

Comparison of ADVIA Centaur[®] and Pharmacia UniCAP[®] tests in the diagnosis of food allergy in children with atopic dermatitis

Contin-Bordes C, Petersen A, Chahine I, Boralevi F, Chahine H, Taïeb A, Sarrazat A, Moreau J-F and Taupin J-L. Comparison of ADVIA Centaur[®] and Pharmacia UniCAP[®] tests in the diagnosis of food allergy in children with atopic dermatitis.

Pediatr Allergy Immunol 2007; 18: 614–620.

© 2007 The Authors

Journal compilation © 2007 Blackwell Munksgaard

In a study comprising 63 children diagnosed with atopic dermatitis, the results of the ADVIA Centaur system was compared with the results obtained with the Pharmacia UniCAP100 system, which has been widely considered as a reference method for seric specific IgE (sIgE) measurements. The individual immunization against the most common food allergens [egg (f1), cow milk (f2), cod (f3), wheat (f4), peanut (f13) and soy bean (f14)] was determined by *in vitro* serum IgE testing and skin prick test (SPT). The comparison of the sIgE titers revealed a good concordance between the Centaur and the UniCAP tests for f1, f3, and f13 (94 %, 91 %, and 96 % respectively). However, the concordance was lower for f2, f4, and f14 (76 %, 77 %, and 77 % respectively) because of discrepancies between the two techniques. When compared with SPT and clinical diagnosis, on the 40 discordant cases found between the Centaur and the UniCAP, the Centaur showed concordance with the patients food reaction and SPT in 34/40 cases, and UniCAP in only 6/40 cases. Accordingly, the Centaur test displayed a statistically significantly better performance on specificity and concordance with SPT for f2, f4, and f14 (concordance/specificity = 70%/71%, 76%/75% and 90%/88% respectively), than the CAP test (49%/54%, 51%/52% and 67%/65% respectively).

Cécile Contin-Bordes^{1,*}, Anita Petersen^{2,*}, Isabelle Chahine¹, Franck Boralevi³, Hikmat Chahine⁴, Alain Taïeb³, Anne Sarrazat¹, Jean-François Moreau¹ and Jean-Luc Taupin¹

¹Laboratory of Immunology, CHU Pellegrin, Amélie Raba Léon, Bordeaux, France, ²ALK-Abelló Department of Development and Support, Stenløse, Denmark, ³Dermatology department of the Children's hospital, Bordeaux, France, ⁴Laboratoire Forte et associés, St Paul les Dax, France

Key words: ADVIA Centaur[®]; atopic dermatitis; food allergy; specific IgE assay; UniCAP100[®] system

Jean-Luc Taupin, Laboratory of Immunology, CHU Pellegrin, Amélie Raba Léon, Bordeaux, France
Tel.: +33 5 56 71 43 31
Fax: +33 5 56 79 60 79
E-mail: jean-luc.taupin@chu-bordeaux.fr

*These authors contributed equally to this work.

Accepted 13 January 2007

The basis for an etiologic diagnosis of allergic disease is the demonstration of a sensitization, namely the presence of allergen-sIgE antibodies. To identify the causative allergen, *in vivo* skin prick tests (SPT) or *in vitro* allergen sIgE measurements are usually performed. A reliable and accurate assessment of sIgE is essential to reach a correct diagnosis (1, 2).

Food allergy is a common problem in infants and small children, and it clearly plays a significant pathogenic role in 40% of children with moderate to severe atopic dermatitis (3, for review see 4). There is an increasing consensus to think that food allergens including cow's milk, egg, and peanut are accounting for most of the reactions found in the pathogenesis of atopic dermatitis in infancy and childhood (5, 6).

Wheat, soy and fish are other common allergens causing food allergy in children. Importantly, elimination of the responsible food leads to marked improvement in eczematous symptoms in the majority of these children. In this paper, we report on the possible influence of the most common food allergens in 63 children diagnosed with atopic dermatitis by measuring *in vitro* sIgE and SPT. A reliable method for determination of food-sIgE levels, together with SPT, may reduce the need for oral food challenges in children with atopic dermatitis (7).

