

Food specific IgE antibodies: a comparative study of AlaSTAT and Pharmacia RAST Phadebas CAP systems in 49 patients with food allergies

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Forty-nine sera from patients with food allergies confirmed by challenge and of 14 with suspected allergy were assayed with the DPC AlaSTAT liquid phase EIA and the Phadebas RAST CAP radioimmunoassay (RIA). Twenty-two foods were tested (four animals, 18 vegetables). Controls were performed on nonatopic subjects (N = 8). Specificity was 100%. Concordance RAST CAP/AlaSTAT was 79.6%; prick tests/AlaSTAT: 76%; prick tests/RAST CAP: 73.9%. AlaSTAT detected specific IgE even in sera with low total IgE (<60 KU/L), while RAST CAP was inconclusive. Dilution studies and reproducibility were excellent. AlaSTAT EIA compares favorably with the Phadebas RAST CAP RIA for measurement of food-specific IgE antibodies.

INTRODUCTION

Food allergy includes immunologically mediated reactions to foods. The majority of immediate reactions to foods are due to IgE-mediated hypersensitivity.¹⁻³ The diagnosis relies on a comprehensive history, prick skin tests with food extracts or with fresh foods, laboratory tests that demonstrate specific IgE, and oral challenges.⁴

A great number of techniques are currently available: Phadebas radioallergosorbent test (RAST), Phadebas RAST CAP, Phadezym RAST, fluorescent allergosorbent

test (FAST), and MAST-CLA.⁴⁻¹⁰ Manufacturers rarely provide evidence of quality controls. Various technical aspects make comparisons between different tests difficult; however, radioimmunoassays could serve as reference in attempts to assess new immuno-enzymatic tests. The units of the Phadebas RAST CAP system have been validated by the World Health Organization. The standard is total IgE. It is therefore advisable to compare new immuno-enzymatic tests with this method.

We report the results of a study with a novel assay, AlaSTAT, using a soluble ligand, allowing for testing serum samples in a liquid phase. We have assayed serum samples of 49 patients with food allergies, most confirmed by challenges and 26 patients with suspected food allergies; the AlaSTAT test was highly reliable. The sensitivity of this test was comparable to the Phadebas RAST CAP System for vegetable and animal allergens.

MATERIAL AND METHODS

Allergens

Twenty-two allergens (four of animal origin and 18 of vegetable ori-

gin) were evaluated: egg white and yolk: 17 patients; fish: 5; shrimp: 3; pork: 2; peanut: 11; hazelnut: 9; apple: 6; celery: 6; soybean: 4; wheat and rye flour: 4; carrot: 2; baker's yeast: 3; kiwi fruit: 2; garlic: 2; and potato, tomato, orange, mango, strawberry, rice, chocolate: 1 each.

Patients

We studied 55 patients, 31 female and 24 males including 42 adults and 13 children aged 12 years or less. The clinical symptoms were either immediate and severe, or atopic dermatitis (Table 1). The diagnosis of "immediate food allergy" was established stepwise: first, skin prick tests and in vitro assay for specific IgE antibodies; then challenges. The prick tests were performed with a fine disposable needle¹² with allergenic extracts (Stallergenes S.A., Fresnes, France), and with fresh pulp of fruits or vegetable. Positive controls were codeine phosphate (9%); negative, diluent. A prick test was considered positive when the wheal was 3 mm in diameter, while the diluent control remained negative.¹²

A labial challenge was carried out with a drop of the fresh food crushed in water, laid on the inferior lip of

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Table 1. Tests and Assays Performed on 49 Patients with IgE-Mediated Immediate Food Allergies (group 1)

