

Comparison of Two Assays for the Determination of Specific IgE in Serum of Atopic and Nonatopic Subjects: the Allergenetics FAST[™] and the Phadezym RAST[®]

A. PÉCOUD, R. PEITREQUIN, J. FASEL and P. C. FREI

Division of Allergy and Immunology, Dept. of Internal Medicine,
Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

The FAST[™] assay (Allergenetics) for the determination of specific IgE has recently been introduced. The results of this test, graded in classes 0 (negative), 1 (equivocal) and 2 to 6 (positive) were compared with those of another commercially available test (Phadezym RAST[®], Pharmacia), graded from class 0 (negative) and classes 1 to 4 (positive). In 52 adults suffering from rhinitis/asthma, a total of 114 positive skin prick tests (SPT) were obtained for common respiratory allergens. In 67 % of these tests the Phadezym RAST was positive and in 63 % the FAST was positive (classes 2 to 6). In these patients there were 151 negative SPT: 6 % corresponded to a positive Phadezym RAST (all class 1) and 34 % to a positive FAST (classes 2 to 6). The serum of nine nonatopic volunteers who had negative SPT for 12 common allergens were tested. In none did the Phadezym RAST give any positive results; the FAST was positive in all nine sera, detecting between five and 11 allergens. When both assays were performed on 14 unselected cord blood samples, the Phadezym RAST was positive in three samples (with class 1 results to a maximum of two allergens), and the FAST was positive in 12 samples, detecting between one and seven allergens. Thus, in our hands, the FAST gave an abnormally high number of positive results in patients with negative SPT, in nonatopic volunteers, and in cord blood.

Key words: cord serum; nonatopic; RAST/FAST[™].

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CLINICAL ASPECTS

The Allergenetics FAST[™] and the Phadezym RAST[®] gave the same proportion of positive results when the skin prick test for the corresponding allergen was positive. However, unlike the Phadezym RAST, the FAST gave frequent positive results when the skin test was negative, in nonatopic subjects and in cord serum.

In 1967, Wide et al. described the radio-allergo-sorbent-test (RAST), an *in vitro* method for detecting the presence of IgE molecules specific for various allergens in the serum of atopic patients (12). Since then, some modifications have been introduced, one of them being the use of an enzymeimmunoassay (Phadezym RAST[®]) instead of the radioimmunoassay originally described. Recently, a new technique has

been commercialized (FAST[™], Allergenetics, Mountain-View, California) which has the following modifications: a) the polystyrene walls of the microtiter wells are directly coated with the allergen, instead of using, as the solid phase, cellulose discs coated with allergens, b) an enzyme labelled anti-IgE of the monoclonal type, and c) a fluorescent substrate to reveal the reactions (10). These modifications have re-

sulted in a shorter manipulation time. In 1984, this technique was introduced in Switzerland. The finding of many more positive results with the FAST than we were accustomed to with the Phadezym RAST, prompted us to compare the results of both assays on the same serum samples in atopic and nonatopic subjects.

MATERIAL AND METHODS

Subjects

The comparison between the two different assays was performed on the serum of three groups of subjects:

1) A first group consisted of 52 adult patients referred to our outpatient clinic for an allergic investigation of rhinitis (28) and asthma (24). There were 30 females and 22 males, with a mean age of 30 ± 7 years (mean ± 1 SD; range 16–45). Eight to 20 skin tests, selected according to the patient's history, were performed on the day of the investigation as described below.

2) A second group of nine nonatopic control volunteers consisting of five males and four females (age range: 20–47) were selected because they could be considered as not allergic, for the following reasons: a) none had any past or present history of respiratory, skin or digestive disease, in which allergy could be involved, b) in each subject, 12 skin prick tests were performed, which were all negative for the following allergens: house dust, *Dermatophagoides farinae*, cat, cow, *Dactylis glomerata* (orchard grass), birch, chenopod, *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Mucor racemosus* and *Alternaria tenuis*, and c) each subject had a serum total IgE concentration lower than 80 IU/ml.

3) A third group consisted of 14 unselected newborns, in whom about 15 ml of cord blood was collected at birth.

