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Development and Performance Evaluation of a Visual Fast Test For the Detection of Specific IgE in Capillary Blood or Heparin Blood

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Original paper

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Key Words
fast allergy test – screening method – capillary blood – heparin blood – type I sensitisation – ELISA

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The number of allergic patients in industrialised countries has increased over the past 30 years. Presently, approximately 30% of the European population is affected. According to estimations, only a fraction of patients with respiratory allergy is diagnosed at an early stage and treated correctly. The European Academy of Allergology and Clinical Immunology (EAACI) has, therefore, initiated a campaign focusing on the early diagnosis of allergy. Early diagnosis requires easy-to-use screening instruments. Thus, a simple visual allergy test for the fast detection of specific IgE in capillary blood was developed. This test is based on the ELISA technique (enzyme-linked immunosorbent assay) and adapted to be carried out at room temperature yielding a result within 30 minutes from only 2-3 drops of whole blood (75-100 µl). Specific IgE for either 12 separate food allergens or 12 separate inhalation allergens is determined in parallel in one test. The current allergen panel covers approximately 90% of the relevant inhalant allergens or food allergens for northern and central Europe. Allergens on the membrane produce a visible sign in the shape of a "+" for positive reactions and a "-" for negative reactions. Correlation of results of the fast allergy test with results of the Pharmacia CAP was demonstrated. The test showed 96 % positive and 98 % negative correlation on average in 331 patients.

In conclusion, the test represents a valid method for screening of elevated specific IgE for the most common inhalation and food allergens.

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Introduction

The occurrence of allergies is increasing in all industrialised countries. To date, almost one third of the European population is affected. Generally, allergies represent one of the major health problems not only in Europe but worldwide [5]. The situation might even become worse, because the prevalence is highest in young people [2]. An overview of the epidemiologic development of allergies in Germany is given by the special report on allergies of the German Federal Bureau of Statistics [15]. As a consequence to secondary diseases resulting from untreated allergies the European Academy of Allergology and Clinical Immunology (EAACI) issued the demand for an early diagnosis and therapy of allergies.

The aim of the paper presented here was the development of a valid, inexpensive and fast visual screening test for the detection of specific IgE antibodies which would match the sensitivity and specificity of already established IgE-detection methods of which the Pharmacia CAP laboratory test represents the standard system with the highest market share. We performed a validation and optimisation of the fast allergy test in comparison to the Pharmacia CAP system together with an independent laboratory and during clinical routine.

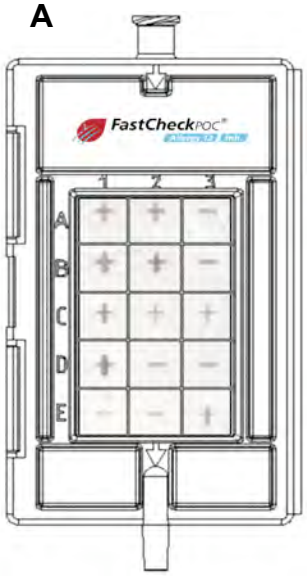
Translation of Reprint

Table 1: Current allergen panels used in the fast allergy test for 12 inhalation or food allergens:

	Inhalation allergens		Food allergens	
	Code	Allergen	Code	Allergen
1	d1	House dust mite	f74	Hen egg
2	t3	Birch pollen	fx74	Fish mix*
3	t4	Hazel pollen	fx73	Meat mix*
4	gx3	Grass pollen mix*	f24	Shrimp
5	w6	Mugwort pollen	f13	Peanut
6	w20	Stinging nettle pollen	f17	Hazel nut
7	e1	Cat epithelia	f14	Soy bean
8	e2	Dog epithelia	f4	Wheat flour
9	k82	Latex	f5	Rye flour
10	m2	Cladosporium herbarum	f31	Carrot
11	m3	Aspergillus fumigatus	f85	Celery
12	m6	Alternaria alternata	f199	Milk

*Content of allergen mixes:
gx3: sweet vernal grass, timothy grass, rye grass, rye, velvet grass
fx73: chicken, pork, beef
fx74: cod, herring, mackerel, plaice

A



B

	1	2	3
A	X	X	
B	X	X	
C	X	X	X
D	X		
E		X	X

A1	House dust mite
A2	Cat epithelia
A3	Stinging nettle
B1	Hazel pollen
B2	Birch pollen
B3	Cladosporium herbarum
C1	Grass pollen mix
C2	Latex
C3	Aspergillus fumigatus
D1	Mugwort pollen
D2	Dog epithelia
D3	Alternaria alternata
E1	Negative control
E2	Borderline control
E3	Positive control

Figure 1: The fast allergy screening test „inhalation“ was performed with heparin blood of a patient with multiple allergies. Part A of the figure shows the original result, scanned directly after stopping the incubation with developer. The right hand table depicts the respective allergen in each field of the grid. Part B features the schematic result; the patient reacts to 8 of 12 allergens, with most prominent reaction to mugwort pollen.

