

A New Rapid Test for the Detection of IgE Antibodies to Inhalant Allergens

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ABSTRACT

*The purpose of this study was to investigate if the new IgE-quick[®], a test based on an enzyme-linked immunoabsorbent assay method agrees with the established Phadezym-RAST[®] and the skin prick test. Both *in vitro* methods correlated very well ($r = 0.96$) as well as to the skin prick test (SPT) (RAST/SPT $r = 0.93$, IgE-quick/SPT $r = 0.91$). The new IgE-quick can be recommended for detection of antigen-specific antibodies, as it correlates to skin prick tests and RASTs to a large extent.*

Immediate allergies (Type I, Gell and Coombs) are associated with the presence of IgE antibodies in serum against one or more allergens.^{1,2} Identification of specific IgE antibodies can be made by radioimmunologic or enzyme-immunologic assay methods.³ Many times it has been shown that both methods are comparable with each other but both take a considerable time before one arrives at results. Besides which, one needs either a y-counter or special photometer in addition to the normal laboratory apparatus. Since these constitute a relatively large laboratory investment, the detection of specific IgE antibodies has until now only been possible in specially equipped laboratories.

The development of a rapid test method based upon an enzyme-linked immunosorbent assay now makes it possible to detect specific IgE antibodies to the most common allergens in central Europe within 2 hours in any consulting room.⁴

We compared this new rapid test method (IgE-quick[®], Quidel, San Diego, CA) with one of the established enzyme-immunologic methods (Phadezym-RAST[®], Pharmacia, Uppsala, Sweden) and with case histories and skin prick tests in patients with allergies to inhalants and in control persons. The aim of this study was to investigate the agreement between the *in vivo* and both *in vitro* methods for each allergen tested. Both *in vitro* methods for identification of allergen-specific IgE corresponded to the *in vivo* detected sensitivity to a large extent and the *in vitro* methods correlated among themselves.

PATIENTS AND METHODS

Patients

Investigations were carried out in 75 patients with inhalant allergies and in 25 control persons in whom a case history gave no suggestion of any allergy (mean age: 25 ± 12 years; 57 men, 43 women).

To identify sensitivity *in vivo* and *in vitro*, the same allergen panel of the most frequent inhalant allergens in central Europe was used (Table I).

In Vivo Determination of Allergen-Specific IgE Antibodies by Skin Test

Skin tests were carried out by a skin prick test (SPT) on the inner side of the forearm, using standardized allergens from Allergopharma (Reinbek, FRG) and ALK (Copenhagen, Denmark). A positive result was taken when the weal was at least as large as that obtained with the histamine control solution 15 minutes after application.

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TABLE I

Standardized Allergens from Allergopharma and ALK
Used for the Skin Prick Test

Allergen
Timothy grass
Mugwort
Birch
<i>Alternaria</i>
Rye
Cat
Dog
<i>Dermatophagoides pteronyssinus</i>
<i>Dermatophagoides farinae</i>

In Vitro Determination of Allergen-Specific IgE Antibodies by Immunologic Methods

For determination of specific IgE antibodies serum samples were obtained from all persons and stored at -20°C until used.

1. *Phadezym-RAST*[®]. The Phadezym-RAST (Pharmacia) was used to detect specific IgE antibodies. In this method, the patient's serum is incubated with a paper disc upon which the allergen is covalently coupled. After washing, the paper disc/allergen/IgE complex is retained in the test tube. An anti-IgE, which is labeled with the enzyme β -galactosidase, is then allowed to react during a second incubation with the bound IgE. After washing away the excess reagents, a paper disc/allergen-IgE/anti-IgE-enzyme complex remains in the tube. When a reducing agent (glutathione) is added, the enzyme is set free and reacts with an *o*-nitrophenyl- β -galactoside substrate, to produce yellow-colored *o*-nitrophenol and colorless galactose. This enzymatic hydrolysis is stopped by adding sodium carbonate. The absorption measured at 420 nm is directly proportional

to the allergen-specific IgE present and can be estimated in RAST classes (0–4). A reaction is regarded positive if RAST class 1 is exceeded. This method of testing requires 24 hours.

2. *IgE-quick*[®]. For this investigation IgE-quick (Quidel), central European version, was used. In this method 9 allergens listed in Table I, plus a negative control, are attached in a row onto a dipstick. This dipstick is incubated with 1 ml of the patient's serum for 1 hour. After washing with cold tap water, the dipstick is once more incubated with an alkaline phosphatase-conjugated anti-IgE antibody. After a second washing, it is incubated with the Quidel enzyme substrate solution. If there are specific antibodies present in the patient's serum sample, a blue color develops on the respective allergen-coated square on the dipstick. For evaluation, the blue coloration of each allergen square is compared with that of the negative square. The density of the color is directly proportional to the amount of specific IgE antibody present in the serum. A positive result is taken if any color is deeper than the negative control. This method takes 2 hours.

RESULTS

Skin Tests

Seventy-five patients showed positive reactions on a skin test to one or more allergens listed in Table I. None of the control subjects showed any positive skin test responses.

The positive skin test reactions are listed in Table II. Twenty-four patients were positive to pollens, 17 to pollens and house dust mites, 16 to house dust mites alone, 9 to pollens, mites and animal dander, 2 to mites and animals, 1 to pollens and *Alternaria tenuis*, and 1 to mites, pollens and *Alternaria* (Fig. 1).