At present, the Pharmacia CAP is the most widely accepted reference method for sIgE measurements. It has been shown that quantification of food-sIgE in the UniCAP is a useful test for diagnosing symptomatic allergy to egg, milk,

peanut and fish in the pediatric population and could eliminate the need to perform double-blind, placebo-controlled food challenges in a significant number of children (8). However in a recent study, the UniCAP failed to accurately determine sIgE to wheat and soy (9). Determination of sIgE has been introduced on the ADVIA Centaur technology platform (Bayer Diagnostics, Puteaux, France), a newly developed immunochemiluminometric *in vitro* assay for determination of sIgE antibody using PMP coupled with anti-IgE as solid phase and liquid biotinylated allergen-extracts (10). The aim of this study was to compare the results of the sIgE assay obtained either from the ADVIA Centaur or from the UniCAP systems with the six primary food allergens: hen's egg white, cow's milk, cod fish, wheat, peanut and soy bean. The results from both *in vitro* sIgE methods were compared with SPT results to determine which of the two *in vitro* tests shows the best performance with respect to the allergy diagnosis based on the patient's clinical history and SPT.

Patients and methods

Patients

The study enrolled 63 referrals to the Department of Dermatology at the Children's hospital in Bordeaux between January 2003 and June 2003 with informed consent from the parents. The children were between 4 months and 17 yr (median 2 yr and 2 months) old, and the majority were boys (boys/girls ratio = 1.55). All the children presented clinical signs of atopic dermatitis. Some of the children also presented a case history of allergic reaction after food introduction. The clinical diagnoses were supported by skin test and determination of serum sIgE towards the primary food allergens: egg, milk, cod, wheat, peanut and soy. Patients were segregated in three groups based on the following parameters:

- Group 1: One isolated clinical sign (atopic dermatitis)
- Group 2: One isolated clinical sign (atopic dermatitis) and an apparent allergic reaction after food uptake or a positive skin test towards the concerned allergen.
- Group 3: One isolated clinical sign (atopic dermatitis) and an apparent allergic reaction after food uptake as well as a positive skin test towards the concerned allergen and/or a positive labial or oral provocation test. (Only three patients from group 3 received an oral provocation test at the time of the study).

For most of the patients, the apparent allergic reaction (vomiting or asthma) after food uptake was caused by an acute IgE mediated reaction as no clinical exacerbation of the cutaneous lesions were recorded.

Skin prick test

Skin prick tests were performed following the established guidelines excluding the patients under therapeutical regimen using antihistamines of any type. Allergen extracts used were egg white (f1), milk (f2), cod (f3), wheat (f4), peanut (f13) and soy (f14), and all were purchased from Laboratoire Stallergènes (Paris, France), except for f2 which was the native allergen (fresh cow's milk). Allergen extracts, along with appropriate negative (saline) and positive (histamine: 10 mg/ml) controls, were applied on the forearm using the prick technique. Other allergens were tested on the basis of the child's history, but are not included in the results of this study. Allergens eliciting a mean weal diameter of 3 mm or greater than the negative control were considered positive. In 13 cases, SPT could not be carried because of anti-histaminic treatment or dermographism.

Serum samples

Serum obtained from each child initial medical examination was divided into two aliquots. One aliquot was stored at 4°C (for no longer than a week) until it was analyzed for sIgE with the UniCAP100, our routine assay for sIgE measurement in the clinical laboratory. The second aliquot was immediately frozen for further sIgE analysis on the ADVIA Centaur.

Allergens/assay procedures

Serum IgE titers were measured in all patients for the following six allergens: egg white (f1), cow's milk (f2), cod (f3), wheat (f4), peanut (f13) and soy bean (f14). All patient samples were also tested using the ADVIA Centaur system, however only a selection of these were also tested in the Pharmacia CAP system depending on the quantity of serum available from the children.