Skin prick test (SPT)

The skin prick tests were performed on the volar side of one or both forearms, by means of a disposable prick tester (Allterprick, Scan-

medica, Zwanenburg, Holland). No test was performed if the patient was taking any medication known to interfere with the results of SPT. The tests were performed using one or two commercially available preparations (Bencard Allergy Unit, Beecham, London, UK and Pharmalgen, Pharmacia, Uppsala, Sweden) both of which are used routinely in our allergy clinic. Tests which showed discrepant results between these two commercial preparations were not taken into consideration in this study. The results of the SPT were graded according to the recommendations of the Northern Society for Allergology: a test was negative if it produced a reaction similar to that produced by the vehicle solution (negative control) and was positive at 3+ if the magnitude of the wheal induced was identical to that induced by 0.1 % histamine (positive control) (1). In our routine practice of allergy as well as in this study, SPT's which gave a negative or a 1+ result were considered to be negative, whereas all results 2+ and above were considered as positive.

Determination of specific IgE in the serum

All blood samples were drawn and allowed to clot at room temperature. After separation, the serum was frozen and kept at -20°C for a period from 1 week to 2 months. No serum was frozen and thawed more than three times during the whole study. All sera were assayed by the two following techniques:

a) *The enzyme-linked immunosorbent test Phadezym RAST (Pharmacia)*. This test became commercially available in 1982 and has been used since for routine testing in our laboratory. The test was performed by strictly following the manufacturer's instructions. Briefly, cellulose discs coated with allergens were incubated for 3 h with the serum to be tested. After washing, the discs were incubated overnight with an alkaline galactosidase-labelled goat antihuman IgE. After a second wash, the system was allowed to react with a substrate, and color intensity was measured by spectrophotometry. The results were graded as class 0 (absent or undetectable),

classes 1 (low), 2 + (moderate), 3 + (high) and 4 + (very high levels of specific IgE). For comparison with SPT, the results of the tests were considered either negative (0) or positive (classes 1 + to 4 +) since even class 1 + results have been shown to be indicative of the presence of specific IgE (5, 8).

b) *The fluorescence allergeo-sorbent-test FAST (Allergenetics)*. Again the test was performed by strictly following the manufacturer's instructions (Allergenetics form 146, December 1982). Briefly, microtiter wells coated with allergens were washed then filled with serum; after 2 h at room temperature the wells were washed and the enzyme-antibody conjugate (alkaline phosphatase-labelled monoclonal antihuman IgE) was added. After 2 h, another wash was performed, the fluorescent substrate (4 methylumbelliferyl phosphate) added, and the fluorescence was read by means of a "Microfluor Automatic Reader" (Dynatech, Kloten, Switzerland). As proposed by the manufacturer, results were graded 0 (< 0.02 IU/ml, very low levels or below detection), classes 1 (0.02–0.1 IU/ml or equivocal), 2 (0.1–0.5 IU/ml), 3 (0.5–2.5 IU/ml), 4 (2.5–12.5 IU/ml), 5 (12.5–62.5 IU/ml) and 6 (> 62.5 IU/ml). Classes 2 to 6 indicate increasing levels of allergen specific IgE.

Each day an analysis was performed, a standard curve was constructed from the fluorescent signals of the blank and the standard concentrations. These were found to be very similar to the data provided by the manufacturer, and the slopes of the curves were highly reproducible from one day to another.

The serum total IgE concentration was determined by means of the paper-radio-immuno-sorbent-test (PRIST) (Pharmacia, Uppsala, Sweden). With this technique, values superior to 120 IU/ml (about 200 ng/ml) were considered to be suggestive of atopy (14).

All the assays mentioned above are meant to be used in the routine laboratory practice, therefore no technical duplicates were performed with any of the assays.

RESULTS

The correlation between the results of the Phadezym RAST, the FAST and the results of the SPT performed in 52 patients suffering from asthma and rhinitis is shown in Tables 1 and 2. An analysis of the 114 positive SPT found in these patients is shown in Table 1: when all allergens are considered together, specific IgE for the corresponding allergen were detected in

Table 1

Correlation between 114 positive skin prick tests (SPT) and the detection of specific IgE by Phadezym RAST[®] or FAST[®]

Allergen	Pos. SPT	Number of		
		Pos. Phadezym RAST classes 1–4	Equiv. FAST class 1	Pos. FAST classes 2–6
House dust	19	9	5	11
Mite <i>D. farinae</i>	17	11	8	7
Cat epithelium	11	8	3	8
Dog epithelium	3	3	0	3
Horse epithelium	4	1	1	2
<i>Dactylis glom.</i>	20	19	2	18
Birch	12	9	1	10
Oak	3	1	1	2
Dandelion	3	3	2	1
Plantain	10	6	4	4
Chenopod	7	4	4	1
<i>Aspergillus fum.</i>	2	1	0	2
<i>Mucor racemosus</i>	1	0	0	1
<i>Alternaria tenuis</i>	2	1	0	2
Total	114 (100%)	76 (67%)	31 (27%)	72 (63%)