Patient Number	Sex	Age, yr	Symptoms*	Prick Test, mm†	Challenge‡	IgE, KU/L	RAST, KU/L	Class	AlaSTAT, U/mL	Class	Food Allergen (+)
1	M	30	AO + OS	3.5	History	219	3.04	2	2.05	0	Hazelnut
2	F	39	AO + asthma	5	OC + 10 g	33	<0.35	0	0.23	0	
3	F	38	AO + OS	3	History	384	2.29	2	0.79	1	
4	F	20	Asthma + OS	7	History	22	1.53	2	0.25	0	Apple-1st batch
5	F	24	AO + asthma	4	+++100 g	27	<0.35	0	0.20	0	
6	F	28	AO + OS	7	History	122	7.1	3	0.20	0	
7	M	14	Rhinitis + OS	3	History	75	5.5	3	0.35	0	Apple-1st batch
8	M	32	AO + OS	11	History	278	2.56	2	0.20	0	Celery
9	F	43	OS	5	OC + 50 g	14	0.53	1	0.20	0	
10	F	35	AO + OS	3	History	93	4.10	3	0.20	0	
11	F	46	Anaphylaxis + asthma + rhinitis	6	OC + 2 g	1043	24.1	4	1.06	1	Celery
12	M	23	Pruritus + asthma	5	SOC + 5 g LC+	713	3.4	2	1.76	2	
13	F	33	AO+	10	LC+	164	2.1	2	0.20	0	
14	F	32	AO + OS	5	History	217	<0.35	0	0.20	0	Wheat flour
15	F	28	AO + OS	3	History	122	3.3	2	0.64	1	
16	M	31	Asthma	0	BC + 10 ⁻⁶	734	8.4	3	0.20	0	
17	F	27	Asthma + AD	0	OC + 30 g	>1000	5.2	3	2.17	2	Rye
18	M	24	Asthma + rhinitis	3	OC + 10 g	104	6.6	3	1.06	2	
19	F	28	Asthma + AD	5	OC + 10 g	>1100	<0.35	0	19.4	4	
20	F	8	AD	7	OC + oil 5 mL	1880	>100	5	51.8	5	Peanut
21	M	4	AD + AO	6	OC + 10 mg LC+	195	>100	5	60	5	
22	F	6	AD + asthma + AO	10	OC + 10 mg LC+	473	>100	5	58	5	
23	M	5	AD + asthma	6	OC + oil 5 mL	>1100	>100	5	46	5	Soybean
24	M	1	AD	3	OC + 5 g	57	0.64	1	6.10	3	
25	F	14	AD	3	OC + 50 g	>2000	2.13	2	1.4	1	
26	F	46	Urticaria + OS	0	OC + 70 g	1043	0.40	1	1.6	2	Garlic
27	M	27	AD + laryngeal AO	8	History++		12.5	3	61.18	5	
28	F	23	AO + urticaria	5	OC + oil 30 mL		33.5	4	15.5	4	
29	M	11	Urticaria	4	OC + 1 g	514	<0.35	0	0.31	0	Choco-late
30	F	18	AO + urticaria	N/A	OC + 1 g	117	7.6	3	0.41	1	
31	F	33	AD	3	OC + 50 g EV+	1370	<0.35	0	0.66	1	
32	M	14	Anaphylaxis	6W	OC + 1 mg	>1100	>100	5	55.69	5	Egg
				6Y			31.2	4	60.45	5	
33	M	1	AO + asthma	3W	LC+	38	>54	5	60.60	5	
34	M	31	Asthma + sinusitis	7W	BC+	734	28.6	4	15.64	4	Egg
					LC+		35.4(Y)	4	22.41(Y)	4	
35	M	5	AO	3W	History		5.4	3	13.99	3	
36	F	22	AO + asthma	3W	OC + 1 g	146	9.9	3	6.45	3	Egg
							5.6(y)	3	10.36	3	
37	F	11	AO + conjunctivitis	4W	OC+	1538	1.48	2	1.34	1	
							0.48(Y)	1	1.01(Y)	1	Egg
38	M	37	AO + urticaria	0W	OC + 30 g	513	2.75	2	0.20	0	
39	M	45	Chronic urticaria	0W	OC+	7	<0.35	0	0.20	0	Egg
				0Y	EV+		<0.35	0	0.20	0	
40	F	28	AD	3W	EV+	>1100	1.51	2	0.20	0	
41	F	24	Shock + asthma	13W	LC+	994	14.6	4	5.54	3	Egg

Table 1. (Cont'd)

Patient Number	Sex	Age, yr	Symptoms*	Prick Test, mm†	Challenge‡	IgE, KU/L	RAST, KU/L	Class	AlaSTAT, U/mL	Class	Food Allergen (+)
42	F	8	Asthma	12Y 7	LC+ OC + 1 g	968	28.9 28.6	4 4	22.32 60.47	4 5	Fish
43	F	10	Shock	10	History	216	19.7	4	61.14	5	
44	M	7	Asthma + AO + urticaria	6	OC + 1 g LC+	302	4.3	3	48.23	4	Fish
45	M	48	Shock	3	History	59	<0.35	0	1.61	2	
46	M	37	AO + urticaria	3	History	513	1.87	2	0.75	1	Shrimp
47	F	25	AO + urticaria	5	History	238	0.62	1	0.25	0	Shrimp
48	M	25	AO + urticaria	0	LC+	80	<0.35	0	0.87	1	
49	M	27	Generalized eczema	3	LC+	>1100	<0.35	0	0.97	1	Pork

* AO = angioedema, AD = atopic dermatitis, and OS = oral syndrome.

† W = egg white and Y = egg yolk.

