removed by a second washing step. Immunodetection is performed photometrically. In contrast, the ImmunoCAP® System is based on an allergen coated surface with high protein binding capacity(CAP) on which the patient sample is incubated. Specific IgE binding to its corresponding allergen and non specific IgE is removed by washing. Detection of IgE binding is monitored by the use of fluorescence technology. A comparison of both methods is shown in ***Table 1***. Total IgE was measured using the Total IgE EIA(08101FL).

Statistical analysis

Statistics including Fisher's exact, Chi-square and kappa agreement tests, were carried out using excel plug-in Analyse-it(Version 1.62). Kappa agreement > 0.4 was considered as moderate, > 0.6 as high and > 0.8 as very high. $P < 0.05$ were defined as significant. Receiver operating characteristic(ROC) analysis including area under the curve(AUC) was performed and positive-(PPV) and negative predictive value(NPV) as well as test efficiency was determined for each allergen.

RESULTS

Specific IgE

Prevalence of positive test results varied between 0/18(f23), 0/20(f24) and 11/20(e5) for ALLERG-O-LIQ and between 3/18(f23) and 11/20(d1/d5) for ImmunoCAP®. The qualitative agreement between both methods was found between 75%(f24) and 100%(d2) depending on the allergen(***Table 2***). Overall qualitative agreement for inhalant(n = 140), food(n = 78) and all allergens(n = 218) tested was 92.1%(kappa = 0.84), 83.3%(kappa=0.58), 89.0%(kappa=0.77), respectively. Sensitivity, specificity, PPV, NPV and DE were found at 88.2%, 95.8%, 95.2%, 89.6%, 92.1%(inhalant allergens), 62.5%, 92.6%, 78.9%, 84.7%, 83.3%(food allergens) and 81.5%, 94.4%, 91.5%, 87.5%, 89.0% (all allergens)(***Table 3***). ROC and comparative descriptive analysis show good discrimination(AUC=0.922) between ImmunoCAP® positive and negative samples when using the results of sIgE test of ALLERG-O-LIQ (***Fig.1***).

Table 1 **Methodology of ImmunoCAP® and ALLERG-O-LIQ**

	ALLERG-O-LIQ	ImmunoCAP®
Test principle	fluid phase reversed enzyme-immuno-assay(R-EAST)	solid phase fluorescence-enzyme-immuno-assay(FEIA)
Test system	ELISA based technology	closed immunoassay system performed in single wells(CAPs)
Allergens	fluid phase allergens	solid phase allergens
Test procedure	1. IgE binds to anti-IgE 2. Allergen binds to sIgE	1. sIgE binds to allergen 2. Anti-IgE binds to sIgE
Reporter molecule	streptavidin-HRP	β-galactosidase coupled anti-IgE
Detection	photometry	fluorophotometry
Units	k_AU/L	k_AU/L
Calibration	according to WHO 75/702	according to WHO 75/702
Classes	0~6	0~6

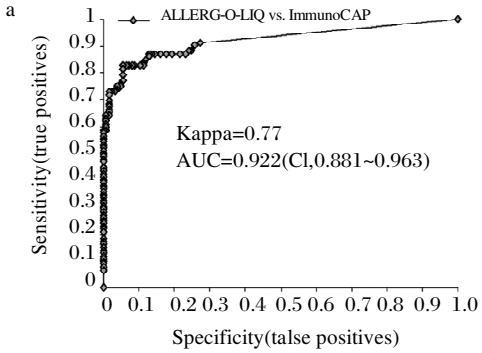
Table 2 **Agreement between ALLERG-O-LIQ and ImmunoCAP®**

		ImmunoCAP®				ImmunoCAP®		
ALLERG–O–LIQ	d1	+	–		d2	+	–	
	+	10	0	10	+	10	0	10
	–	1	9	10	–	0	10	10
		11	9			10	10	
	d5	+	–		e1	+	–	
ALLERG–O–LIQ	+	9	0	9	+	9	0	9
	–	2	9	11	–	1	10	11
		11	9			10	10	
	e5	+	–		i6	+	–	
ALLERG–O–LIQ	+	8	3	11	+	7	0	7
	–	1	8	9	–	2	11	13
		9	11			9	11	
	m3	+	–		All inhalant	+	–	
ALLERG–O–LIQ	+	7	0	7	+	60	3	63
	–	1	12	13	–	8	69	77
		8	12			68	72	
	f1	+	–		f2	+	–	
ALLERG–O–LIQ	+	7	3	10	+	8	1	9
	–	1	9	10	–	0	11	11
		8	12			8	12	
	f23	+	–		f24	+	–	
ALLERG–O–LIQ	+	0	0	0	+	0	0	0
	–	3	15	18	–	5	15	20
		3	15			5	15	
	foods	+	–		All allergens	+	–	
ALLERG–O–LIQ	+	15	4	19	+	75	7	82
	–	9	50	59	–	17	119	136
		24	54			92	126	