The ADVIA Centaur sIgE assay is a reverse sandwich immunoassay using direct chemiluminescent technology. The monoclonal mouse antihuman IgE antibody is covalently bound to paramagnetic particles (PMP) in a solid phase. The anti-IgE coupled to the PMP captures the sample IgE. Non-IgE is removed by washing. Biotinylated allergen is added in excess and binds

to the allergens-sIgE antibody captured on the solid phase. Streptavidin labeled with acridinium ester (LITE reagent) is added and binds to the biotin-labeled allergen. A direct relationship has been demonstrated to exist between the amount of allergen-sIgE present in the sample and the amount of relative light units emitted by the acridinium ester and detected by the apparatus. Results are expressed in kU/l.

The Pharmacia CAP system uses cyanogen-bromide-activated cellulose derivatives as the solid phase encased in a capsule (ImmunoCAP). The allergen is covalently bound to this hydrophilic carrier polymer, which catches all allergen-sIgE in the sample which is detected directly with a combination of polyclonal and monoclonal anti-IgE (Fc specific) antibodies labeled with beta-galactosidase, generating fluorescence.

Calibrators used by the two assays were adjusted to the same reference standard allowing the direct comparison of the sIgE titers measured with both techniques. The cut off is 0.35 kU/l in both *in vitro* systems. Children were considered sensitized if their sIgE levels were above the cut off.

Statistics

We considered as the reference the SPT. Differences in concordance, sensitivity and specificity between the SPTs and ADVIA Centaur or UniCAP100 sIgE assays for the same allergens were determined and evaluated to see if they were statistically significant. In this study, significance will imply two-sided tests and confidence intervals at 95% confidence. McNemar's tests were performed under the assumption, that the difference between the two *in vitro* methods on the same serum sample is independent of the actual level of sIgE in the serum sample. For statistical calculations, we only included samples for which all three method results for each allergen were available.

Results

Clinical outcomes

Twenty of 63 children (31.7 %) were included in group 1, 32 of 63 (50.7 %) in group 2, and 11 of 63 (17.4 %) in category 3. For f1, f2, f3, f4, f13 and f14, the results of the three methods were available for 49, 41, 44, 48 and 43 children respectively. Of note, in group 1, 11 of 20 children (17.4 %) were prescribed SPT even in the absence of allergic reaction after food challenge but because of severe, treatment-resistant

atopic dermatitis or atopic dermatitis with growth delay (based on the recommendations of our Pediatric Dermatology department). For all these 11 children, the SPT appeared to be negative for all the allergens tested.

In vitro method comparison

Direct comparison between ADVIA Centaur and UniCAP100 for the six food allergens is presented as scatter plots in Fig. 1. The number of discordant samples (being positive in one method and negative in the other) for each allergen and the dose in kU/l is shown in Table 1 and compared with the SPT results and the patient's apparent food reaction to the concerned allergen. There was a good correlation and concordance between the two methods for f1, f3 and f13. However, there was a poorer correlation and a lower concordance between the two methods for f2, f4 and f14. The discordance between the two methods was in most of the cases due to discrepancy between the UniCAP and SPT results. Of the 40 discordant samples, the ADVIA Centaur data were in agreement with the SPT outcome in 34 cases whereas the results from the CAP were in agreement with SPT in only six cases.

Comparisons between sIgE assays and SPT taken as the reference

Contingency tables describing positive/negative results on SPT and sIgE measured by the Centaur and the UniCAP systems are listed in Fig. 2. Comparison of each *in vitro* test to SPT for sensitivity, specificity and concordance at 0.35 kU/l cut off, are reported in Table 2A and Table 2B. Statistically significant differences regarding specificity and concordance in reference to SPT were noticed between UniCAP100 and ADVIA Centaur. The best specificity and concordance to SPT were achieved using the Centaur test for the allergens f2 (ADVIA/UniCAP specificity: 70%/49%, $p = 0.021$; concordance to SPT 71%/54%, $p = 0.004$), f4 (ADVIA/UniCAP specificity: 76%/51%, $p = 0.002$; concordance to SPT 75%/52%, $p = 0.002$), and f14 (ADVIA/UniCAP specificity: 90%/67%, $p = 0.002$; concordance to SPT 88%/65%, $p = 0.002$).