‡ History = strongly positive history (see text), OC = oral challenge, LC = labial challenge, BC = bronchial challenge, EV = elimination.

Table 2. Comparison Phadebas RAST CAP System/AlaSTAT in 14 Atopic Patients with Either Positive Prick Test to a Food Allergen or a Subjective Positive History

Patient Number	Sex	Age, yr	Symptoms	Prick Test, mm	IgE, KU/L	RAST, KU/L	Class	AlaSTAT, U/mL	Class	Food Allergen
1	M	30	AO + asthma	3	219	3.7	3	5.3	3	Orange
				0		2.83	2	2.07	2	Soybean
				3		3.4	2	4.65	3	Potato
15	F	28	AO + OS	0	122	0.35	0	0.20	0	Soybean
				0		1.1	2	2.32	2	Carrot
				3		0.6	1	0.20	0	Hazelnut
9	F	43	AO + OS	3	14	<0.35	0	0.37	1	Kiwi fruit
13	F	33	AO	7	164	3.7	3	1.18	1	Carrot
				12		1.48	2	0.48	1	Hazelnut
				2		1.7	2	0.20	0	Peanut
14	F	32	AO + OS	N/A	217	0.36	1	0.77	1	Mango
25	F	14	AD	N/A		2.52	2	0.64	1	Rice
						1.94	2	0.46	1	Hazelnut
						8.40	3	4.32	3	Baker's yeast
						0.57	1	0.37	1	Pork
30	F	18	AO + OS	3	117	1.7	2	0.27	0	Hazelnut
				3		10.8	3	0.45	1	Apple
				3		4.7	3	2.57	2	Tomato
50	F	51	Urticaria	2	188	<0.35	0	0.27	0	Celery
51	F	26	Urticaria	0	>1100	27.2	2	0.20	0	Strawberry
19	F	27	AD	2	>1100	2.30	2	0.68	1	Baker's yeast
52	M	19	AD	4	>1100	8.50	2	1.74	2	Baker's yeast
53	F	45	AO	3	46	<0.35	0	0.20	0	Kiwi fruit
54	F	32	Urticaria	Neg	21	<0.35	0	0.20	0	Fish
55	F	6	AD	Neg		14.1	3	1.53	2	Peanut
				2		6.3	3	1.37	1	Soybean

the mouth, half-opened. The reaction occurred within 15 minutes: swelling of the lip and urticaria on the adjacent skin, and sometimes of the cheek with conjunctivitis, have been observed.² Oral challenges

were carried out when the labial test was negative or inconclusive: single blind or double blind, placebo-controlled, challenges took place in the hospital clinic.^{3,13} They were performed in the morning on an empty

stomach. The native food was included in a colorless opaque gelatin capsule, or was mixed with apple compote. The selection of the initial challenge dose was based on clinical symptoms and levels of food specific

IgE antibodies (Tables 1 and 2). The challenges were considered positive when the following symptoms occurred within one hour after ingestion: rash, urticaria, angioedema, conjunctivitis, rhinitis, asthma, tachycardia and/or hypotension, reduction of peak respiratory flow rate (FEV₁), diarrhea, vomiting, and severe abdominal pain. Each patient was kept under strict observation for 24 hours.

None of the patients who underwent these challenges had a delayed reaction. Of 49 patients with demonstrated immediate allergy, 14 patients had suffered life threatening anaphylaxis or had typical symptoms at least 3 times after ingesting the offending food alone. These patients were not submitted to oral challenge, as the clinical symptoms made the diagnosis obvious; 34 patients were challenged: eight were challenged by labial application; two had single blind bronchial challenges; 24 had oral challenges: seven children had single blind oral challenges; ten adults had double blind oral challenges; and seven adults had open oral challenges (amounts of food: 30–100 g). Patient 40 had his/her diagnosis confirmed by successful elimination.

In Vitro Assays

The sensitivity of both in vitro assays, Phadebas RAST CAP System and AlaSTAT, was tested on serum samples of patients with immediate reactions (group 1). The same study was conducted on 25 serum samples of patients with suspected immediate allergy with strongly positive history and prick tests but no challenge.

The specificity of the AlaSTAT assay was confirmed by running 16 determinations for each of 27 allergens. Eight determinations were run on sera of nonatopic subjects (Phadiatop negative), and eight on sera of atopic patients (positive prick tests and/or RAST to house dust mites, cat epithelia, grass pollens). Each serum was tested against nine allergens.

The intra-assay and inter-assay reproducibility was evaluated by ten determinations on serum samples selected: either with different total IgE levels (14–1538 KU/L by Pharmacia RIA), or variable specific IgE levels: 7 class 0; 6 class 1; 2 class 2; 3 class 3; 4 class 4. Dilutions 1/2–1/64 were conducted on 3 AlaSTAT class 5 sera, and 1/2–1/8 on 11 class of 4–5 sera.