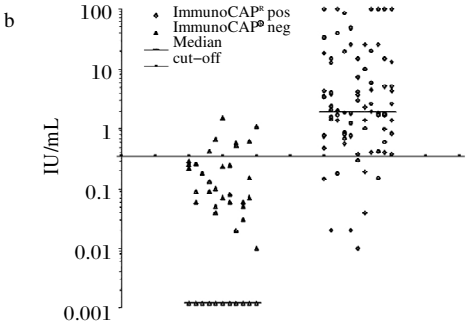
d1:*Dermatophagoides pteronyssinus*; d2:*Dermatophagoides farinae*; d5:Blomia; e1:Cat epithelia; e5:Dog epithelia; i6:German cockroach; m3: Aspergillus fumigatus; f1:egg; f2:cow's milk; f23:crab; f24:shrimp.

Table 3 **Assay performance data of the ALLERG-O-LIQ System for specific IgE compared to ImmunoCAP®**

	d1	d2	d5	e1	e5	i6	m3	f1	f2	f23	f24
n	20	20	20	20	20	20	20	20	20	18	20
Kappa statistic	0.90	1.00	0.80	0.90	0.60	0.79	0.89	0.60	0.90	–	–
2-tailed *P*	0.0001	< 0.0001	0.0003	0.0001	0.0059	0.0003	0.0001	0.0062	0.0001	–	–
P	0.0001	< 0.0001	0.0003	0.0001	0.0098	0.0005	0.0001	0.0020	0.0001	–	–
Sensitivity	90.9%	100.0%	81.8%	90.0%	88.9%	77.8%	87.5%	87.5%	100.0%	0.0%	0.0%
Specificity	100.0%	100.0%	100.0%	100.0%	72.7%	100.0%	100.0%	75.0%	91.7%	100.0%	100.0%
Agreement	95.0%	100.0%	90.0%	95.0%	80.0%	90.0%	95.0%	80.0%	95.0%	83.3%	75.0%
Positive predictive value	100.0%	100.0%	100.0%	100.0%	72.7%	100.0%	100.0%	70.0%	88.9%	–	–
Negative predictive value	90.0%	100.0%	81.8%	90.9%	88.9%	84.6%	92.3%	90.0%	100.0%	83.3%	75.0%
Efficiency	95.0%	100.0%	90.0%	95.0%	80.0%	90.0%	95.0%	80.0%	95.0%	83.3%	75.0%



Receiver operating characteristics(ROC) analysis of the specific IgE results



Comparative descriptive analysis of the specific IgE results

(a)ROC analysis and comparative descriptive analysis (b)show good differentiation between ImmunoCAP® positive and negative samples using the specific IgE test of ALLERG-O-LIQ as expressed by the area under the curve(AUC) of 0.922(Confidence interval CI = 0.881 to 0.963) and a qualitative kappa agreement value of 0.77, respectively. In the comparative descriptive analysis (b) values below 0.001 k_AU/L are shown as 0.001 k_AU/L and values above 100 k_AU/L as 100 k_AU/L.

Fig. 1 Receiver operating characteristics(ROC) analysis and comparative descriptive analysis of the specifife IgE results

Total IgE

Serum samples derived from 79 patients were tested for total IgE by ALLERG-O-LIQ Total IgE and ImmunoCAP®(***Table 4***). The agreement between the total IgE results was found at $r = 0.87$($P < 0.0001$; according to Pearson). Mean and median values were 329.7 k_AU/L and 121.2 k_AU/L, 570.8 k_AU/L and 137.0 k_AU/L for ALLERG-O-LIQ and ImmunoCAP® respectively. Results are shown in ***Fig. 2***.