Discussion

Results of testing surveys available in the literature already suggest that not all *in vitro* tests for sIgE measurements yield similar results (11). In

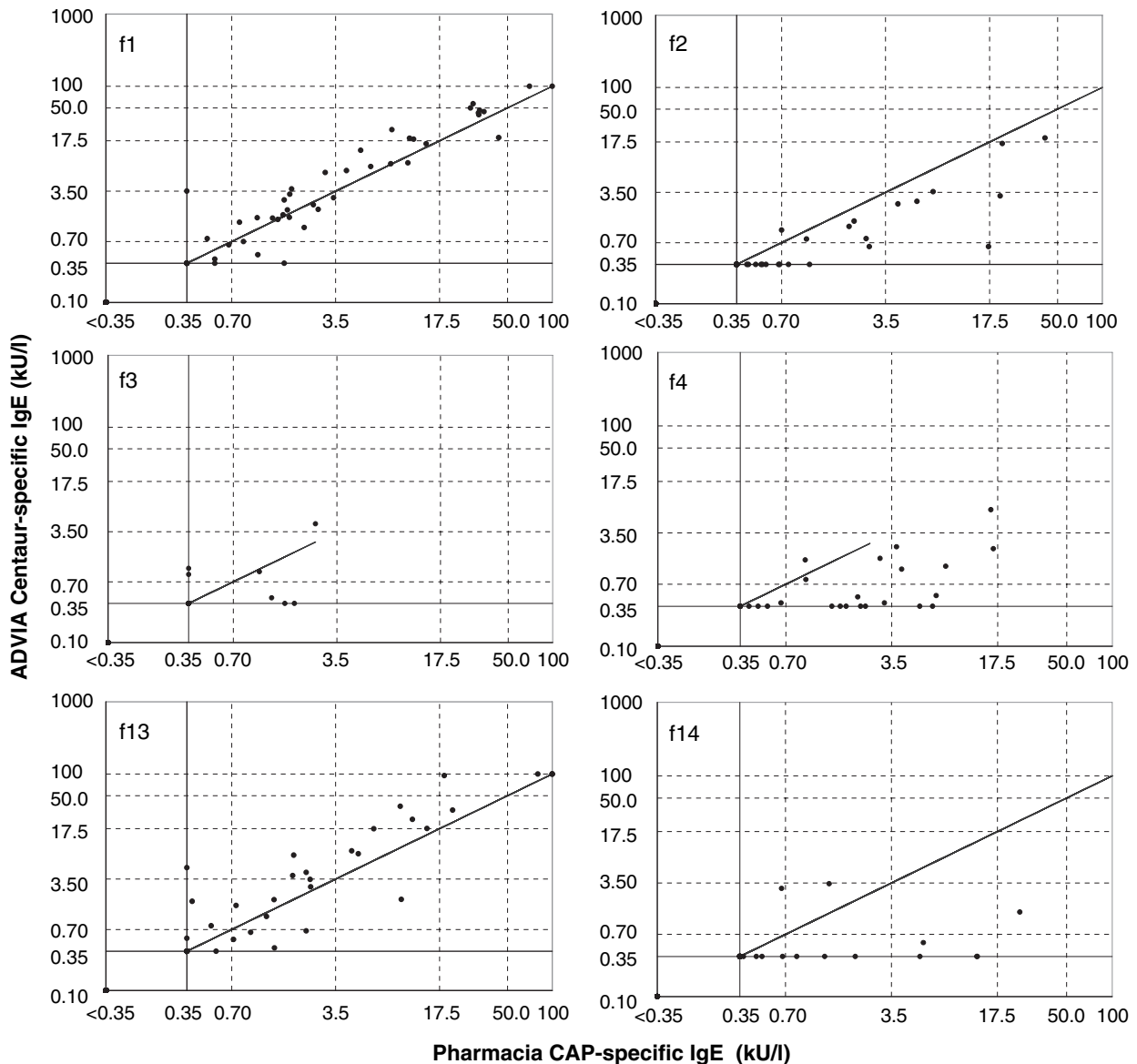


Fig. 1. Comparison between the results obtained with the ADVIA Centaur sIgE assay and Pharmacia CAP system for the six most common food allergens (f1, f2, f3, f4, f13, f14). The cut off (0.35 kU/l) is shown as a solid line whereas the dotted grid lines indicate the allergy classes.