Assays

Briefly, to perform the AlaSTAT test, 50 μ L of the patient serum sample is first incubated in a ligand-coated tube for one hour with 100 μ L of an individual allergen prepared initially with soluble dextran or soluble Ficoll, and amino acid copolymers; a CDI activation and coupling with amino acid copolymer and allergen or by 1,4-butane dioldiglycidyl ether activation and coupling.¹⁴ During this liquid phase reaction, IgE specific for the given allergen, if present, binds to the allergen on the liquid matrix. The allergen-IgE complex thus formed in the liquid phase is then reacted for one hour with 50 μ L of avidin which, during this second incubation period, binds the biotin-matrix-allergen-IgE complex and also the biotin on the coated-tube, thereby forming a bridge between the solid phase and the reacted IgE. IgE antibodies not specifically bound to the biotin-coated tube are removed by washing the test tube. The amount of specific IgE that is immobilized on the tube is quantitated by adding 200 μ L of horseradish peroxidase-labeled monoclonal anti-IgE antibodies which recognize epitopes of the epsilon chain of the IgE molecule. This reaction is allowed to proceed for one hour, after which excess enzyme is washed away. Five hundred microliters of enzyme substrate is then added and allowed to react with the bound enzyme for 15 minutes, producing a color directly proportional to the amount of endogenous IgE specific for the allergen. A semiquantitative result in terms of a class number is

assigned to the sample with respect to the chosen allergen by comparing the color generated in the sample tube with the color produced in a series of standards (designated A, B, C, and D) processed in the same assay against the reference allergen, timothy grass pollen. The AlaSTAT specific IgE assay is calibrated to the Phadebas RAST and employs a similar classification system.

The Phadebas RAST CAP assay was performed by J. P. Nicolas, MD, at the medical biochemistry laboratory, according to the manufacturer's instructions.

For statistical evaluation we used the Student paired test.

RESULTS

Four hundred sixteen tests (allergens = 27) were negative: 24 nonatopic sera, and 24 atopic sera. Specificity was 100%.

In patients of group I (confirmed immediate allergy) prick test sensitivity was 87.75%. AlaSTAT sensitivity was 76% for animal food allergens (RAST: 76%) and 73% for vegetable food allergens (RAST: 76.9%). We had a problem with the first batch of AlaSTAT apple allergen: all four sera tested negative initially; however, these four sera tested positive with a second batch.

Concordance prick tests/AlaSTAT was 76% (prick tests/RAST: 73.6%). The overall concordance AlaSTAT/RAST was 79.6%. For animal food allergens, class concordance was 14/25; one class up was found 6 times with AlaSTAT, five with RAST. For vegetable food allergens, class concordance was 11/26; RAST was one class up 10 times, AlaSTAT 5 times (Fig 1). We found a relation between the level (class) of specific IgE and the triggering dose of the food allergen (Fig 2): the higher levels of specific IgE corresponded to <500 mg challenge doses in 77% of cases. Some patients who reacted to minimal amounts of food could have low levels of specific IgE.

When comparing RAST and AlaSTAT as used on patients'

AlaSTAT

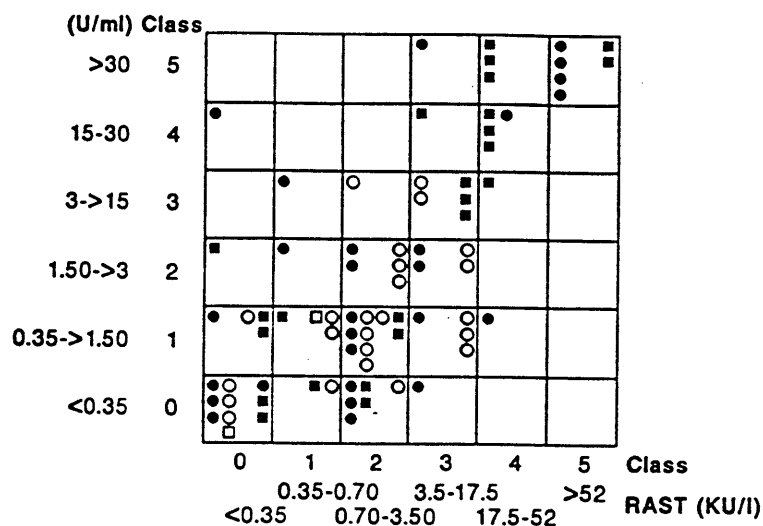


Figure 1. Comparison of classes of food-specific IgE antibodies as measured by AlaSTAT and Phadebas RAST Cap System. Group 1 = ■ animal and ● vegetable and group 2 □ = animal and ○ vegetable.