Material and Methods

Technical Basics

Test Development

The development of the rapid allergy test was based on the classical ELISA technique. In most available laboratory test systems allergens are bound to a solid phase. The patient's serum is incubated with the allergens. If the sample contains IgE antibodies that are specific for the immobilised allergens, they will bind to the allergens. Subsequently, they are detected by a specific anti-IgE antibody that is conjugated with an enzyme, a fluorescence dye or other molecule, that gives a measurable signal for quantification. The derived intensities are translated into numbers using a reference line.

The rapid allergy test follows the same principle. Through optimisation of the employed chemicals and the combination of several steps the result is a test that is run with capillary blood at room temperature and gives valid results within 30 minutes. Optimal adjustment of the applied components reduces the quantity of required sample material to only 2–3 drops of blood (75–100 µl).

Twelve allergens – either 12 food allergens or 12 inhalation allergens (table 1) – and 3 controls are located on a membrane in a grid of three by five positions. Every single allergen can be differentiated. In addition, every position (a total of 15) has an internal control. After processing, this internal control results in a horizontal bar. In case the sample contains IgE that binds to the allergen at the given grid position, a vertical bar appears together with the horizontal internal control bar to form a plus sign. If no IgE is bound, only the internal control bar becomes visible as a minus sign that is easily interpreted (figure 1). The allergens selected for both panels represent the prevalence of sensitisation in north and central Europe and can be replaced by different allergens for other geographic regions.

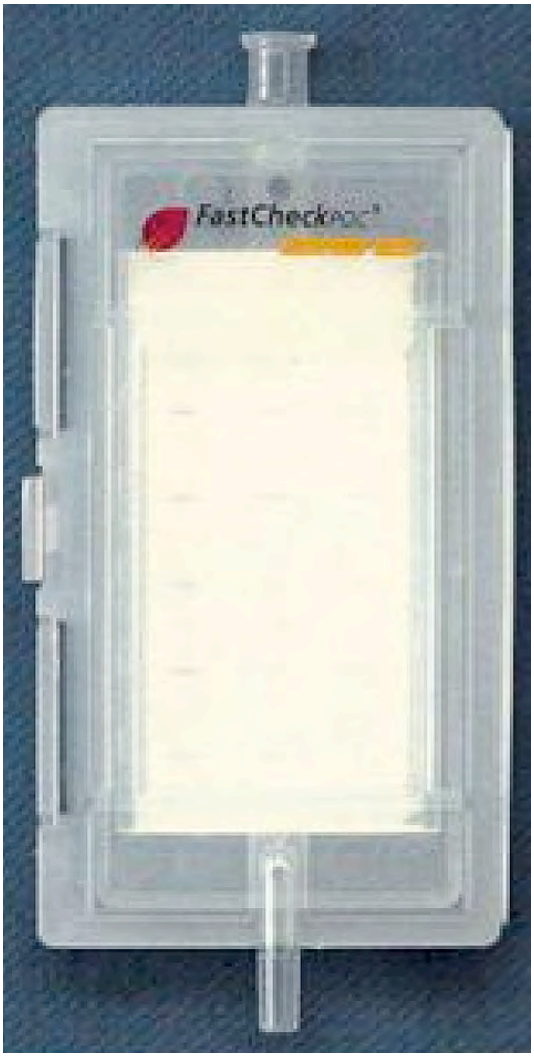


Figure 2: The fast allergy test housing holds the test membrane with allergens and controls inside a flat incubation chamber. A latch keeps the lid in a half open position during sample incubation and assures tight fitting of the lid when closed.
The device is closed completely before conducting washing steps and substrate incubation. Washing solution and substrat are added through the upper Luer lock using a syringe.

Development of the Test Device

The test device (figure 2) ensures a simple handling of the test. Contact with sample material occurs only for a short time and the results can be read without any technical help. The test device housing is designed to enable washing steps and the addition of substrate to be performed through a port by using a syringe. The housing is opened only once upon addition of the sample. The incubation chamber containing the membrane has a very low volume and 400 µl of test solution containing the 75-100 µl of blood (3 drops) are sufficient to entirely cover the test membrane. The lid of the housing is equipped with a gridded window to facilitate the read-out and to assign results.