TABLE II

Skin Test Reactions to Different Allergens

Allergen	Positive	Negative
Timothy grass	48	52
Mugwort	27	73
Birch	24	76
<i>Alternaria</i>	2	98
Rye	38	62
Cat	17	83
Dog	4	96
<i>Dermatophagoides pteronyssinus</i>	43	57
<i>Dermatophagoides farinae</i>	37	63

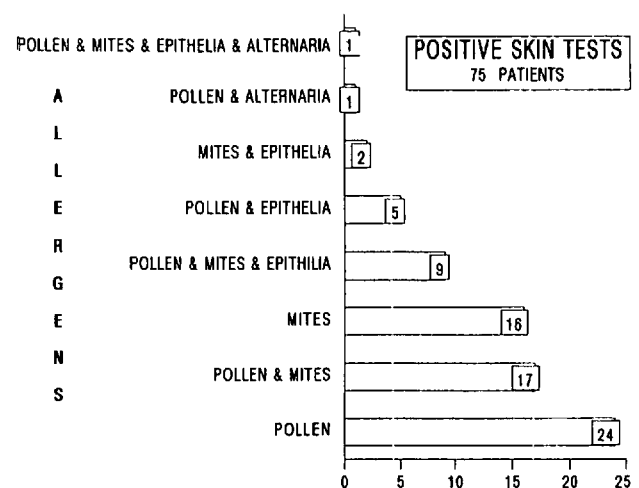


Figure 1. Distribution of skin test reactions in 75 patients.

TABLE III

Percent Correlation between the Different *in Vitro* Methods and Skin Reactions

A. RAST		% Correlation with Skin Tests	
Allergen	Positive/Positive Negative/Negative	False	
		Positive	Negative
Timothy grass	91	1	8
Mugwort	86	0	14
Birch	95	1	4
<i>Alternaria</i>	99	0	1
Rye	94	0	6
Cat	90	2	8
Dog	98	0	2
<i>Dermatophagoies pteronyssinus</i>	89	0	11
<i>Dermatophagoides farinae</i>	94	0	6
Total Number	836	4	60

B. IgE-Quick		% Correlation with Skin Tests	
Allergen	Positive/Positive Negative/Negative	False	
		Positive	Negative
Timothy grass	89	1	10
Mugwort	87	2	11
Birch	93	2	5
<i>Alternaria</i>	98	1	1
Rye	92	0	8
Cat	87	2	11
Dog	98	0	2
<i>Dermatophagoides pteronyssinus</i>	86	0	14
<i>Dermatophagoides farinae</i>	93	0	7
Total Number	823	8	69

Allergen-Specific IgE Antibodies

Results of allergen-specific IgE antibody determination with both methods used—the Phadezym-RAST and the IgE-quick—were in good agreement with the skin test results for the allergens used in this study. This agreement was seen in positive IgE antibody/positive skin test and in negative IgE antibody/skin test patients. Correlations between Phadezym-RAST and SPT were 86% for positive and 89% for negative results. For IgE-quick and SPT the correlations were 86% for positive and 98% for negative results. The results are shown in Table III and Figure 2.

False-positive *in vitro* results with negative skin tests were seen in 4 instances with Phadezym-RAST and in 8 instances with IgE-quick. There were 60 false-negative Phadezym-RAST results, while 69 positive skin tests and negative results were obtained with IgE-quick.

Expressed as a percentage of total investigations made

with each method, there were less than 1% false-positive and false-negative results using the both *in vitro* methods. The two methods did not differ from each other significantly.

DISCUSSION

Our results in this study demonstrate that both *in vitro* methods corresponded to the *in vivo* detected sensitivity against the tested allergens to a large extent. The correlation between the two *in vitro* tests was also very high. These data suggest that the new easy to handle and very quick *in vitro* test—IgE-quick—can be recommended to detect allergen-specific IgE antibodies.

Apart from the advantage of saving time to get a result, no special laboratory equipment is required. The handicap of IgE-quick is the offered panel of allergens. Investigations are underway to add different allergens to the offered panel of allergens, especially nutritive allergens.

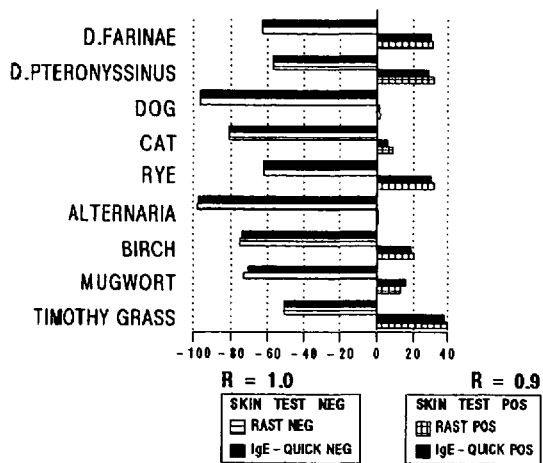


Figure 2. Percent correlation between the different in vitro methods and positive and negative skin reactions.

Finally, we should like to point out that only after evaluation of all diagnostic parameters, especially case histories and skin tests, can the clinically relevant allergen be identified. Allergen-specific immunotherapy, if necessary, should not be started before this procedure has been carried out. The availability of *in vitro* methods should not lead anyone to start immunotherapy without manifest clinical evidence identifying a specific allergen.

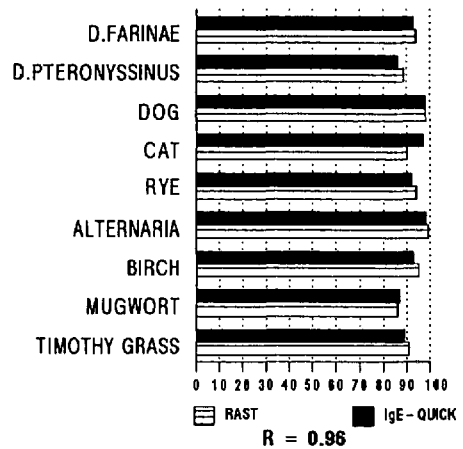


Figure 3. Correlation between the two in vitro methods for the different allergens tested.

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