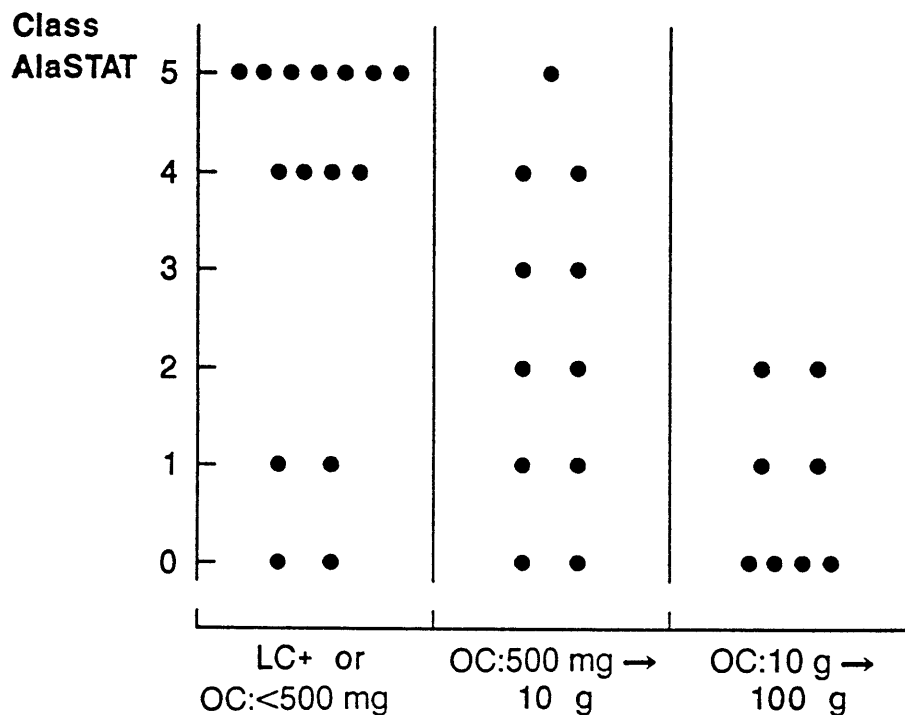


Figure 2. Comparison between the class of food-specific IgE (as measured by AlaSTAT) and the reactive threshold dose in the course of the oral challenge.

serum samples of group 2, we found similar results: RAST: 80.7% and AlaSTAT: 73.1% (NS). The AlaSTAT assay detected specific IgE in sera with low total IgE: 59 KU/L (patient 45); 57 KU/L (24); 38 KU/L

L (33); and 14 KU/L (9) (patient 9, Table 3). RAST was negative in 45 and 9, and 2 classes lower in patient 24.

Specific IgE antibodies were measured in different serum sam-

ples at increasing dilutions. The graphic plot of specific IgE/dilution shows a linear curve from 1/8. Above 60–65 U/mL AlaSTAT, the curve plateaued (saturation). Conversely, with IgE < 60 U/mL, 1/2 dilutions (1/4, 1/8) showed a 50% reduction (Figs 3 and 4).

The coefficients of variation (CV) (reproducibility) were 1.68% to 9.9% for Classes II, III, IV, and V (Table 3). The CVs of classes 0 and 1, 10.9% to 20.8%, were not associated with a change of class in 12/13 serum samples. Coefficients of variation were similar in inter-assay and intra-assay studies. All different allergens that were evaluated were very similar, except the first batch of "apple": CV: 20.8%; the second batch of "apple" had a CV: 5.2% to 7.6%. The CV was independent of total IgE levels.

DISCUSSION

Specific IgE antibodies have a major role in food allergies. IgG antibodies have been identified in many subjects, but their exact role remains controversial.^{15,16} Lymphocyte-mediated hypersensitivity has been associated with cow milk sensitivity in infants^{1,17}; therefore, the identification of specific IgE antibodies is important. A valid evaluation of an in vitro assay, designed for the measurement of specific IgE antibodies to foods, requires the availability of serum samples from patients with demonstrated immediate and/or severe food allergy. The double blind, placebo-controlled food challenge (DBPCFC) remains the "gold standard."^{3,13} We consider a combination of typical symptoms, occurring at least 3 times rapidly after the isolated ingestion of a given food and confirmed by positive prick tests to be as conclusive as the DBPCFC for a positive diagnosis.