the present study we compare a new method (ADVIA Centaur sIgE assay) with our current method (Pharmacia UniCAP100) that is considered as a reference in routine clinical laboratories. This study analyzes the performances of the tests in a routine group of children, suffering from atopic dermatitis suspected to be due to food allergies at various stages of severity. Implications of food allergy in clinical signs and symptoms were primarily suspected on the basis of anamnesis and SPT in most children included in this study. Because pathogenesis secondary to food allergy in atopic dermatitis is bound to different immunological mechanisms,

not only IgE-mediated ones, 100% agreement between clinical tests and the clinical diagnosis does not exist for food allergy (12). The detection of sIgE antibodies clearly established that a sensitization has occurred, but does not always indicate the presence of an allergic disease (13). Sometimes food sensitization also persists, in children with atopic dermatitis, even though food tolerance has been reached.

Our method comparison revealed that sIgE values obtained with the ADVIA Centaur system showed a good correlation with those obtained from the UniCAP100 system for f1, f3 and f13, while such correlation was much lower for f2, f4

Table 1. Analysis of discordant results between ADVIA Centaur and Pharmacia Unicap for the six most common food allergens (f1, f2, f3, f4, f13, f14) in reference to SPT. Concordant sIgE results to SPT and allergic manifestations are in bold.

Allergen code	Number of discordant samples (%)	Number of concordant results between UniCAP and SPT*	Number of concordant results between Advia Centaur and SPT*			
(A) General						
f1	3 (6)	2	1			
f2	10 (24)	1	9			
f3	4 (9)	2	2			
f4	10 (23)	0	10			
f13	3 (4)	1	2			
f14	10 (23)	0	10			
Allergen code	Patient number	UniCAP kU/l	ADVIA Centaur kU/l	SPT*	Allergic reaction after allergen uptake**	Group of food allergy reactivity***
(B) Detailed						
f1	39	0.54	<0.35	+	+	2
	58	1.58	<0.35	—	—	1
f2	67	<0.35	3.51	—	—	2
	15	0.68	<0.35	—	—	2
	18	0.55	<0.35	—	—	2
	25	1.08	<0.35	—	—	3
	32	0.67	<0.35	+	+	2
	44	0.52	<0.35	—	—	3
	47	0.47	<0.35	—	—	2
	54	0.42	<0.35	—	—	3
	55	0.78	<0.35	—	—	2
	60	0.41	<0.35	—	—	2
f3	61	0.51	<0.35	—	—	2
	17	< 0.35	0.89	—	—	2
	27	1.56	<0.35	—	—	2
	33	1.81	<0.35	—	—	2
f4	38	<0.35	1.08	—	—	1
	1	2.35	<0.35	—	—	2
	17	5.36	<0.35	—	—	2
	18	2.18	<0.35	—	—	2
	25	0.4	<0.35	—	—	3
	35	1.75	<0.35	—	—	2
	38	6.51	<0.35	—	—	1
	44	1.6	<0.35	—	—	3
	48	1.41	<0.35	—	—	2
	60	0.53	<0.35	—	—	2
f13	66	0.46	<0.35	—	—	1
	41	< 0.35	0.53	—	—	1
	48	< 0.35	5.04	+	+	2
f14	66	0.55	<0.35	—	—	1
	1	1.27	<0.35	—	—	2
	7	2.02	<0.35	—	—	1
	12	0.37	<0.35	—	—	1
	20	5.39	<0.35	—	—	2
	27	12.8	<0.35	—	—	2
	44	0.83	<0.35	—	—	3
	55	0.49	<0.35	—	—	2
	65	0.45	<0.35	—	—	3
	67	0.67	<0.35	—	—	2
	68	12.9	<0.35	—	—	2

*The SPT was performed at the time of the blood collection.