67 % of the comparisons by the Phadezym RAST and in 63 % by the FAST (if only classes 2 to 6 are considered). With both techniques, the highest correlation was achieved with grass pollens (orchard grass: almost 100 %) and the lowest for house dust (about 50 %). Table 2 shows the discrepant findings, i.e. the associations of a negative SPT with a positive Phadezym RAST or FAST. Major differences were found between both *in vitro* assays: whereas results obtained with the Phadezym RAST were positive in 6 % (all of them class 1) of the negative SPT, the FAST assay gave a positive result in 34 % of these comparisons (classes 2 to 6). The number of discrepant results was particularly high with house dust, cat, dog and some molds.

When the serum samples of nine nonallergic volunteers, who were all SPT negative for the 12 allergens tested, were assayed, major differences were also found between both techniques (Table 3, upper section). While none of the subjects gave a positive result with Phadezym RAST, the FAST gave some positive results in all subjects: between five and 11 allergens were positive, if one considers only the results of classes 2 to 6. Table 4 shows that the majority of the positive

FAST results were found in classes 2 to 4; 7 % of them were found in classes 5 and 6. These positive results were evenly distributed among the 12 allergens tested.

The lower section of Table 3 shows that major differences were also found when the presence of specific IgE to the same 12 allergens was tested by both techniques in 14 cord blood samples. The Phadezym RAST detected some specific IgE in three samples (all class 1). The FAST showed some specific IgE in 12 of the 14 samples, directed against one to seven allergens (classes 2 to 6 considered). As shown in Table 4, positive results were found mainly in FAST classes 2 and 3. Again, positive results were evenly distributed among the 12 allergens tested.

DISCUSSION

This study compared two commercially available techniques for the routine practice of allergy. For the clinician, the presence or absence of specific IgE in sera is more relevant than the actual concentration of these IgE. Therefore, in this study, the results of both assays were considered to be either positive or negative, i.e. indicative of the presence of specific IgE or not.

Table 2

Discrepancy between 151 negative skin prick tests (SPT) and the detection of specific IgE by Phadezym RAST® or FAST™

Allergen	Neg. SPT	Number of			Pos. FAST classes 2-6
		Pos. Phadezym RAST classes 1-4	Equiv FAST class 1		
House dust	21	0	9		10
Mite <i>D. farinae</i>	22	0	6		7
Cat epithelium	7	1	2		5
Dog epithelium	5	1	1		4
Horse epithelium	1	0	0		0
<i>Dactylis glom.</i>	5	2	1		3
Birch	10	1	2		1
Oak	1	0	0		0
Dandelion	1	0	1		0
Plantain	3	0	1		1
Chenopod	1	0	1		0
<i>Penicillium not.</i>	16	1	9		4
<i>Cladospor. herb.</i>	16	0	10		2
<i>Aspergillus fum.</i>	13	1	3		5
<i>Mucor racemosus</i>	15	2	8		7
<i>Alternaria tenuis</i>	14	0	10		3
Total	151 (100%)	9 (6%)	63 (42%)		52 (34%)

Technical problems in the manipulation of the assays seem unlikely since both assays were performed by strictly following the manufacturers' instructions. For both tests the standard curves obtained were similar to the data provided by the manufacturers, and their slopes were highly reproducible from one day to another.

In our patients, 67 % of the positive skin tests correlated with a positive Phadezym RAST:

this figure is similar to that usually reported by many groups (2, 11, 13) including our own laboratory (7, 8). In these studies the same techniques was used, except that bound antibody was revealed by an isotope instead of the enzyme system. In the present study the degree of correlation varied with the allergen tested: the highest correlations were obtained with pollens (more than 95 %) and the lowest with house dust (47 %), a finding which was also

Table 3

Results of the determination of specific IgE to 12 allergens* in nine nonatopic control subjects (with neg. SPT) and in 14 unselected cord blood samples

	Total IgE IU/ml	Number of allergens (of 12) detected by:		
		Phadezym RAST Classes 1-4	FAST Class 1	FAST Classes 2-6
Subject R.P.	53	0	1	8
M.O.	44	0	2	5
B.M.	67	0	3	8
J.G.	17	0	1	8
L.B.	14	0	3	5
D.B.	53	0	3	7
M.P.	5	0	0	11
M.B.	20	0	0	11
J.S.	74	0	4	7
Cord blood No. 1	< 0.5	2	6	3
2	0.8	0	2	0
3	< 0.5	0	1	1
4	< 0.5	0	0	0
5	< 0.5	0	1	2
6	1.2	0	3	3
7	< 0.5	0	1	4
8	< 0.5	2	4	7
9	< 0.5	0	0	4
10	< 0.5	1	3	4
11	< 0.5	0	2	6
12	< 0.5	0	3	6
13	< 0.5	0	3	4
14	< 0.5	0	2	7

* Allergens tested: house dust, *Dermatophagoides farinae*, cow, cat, *Dactylis glomerata* (orchard grass), birch, chenopod, *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Mucor racemosus*, and *Alternaria tenuis*.