Prick tests to food extracts are quite sensitive.³ We did confirm this assertion in our study: 88%. The specificity of the prick tests has been considered highly variable by some groups and the role of in vitro assays has been emphasized. The sensitiv-

Table 1. (Cont'd) Tests and Assays Performed on 49 Patients with IgE-Mediated Immediate Food Allergies (group 1)

Allergen	Class 0		Class 1		Class 2		Class 3		Class 4 and 5	
	IgE, KU/L	CV%	IgE, KU/L	CV%	IgE, KU/L	CV%	IgE, KU/L	CV%	IgE, KU/L	CV%
Intraassay										
Shrimp	238	19.9	122	18.8	1043	9.9				
Apple	75	13	164	20.8						
Egg (class 0 or 1)			1538	13			994	10.5	>1000	1.7
Crab			nd	14.1						
Peanut							57	3.5		
Interassay										
Celery	188	17.2	713	10.9						
Egg							14.6	8.9	>1100	2.65
Shrimp	80	13.6							734	6.5
Pork	>1000	11.6								
Chocolate	1370	18.8								
Kiwi fruit	14	15.5								
Hazelnut			219	11.9						
Soybean									nd	6.7

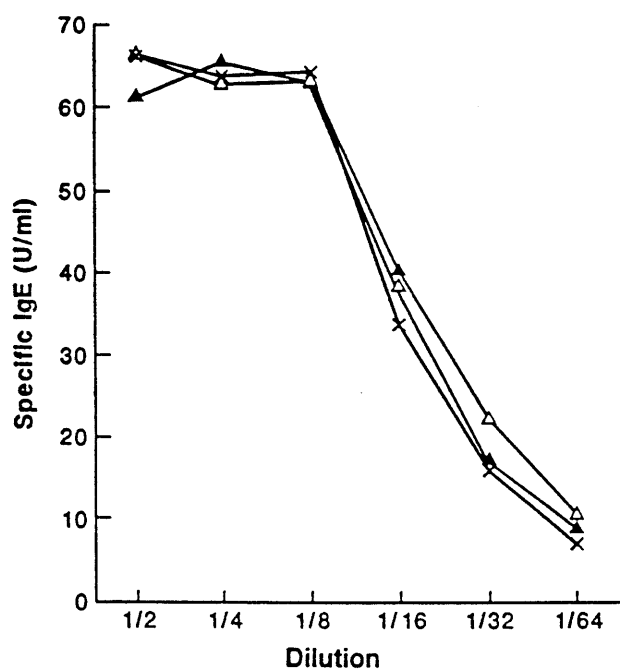


Figure 3. Dilution of specific IgE antibodies with AlaSTAT.

ity of most in vitro assays has generally been considered to be inferior to that of prick tests; two exceptions could be the pediatric Phadiatop, and the Fx5 multitest (six foods) which would be equivalent to skin tests.^{8,9,18,19} Indeed, the in vitro measurement of food-specific IgE antibodies raises certain issues: some allergens, ie, of vegetable ori-

gin, are labile and their extracts lack allergenicity. This was the case for the first batch of "apple." Conversely, vegetable allergens may be very rich in lectins, which can bind to IgE and decrease the specificity of the assay(s).²⁰ Our study confirms the excellent specificity of AlaSTAT.

We did not observe any false pos-

itive reactions with 27 allergens in 24 serum samples of atopic patients, 13 of them containing IgE to grass pollen, 1 to birch pollen, 13 to *Der-matophagoides pteronyssinus*, and 1 to cat epithelia. The coupling of food allergens to the AlaSTAT matrix apparently reduces the exposure of epitopes common to fruits/vegetables and grass pollens.²¹

The binding of allergen(s) to a bidimensional solid phase (paper disk) could be associated with a smaller number of exposed epitopes; moreover, because amino groups are required for cyanogen bromide coupling to cellulose allergosorbents, allergens that contain significant amounts of carbohydrates (eg, foods) will not be represented. It is hoped that the tridimensional presentation (eg, CAP System) will improve the sensitivity of the solid-phase assay. In the present study, we found the sensitivity of the Phadebas RAST CAP System to be 76%, identical to that published previously⁶ for nitrocellulose disks. The increased sensitivity of the CAP Systems for inhalants remains to be demonstrated for foods.²²

The AlaSTAT technology is a liquid-phase assay that uses a soluble backbone or polymer for linking allergen molecules, such that their