**The allergic manifestation after food intake of the considered allergen were recorded from the case history at the time of blood collection.

***See Patients and methods section for group definition.

f1		SPT			
		+	-		
ADVIA Centaur	+	22	17	39	
	-	2	8	10	
		24	25	49	
f2		SPT			
		+	-		
ADVIA Centaur	+	3	11	14	
	-	1	26	27	
		4	37	41	
f3		SPT			
		+	-		
ADVIA Centaur	+	1	4	5	
	-	0	39	39	
		1	43	44	
f4		SPT			
		+	-		
ADVIA Centaur	+	2	10	12	
	-	1	31	32	
		3	41	44	
f13		SPT			
		+	-		
ADVIA Centaur	+	12	17	29	
	-	1	18	19	
		13	35	48	
f14		SPT			
		+	-		
ADVIA Centaur	+	0	4	4	
	-	1	38	39	
		1	42	43	
f1		SPT			
		+	-		
Pharmacia CAP	+	23	17	40	
	-	1	8	9	
		24	25	49	
f2		SPT			
		+	-		
Pharmacia CAP	+	4	19	23	
	-	0	18	18	
		4	37	41	
f3		SPT			
		+	-		
Pharmacia CAP	+	1	4	5	
	-	0	39	39	
		1	43	44	
f4		SPT			
		+	-		
Pharmacia CAP	+	2	20	22	
	-	1	21	22	
		3	41	44	
f13		SPT			
		+	-		
Pharmacia CAP	+	11	17	28	
	-	2	18	20	
		13	35	48	
f14		SPT			
		+	-		
Pharmacia CAP	+	0	14	14	
	-	1	28	29	
		1	42	43	

Fig. 2. Results for the sIgE blood titers measured by the ADVIA Centaur method and the Pharmacia CAP method are depicted in 2 × 2 contingency tables with positive/negative classes.

and f14. The ADVIA Centaur revealed a statistically significant better performance on specificity and concordance with SPT on f2, f4 and f14 when compared with UniCAP.

In the cases of discrepancy between the two *in vitro* systems the Centaur agrees with the SPT in 34 of 40 cases. Interestingly, for cow's milk, wheat and soy the UniCAP100 system exhibited positive results which were not confirmed by the patients' clinical history of food reactions, SPT or the ADVIA Centaur test. One hypothesis which might account for this observation may lie in the assay architecture of the two assays. The UniCAP is designed as a normal catching antibody immunoassay with solid phase bound allergens. This type of assay design requires a high allergen load on the solid phase, because all specific antibodies will bind to the allergen

Table 2. Correlation between ADVIA Centaur (A) or the Pharmacia CAP (B) sIgE assays with skin prick test taken as the reference. Positive/negative concordance was determined at 0.35 kU/l cut off. Sensitivity, specificity, concordance and 95 % confidence intervals were calculated at 0.35 kU/l cut-off using Mc Nemar's statistical test

Allergen	Number of samples	Sensitivity (%)	Confidence interval	Specificity (%)	Confidence interval	Concordance (%)	Confidence interval
(A) ADVIA Centaur							
f1	49	92	73–99	32	15–54	61	46–75
f2	41	75	19–99	70*	53–84	71 ***	54–84
f3	44	100	5–100	91	78–97	91	78–97
f4	44	66	9–99	76 **	60–88	75 **	60–87
f13	48	92	64–100	51	34–69	60	47–76
f14	43	-	-	90 **	77–97	88 **	75–96
(B) Pharmacia UniCAP							
f1	49	96	79–100	32	15–54	63	48–77
f2	41	100	47–100	49	32–66	54	37–69
f3	44	100	5–100	91	78–97	91	78–97
f4	44	67	9–99	51	35–67	52	37–68
f13	48	85	55–98	51	34–69	63	45–74
f14	43	-	-	67	50–80	65	49–79