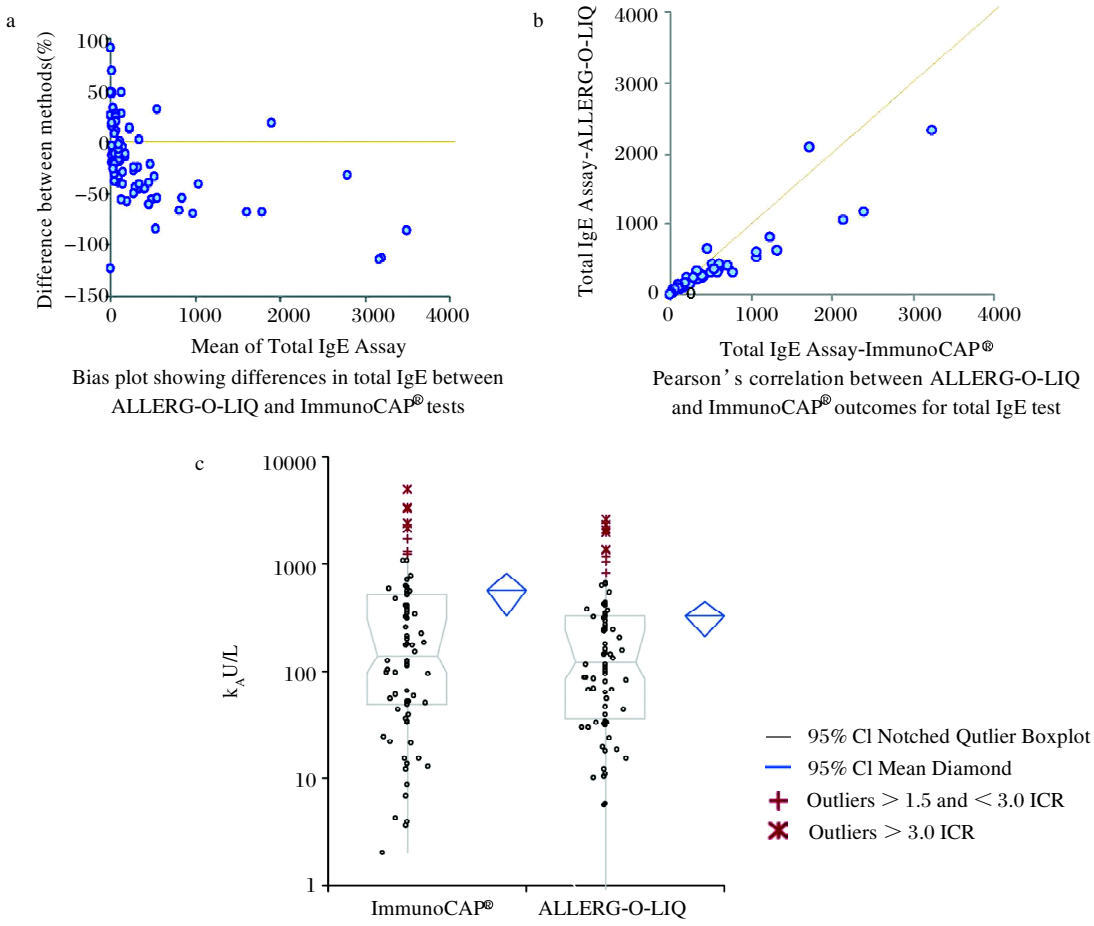
DISCUSSION

After the identification of IgE in 1967 directed research yielded in significant improvements in the diagnosis of type I allergies[10,11]. State of the art allergy diagnosis includes the patient's history, SPT and *in-vitro* tests for the detection of sIgE such as EAST or REAST. Furthermore, provocation challenges and/or cellular tests such as the basophile degranulation test are needed in the case of food allergies. Mircoarrays using purified, recombinant or synthetic allergens have been used in allergy research and represent a promising tool for allergy diagnostic in the future[12-14].

In previous studies the technical performance and clinical usefulness of different *in-vitro* tests for the detection of specific IgE have been analyzed and

Table 4 **Overview of discrepant samples(sIgE)**

ID	Allergen code	Gender	Age	ImmunoCAP® k_AU/L	ALLERG-O-LIQ k_AU/L	Immuno CAP®Total IgE k_AU/L
657	d1	M	35	**0.40**	0.00	110
657	d5	M	35	**0.55**	0.18	110
718	d5	F	30	**1.42**	0.00	295
294	e1	F	47	**2.67**	0.15	983
312	e5	M	42	0.35	**1.09**	**439**
212	e5	M	12	0.35	**0.68**	**753**
542	e5	M	12	0,66	0.19	477
390	e5	F	25	0.35	**1.51**	**726**
714	i6	M	6	**5.02**	0.00	192
859	i6	M	59	**0.38**	0.14	259
727	m3	M	30	**0.39**	0.02	592
791	f1	F	2	**0.41**	0.30	46
831	f1	M	5	0.35	**0.52**	**34**
605	f1	F	8	0.35	**0.63**	**63**
474	f1	M	4	0.35	**0.42**	**3**
856	f2	M	6	0.35	**0.58**	**113**
598	f23	F	20	**5.12**	0.01	488
384	f23	M	16	**0.40**	0.00	n.d.
111	f23	F	23	**1.92**	0.00	477
855	f24	F	3	**0.52**	0.00	120
598	f24	F	20	**7.27**	0.00	488
432	f24	M	27	**2.53**	0.04	333
384	f24	M	16	**0.59**	0.02	n.d.
111	f24	F	23	**2.45**	0.00	477
Number pos/neg in other method				17/24	7/24	
Number>1 k_AU/L/neg in other method				8/24	2/24	