Table 4

Distribution of the FAST® results according to FAST classes in the nine nonatopic subjects and in 14 cord blood samples*

	Total No. tests*	% of the results in FAST class:						
		0	1	2	3	4	5	6
Nonatopics	108	19	16	29	19	10	5	2
Cord serum	168	51	18	20	9	1	1	0

* The 12 allergens tested are described under Table 3.

reported in previous studies published on the RAST (2, 13). On the same serum samples, the FAST gave similar results (63 %), if only the definitely positive results (classes 2 to 6) were considered.

A major difference, however, was noted when both techniques were used to detect specific IgE in the serum of patients in whom the skin prick test for the corresponding allergen was negative. In these conditions, the Phadezym RAST detected 6 % of positive results (all class 1), whereas FAST detected 34 % positive results (classes 2-6), a value which was similar for all the allergens considered. With the RAST, Wühtrich et al. reported results very close to our findings with Phadezym RAST: of 607 negative skin tests for various allergens (which included those tested in the present study), 64 (10.5 %) corresponded to a positive RAST (13). The high number of FAST, clearly positive for allergens which gave negative skin tests, that was found in the present study was also found in the study of Seltzer et al. (10): using a scale from 0 to 4 FAST classes, they found 59 positive FAST (classes 1 to 3) out of 165 skin tests negative for various allergens (36 %).

It might be argued that the sensitivity of the FAST is higher than that of the Phadezym RAST and that, in those patients suffering from diseases with a possible allergic etiology, the skin tests were falsely negative. However, technical problems in performing the skin tests are unlikely since all these tests were compared with a positive (histamine) and a negative (vehicle) control, they were performed in patients who were not taking any drugs, and they gave identical results whenever they were performed with an allergen preparation from two commercial origins. In addition, in no case did SPT give results contrary to the patient's history.

However, the results of the FAST determinations in nonallergic volunteers constitute the main reason for believing that positive FAST which corresponded to negative SPT could be false positive. The nine subjects we selected had no disease, particularly no allergic disease, their SPT were clearly negative for the allergens com-

monly encountered in our region, and the total IgE concentration was in the range of values not considered indicative of atopy. None of the subjects had any allergen positive with the Phadezym RAST. However, all of them had a FAST clearly positive for a minimum of five and a maximum of 11 allergens.

One may raise the hypothesis that the FAST is so sensitive that it can detect traces of specific IgE that might possibly be found in nonallergic subjects. However, this is unlikely considering the results of the FAST determinations in cord blood, where, usually, only very low amounts of total IgE can be found (3, 4). Using the RAST (Pharmacia), Michel et al. tested 136 cord sera with four allergens (*Dermatophagoides pteronyssinus*, orchard grass, timothy grass and cow milk): they found only three positive results (all 3 for cow milk) (6). In the present study the Phadezym RAST also detected low amounts of specific IgE (class 1) for an occasional allergen (in 3 cord sera) but the FAST detected some in 12 of the 14 cord sera, the tests being clearly positive for as many as seven allergens in some samples.

Our study aimed at analyzing the results provided by these assays for the clinician; we did not explore therefore why the FAST gave a surprisingly high number of positive results in nonatopic volunteers and in cord serum. Recently, Schröder et al. (9) also found a poor correlation between the FAST and Phadebas RAST in serum containing low values of specific IgE. Taken together, these data suggest that in the lower part of the calibration curve, there might be some nonspecific binding of the anti-IgE to the allergen-coated polystyrene wall of the wells.

In conclusion, we have analyzed 541 pairs of results provided by two commercial kits sold in Switzerland for the determination of allergen-specific IgE in serum, and our data show that both assays gave the same proportion of positive tests in patients with positive skin tests, but one of them, the FAST, gave frequent positive results in patients with negative skin tests, in nonatopic subjects and in cord blood. The positive results of the FAST should therefore be interpreted with caution.

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Address:

A. Pécoud, M.D.

Division of Allergy and Immunology

Department of Medicine

CHUV - CH-1011 Lausanne

Switzerland

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