*p = 0.021, **p = 0.002, ***p = 0.004. Bold numbers and asterisks indicate statistically significant differences between the two *in vitro* methods in favor of ADVIA Centaur.

regardless of the antibody isotype. The ADVIA Centaur is a reversed-phase immunoassay with anti-IgE antibodies coupled to PMP which captures in a first step all IgE allowing the subsequent sIgE detection with biotinylated allergens in liquid phase. In this second setting, a much lower amount of allergen is needed and the low allergen concentration enables the assay to adjust detection of antibodies based on their affinity and thereby excluding responses from low affinity polyreactive IgE. In our study, multiple low false positive results of the discordant samples were seen in the UniCAP. This may reflect the high allergen load of the UniCAP which allows the detection of antigen-specific low affinity IgE, which is not relevant for the food reaction of the patients. This is in agreement with a previous study which emphasized the failure of soy and wheat allergy to be accurately predicted by the UniCap technique (9). However, we can not exclude that the architecture of the UniCAP test might allow the detection of antigen-specific very low affinity IgE which could have an impact in the future development of the allergic process. We conclude that although the two *in vitro* tests seem to perform equally on egg, cod and peanut, the ADVIA Centaur may provide a more reliable test for sIgE against milk, wheat and soy.

Acknowledgments

We thank the department of Paediatric Dermatology of the Bordeaux University Hospital and all the patients who accepted to participate in this study. We are indebted to Emmanuel Bourgeois and Isabelle Bazot for technical assistance on sIgE measurements with the ADVIA Centaur.

We thank Jesper Fromberg Nielsen (ALK-Abelló, Stenløse, Denmark) for our helpful discussions. We would like to thank Prof. Laurence Bordenave for her help in using ADVIA Centaur.

References

1. YMAN L. Standardization of *in vitro* methods. *Allergy* 2001; 56: 70–4.
2. SZEINBACH SL, BARNES JH, SULLIVAN TJ, WILLIAMS PB. Precision and accuracy of commercial laboratories' ability to classify positive and/or negative allergen-specific IgE results. *Ann Allergy Asthma Immunol* 2001; 86: 373–81.
3. EINGENMANN PA, SICHERER SH, BOROKOWSKI TA, COHEN BA, SAMPSON HA. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998; 101: 8–14.
4. SAMPSON HA. The evaluation and management of food allergy in atopic dermatitis. *Clin Dermatol* 2003; 21: 183–92.
5. RANCE F, GRANDMOTTET X, GRANDJEAN H. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. *Clin Exp Allergy* 2005; 35: 167–72.
6. HILL DJ, HOSKING CS. Food allergy and atopic dermatitis in infancy: an epidemiologic study. *Pediatr Allergy Immunol* 2004; 15: 421–7.
7. ROEHR CC, REIBEL S, ZIEGERT M, SOMMERFELD C, WAHN U, NIGGEMANN B. Atopy patch tests, together with determination of specific IgE levels, reduce the need for oral food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 2001; 107: 548–53.
8. SAMPSON HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107: 891–6.
9. PERRY TT, MATSUI EC, KAY CONOVER-WALKER M, WOOD RA. The relationship of allergen-specific IgE levels and oral food challenges outcome. *J Allergy Clin Immunol* 2004; 114: 144–9.

10. PETERSEN AB, GUDMANN P, MILVANG-GRØNAGER P, et al. Performance evaluation of a specific IgE assay developed for the ADVIA Centaur® Immunoassay System. *Clin Biochem* 2004; 37: 882–92.
11. WILLIAMS PB, BARNES JH, SZEINBACH SL, SULLIVAN TJ. Analytical precision and accuracy of commercial immunoassays for specific IgE: establishing a standard. *J Allergy Clin Immunol* 2000; 105: 221–1230.
12. PAGANELLI R, ANSOTEGUI IJ, SASTRE J, et al. Specific IgE antibodies in the diagnosis of atopic diseases. *Allergy* 1998; 53: 763–8.
13. VAN REE R, AALBERSE RC. Specific IgE without clinical allergy. *J Allergy Clin Immunol* 1999; 103: 1000–1.