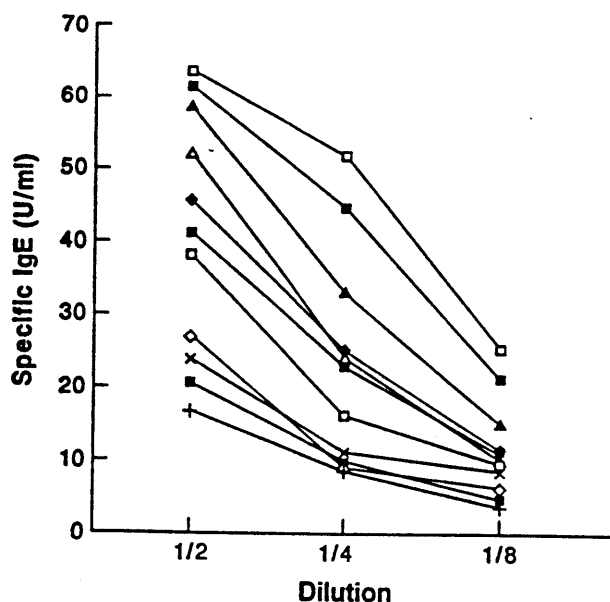


Figure 4. Dilution study of specific IgE antibodies with AlaSTAT.

spatial orientation closely approximates that of the physiologic situation. Furthermore, the identifiable ligand (biotin) is not present on allergen molecules but on the soluble backbone, thus helping to ensure that IgE-binding sites on allergen molecules are preserved. Because of the use of a soluble backbone and amino acid copolymer, an amplification system is achieved that helps to drive the kinetics of the liquid-phase reaction and increase the possible number of immunocomplexes that could be immobilized on a solid support. The overall result of this amplification is that the AlaSTAT assay is complete in 3½ hours or less without loss of sensitivity; the liquid format makes it convenient for either manual performance or complete automation.¹⁴ In our study, the sensitivity of the AlaSTAT assay was identical to that of the Phadebas RAST CAP System. The concordance between these two assays was 79.6% for food allergens (90% for inhalants).⁵ The AlaSTAT technique, applied to food allergens, is more sensitive than other immuno-enzymatic assays.

Another problem found with immuno-enzymatic assays is nonspecific interactions between the en-

zyme of the assay and the food allergen. Depending on the nature of the enzyme, a given assay has a different sensitivity with a vegetable versus animal allergen, as we reported for the Phadezym RAST and the FAST.⁶ We did not find such an interaction with the AlaSTAT assay; sensitivity was 76% for animal allergens and 76% for vegetables (NS). The reproducibility was excellent for classes II, III, IV, and V (1.7% to 9.9%), which suggests good control of the various steps, and consistency of the foods extracts which retain their assigned volume and allergenicity in the test tubes. A CV of 10% to 19% for class 0-1 was acceptable, since there was no change of class for 12/13 serum samples (or 129/130 determinations). The dilution study demonstrated a linear curve of the specific IgE antibodies and confirmed the linearity of the calibration curve. These results are better than those reported with other EIAs.⁷

Finally, we found some degree of relation between the class of AlaSTAT/IgE antibodies and the reactive threshold dose of food used for oral challenge: class 5 was 77% associated with a positive challenge dose <500 mg or with a positive

labial test. Conversely, class 1 (or even 0) could be associated with a small dose inducing a reaction; we emphasize caution, and we *always* give a starting oral challenge dose <500 mg. Before starting the oral challenge, we always perform a "labial" challenge²; if this mucosal challenge is positive, we can stop the procedure, or start the oral challenge with a few milligrams of the offending food.

CONCLUSION

IgE-mediated food allergies are currently implicated in a growing number of syndromes. The increased incidence may be due in part to major changes in modern diets. Processing proteins does influence potential allergenicity. The extreme diversification of food proteins also increases the risk of sensitization. Much remains to be understood regarding the role of additives, changes in binding of proteins to carbohydrates or lipids, etc. We should keep all these considerations in mind.

The initial step for the diagnosis of food allergy is history and prick testing performed by a trained allergist. Commercially available food extracts are poorly reliable, especially those of vegetable origin. We try to use the pulp of the native food. But this most practical method lacks standardization.²³

Interpretation of skin tests with foods remains very challenging, and problems are most frequent; these justify the use of in vitro assays detecting specific IgE antibodies. Currently, the reference assay is the Phadebas RAST CAP System. For the first time, an EIA (AlaSTAT) performed as well as the Phadebas RAST CAP both for vegetable and animal food allergens. We should be able to use this method to investigate the incidence of specific IgE antibodies, and their potential role in enteropathies, other digestive syndromes, asthma, nephrotic syndrome, etc. This assay could also prove useful for selecting the thresh-

old dose of a given food allergen in oral challenges.