Differences are displayed using bias plot (a) and agreement is shown in the Pearson's correlation diagram(b), A correlation coefficient of $r = 0.87$ ($P < 0.0001$) was calculated based using the Pearson's method. Diagram (c) shows a comparative descriptive analysis of the total IgE results obtained by ALLERG-O-LIQ and ImmunoCAP® System. Comparative descriptives show vertical box-plots for each sample, side by side for comparison. The blue line series shows parametric statistics: diamond, mean and the requested confidence interval around the mean; notched line, requested parametric percentile range. The notched box and whiskers show non-parametric statistics: notched box, median, lower and upper quartiles, and confidence interval around the median; dotted line, connects the nearest observations within 1.5 interquartile ranges(IQR) of the lower and upper quartiles. + and ✱, possible outliers-observations more than 1.5 IQR(near outliers) and less than 3.0 IQR(far outliers) from the quartiles. Vertical lines, requested nonparametric percentile range.

Fig. 2 Comparison of Total IgE ALLERG-O-LIQ with ImmunoCAP® total IgE

compared. Although no single method has been officially designated as "golden standard", the Pharmacia CAP System is in worldwide use, and is a de facto standard, to which other methods are compared[11]. Therefore, most studies that were designed to evaluate the accuracy of laboratory methods for the detection of sIgE used the ImmunoCAP® System as the reference method[15-18]. In 2004 the ALLERG-O-LIQ and the ImmunoCAP® System were first compared. The results showed good agreement for inhalant allergens between both methods[16,17]. The Spearman's rho value was 0.95 for birch pollen(t3), 0.90 for timothy grass pollen (g6), 0.82 for mugwort(w6), 0.85 for *D. pteronyssinus* (d1), 0.87 for cat epithelia(e1), 0.82 for dog epithelia (e5). 0.84 for hen's egg(f1), 0.60 for cow's milk (f2), 0.60 for wheat(f4), 0.31 for soybean(f14), 0.68 for hazelnut(f17) and 0.82 for apple(f49).

As in our study the agreement between both methods was significantly higher for inhalant allergens than for food allergens. Kleine-Tebbe and colleagues concluded that the degree of agreement between the two systems is dependent on the complexity of the respective allergen. However, other factors such as total IgE levels or allergen-specific IgG have not been considered.

Recent studies have provided evidence that the number of positive sIgE results and the total amount of sIgE correlated with disease severity and the number of clinical symptoms[19,20].

Despite the fact that they are often promoted as tests for allergy diagnosis, sIgE immunoassays are best regarded as tests for the presence or absence of detectable sIgE. IgE is normally present in the serum, and sIgE

can be found in patients with allergic diseases as well as in about 15% of asymptomatic normal individuals[21]. Additionally, some patients with the classic diseases of IgE-mediated allergic hypersensitivity have easily demonstrable sIgE antibody, and other patients with these diseases do not. Even in a symptomatic individual, a positive sIgE test result in and of itself is not necessarily clinically relevant. Thus, it has traditionally been taught that the result of any test for sIgE-immunoassay or skin test will not in itself determine whether the patient has symptoms of IgE mediated allergic hypersensitivity upon allergen exposure, nor will it in and of itself determine treatment[22].

A recent study emphasized the importance of sIgE density rather than the amount of sIgE with respect to the clinical response[23,24]. Furthermore, it is known from previous literature that low titer of sIgE, especially against allergens of grass pollen and house dust mites do not necessarily have clinical impact. Since the clinical background of sample donors of the present investigation was not available, it remains a matter of further research to investigate the clinical value of ALLERG-O-LIQ. A clinical evaluation that analyzes the clinical accuracy and the applicability of ALLERG-O-LIQ is currently under way.

In the present study we have found a good to excellent agreement between ALLERG-O-LIQ and ImmunoCAP® for the detection of specific and total IgE. The degree of agreement depended on the allergen and was higher in the group of inhalant allergens. The ALLERG-O-LIQ System represents a reliable test for the quantitative detection of specific and total IgE. Further studies are mandatory to investigate the clinical accuracy of the ALLERG-O-LIQ System.

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