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REFERENCES

1. May CD, Bock SA. Adverse reactions to food due to hypersensitivity. In: *Allergy, principles and practice*. Middleton E, Reed CE, Ellis EF, eds. Saint Louis: C.V. Mosby Company, 1978:1159-71.
2. Moneret-Vautrin DA. Food allergy: present problems and perspectives. In: *Advances in allergology and clinical immunology*. Godard PH, Bousquet J, Michel FB, eds. Park Ridge NV: Parthenon Publishing, 1991:473-83.
3. Sampson HA, Metcalfe DD. Immediate reactions to foods. In: *Food allergy*. Metcalfe DD, Sampson HA, Simon RA, eds. Boston: Blackwell Scientific Publications, 1991:92-112.
4. Adler BR, Assadullahi T, Warner JA, et al. Evaluation of a multiple food specific IgE antibody test compared to parental perception, allergy skin tests and RAST. *Clin Exp Allergy* 1991;21:683-8.
5. Alaba O, El Shami ASE. Evaluation of nonspecific IgE binding: comparison of two in vitro allergen-specific IgE assays. In: *Allergy and molecular biology*. Shami ASE, Merrett TG, eds. Oxford: Pergamon Press, 1989:203-14.
6. Moneret-Vautrin DA, Gueant JL, Adbel-Ghani A, et al. Comparative evaluation between two immunoenzymatic techniques (FAST and Phadezym) and the Phadebas RAST in food allergy. *Allergy* 1990;45:104-8.
7. Moneret-Vautrin DA, Kanny G, Gerard H, et al. Determination des IgE spécifiques aux allergènes alimentaires par le MAST-CLA. Correlations avec les tests cutanés et le RAST. *Rev Fr Allergol* 1990;30:225-31.
8. Van Toorenenbergen AW, Oranje AP, Vermeulen AM, et al. De "Phadiatop Paediatric": een bruikbare in vitro-test op een atopische immuunrespons bij zuigelingen en kleuters. *Ned Tijdschr Geneesk* 1991;135:1920-3.
9. Van Toorenenbergen AW, Oranje AP, Vermeulen AM, et al. IgE antibody screening in children. Evaluation of the Phadiatop Paediatric. *Allergy* 1991;46:180-5.
10. Halpern GM. Evaluation of in vitro IgE testing to diagnose atopic diseases. *Clin Rev Allergy* 1989;7:23-48.
11. Pecoud AR. Optimal use of in vitro diagnostic tests in clinical allergy. In: *Progress in allergy and clinical immunology*. Pichler WJ, Stadler BM, Dahinden CA, eds. Hogrefe & Huber Publishers, 1989:428-32.
12. Dreborg S. Skin tests used in type I allergy testing. Position paper. *Allergy* 1989;44[Suppl 10]:1-58.
13. Bock SA. Oral challenge procedures. In: *Food allergy*. Metcalfe DD, Sampson HA, Simon RA, eds. Boston: Blackwell Scientific Publications, 1991:81-95.
14. El Shami AS, Alaba O. Liquid-phase in vitro allergen-specific IgE assay with in situ immobilization. In: *Allergy and molecular biology. Advances in the biosciences*, El Shami AS, Merrett TG, eds. Oxford: Pergamon Press, 1989:191-201.
15. Halpern GM, Scott JR. Non-IgE antibody mediated mechanisms in food allergy. *Ann Allergy* 1987;58:14-27.
16. Berrens L, Homedes IB. Relationship between IgE and IgG antibodies in type I allergy. *Allergy Immunol* 1991;37(3-4):131-7.
17. Kondo N, Agata H, Fukutomi O, et al. Lymphocyte responses to food antigens in patients with atopic dermatitis who are sensitive to foods. *J Allergy Clin Immunol* 1990;86:253-60.
18. Bjorksten B. In vitro diagnostic methods in the evaluation of food hypersensitivity. In: *Food allergy. Adverse reactions to foods and food additives*. Metcalfe DD, Sampson HA, Simon RA, (eds). Boston: Blackwell Scientific Publications, 1991:67-80.
19. Hochreutener H. Klinik und allergologisch-immunologische Parameter bei 40 Kindern von 0-7 Jahren mit atopischer Dermatitis. *Monatsschr Kinderheilkd* 1991;139:618-25.
20. Barnett D, Howden MEM. Lectins and the radioallergosorbent test. *J Allergy Clin Immunol* 1987;80:558-61.
21. Aalberse RC, Koshte Y, Clemens JGJ. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen and hymenoptera venom. *J Allergy Clin Immunol* 1981;68:356-64.
22. Johansson SGO. In vitro diagnosis of atopic allergy. In: *Progress in allergy and clinical immunology*. Pichler WJ, Stadler BM, Dahinden CA, eds. Hogrefe & Huber Publishers, 1989:415-8.
23. Dreborg S. Skin test in diagnosis of food allergy. *Allergy Proc* 1991;12:251-4.

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