Test Procedure

Two to three drops of capillary blood, 100 µl of heparin blood or serum are added to the test solution. The test device is opened and a small amount of washing solution is applied to moisten the membrane. The surplus of the washing solution is removed. The test solution containing the blood is deployed onto the membrane surface and distributed evenly. The incubation time is 15 minutes at room temperature. The solution containig the blood is washed off with tap water. Three washing steps are performed, leaving the washing solution in the device for two minutes each. The washing solution is drained off and 3 ml of substrate is filled into the device using a 5 ml syringe. Following an incubation time of 5–10 minutes the result becomes visible and the test procedure ends by washing off the substrate using tap water.

Comparison of Results Generated by Fast Allergy Test and Laboratory Test

The comparative study was performed at Charité, Klinik für Dermatologie, Venerologie und Allergologie, by ear, nose and throat practice Westpfahl-Wiesener as well as at the research laboratory of DST GmbH. The comparative study enrolled 331 patients. We used Pharmacia CAP (UniCAP 100) as the reference system. The fast allergy test was standardised to reach a lower cut-off of CAP-class 2. When the laboratory test result was a CAP-class 1 and the fast allergy test was negative the results were counted as positive correlation.

Table 2: Comparison of results of allergy testing from fast allergy test and laboratory test

Allergen	CAP positive	Fast Test positive	Positive Correlation	CAP negative	Fast Test negative	Negative Correlation
d1 house dust mite	59	58	98.3 %	139	137	98.6 %
t4 hazel pollen	24	24	100 %	31	30	96.7 %
gx3 grass pollen mix	76	75	98.7 %	38	37	97.4 %
w6 mugwort pollen	62	60	96.8 %	44	43	97.7 %
e1 cat epithelia	33	31	93.9 %	40	40	100 %
t3 birch pollen	80	76	95 %	58	56	96.5 %
k82 latex	12	12	100 %	16	16	100 %
e2 dog epithelia	21	20	95.2 %	47	44	93.6 %
w20 stinging nettle	15	15	100 %	20	20	100 %
m2 Cladosporium	4	4	100 %	35	34	97.1 %
m3 A. fumigatus	4	4	100 %	38	37	97.3 %
m6 Alternaria alternata	15	14	93.3 %	45	45	100 %
f74 hen egg	11	11	100 %	20	18	90 %
fx74 fish mix	14	14	100 %	15	15	100 %
fx73 meat mix	8	7	87.5 %	31	31	100 %
f24 shrimps	10	9	90 %	26	25	96.2 %
f17 hazelnut	33	30	90.9 %	34	34	100 %
f13 peanut	22	19	86.4 %	24	24	100 %
f14 soy bean	14	13	92.8 %	23	23	100 %
f5 rye flour	11	11	100 %	18	18	100 %
f4 wheat flour	18	18	100 %	21	21	100 %
f31 carrot	17	16	94.1 %	18	18	100 %
f85 celery	21	21	100 %	28	28	100 %
f199 milk	15	14	93.3 %	38	36	94.7 %

Patients' blood was used as capillary blood or further processed to obtain serum and heparin blood. Laboratory testing was always performed using serum, the fast test was performed with serum in 82 cases, with capillary blood in 36 cases and with heparin blood in 213 cases.
Correlation of positive results:
N1: number of patients giving a positive result during laboratory testing
N2: number of patients out of N1, reacting positively to the respective allergen in the fast test.
N2/N1 * 100 = positive correlation
Correlation of negative results:
N3: number of patients that did not react to an allergen in laboratory testing
N4: Number of patients out off N3 tested with fast test, that did not react to an allergen.
N4/N3 * 100 = negative correlation

Results

Ease-of-use of the fast allergy test was confirmed during the study. The comparison of the results of the fast allergy test and the reference system Pharmacia-CAP (Unicap 100) show a good correlation. The results of comparing the data from 331 patients generated with the fast allergy test and the UniCAP 100 laboratory test system are listed in table 2. Heparin blood, capillary blood and blood serum samples were concurrently drawn from the patients. For the UniCAP 100 laboratory test only serum was used (as this system requires serum), while capillary blood, heparin blood or serum were used with the fast allergy test. The results of 331 patients were finally included in the evaluation.

For the inhalation panel, the correlation of positive results (see legend of table 2) was between 93.3 % and 100 % while the correlation of negative results was between 93.6 % and 100 %. For pollen (birch, hazel, mugwort, grass pollen mix and stinging nettle), the positive correlation was between 95 and 100 %, in case of epithelia (cat, dog) it reached between 93.9 and 95.2 %, for moulds (Aspergillus fumigatus, Cladosporium herbarum, Alternaria alternata) it was between 93.3 and 100 %, for latex it reached

100 % and for house dust mite it was determined to be 98.3 %. The correlation of negative results (table 2) was between 96.5 and 100 % for pollen, 93.6 to 100 % for epithelia, 97.1 to 100 % for moulds, 100 % for latex and 98.6 % for house dust mite.

The correlation of positively tested samples for the food panel reached a mean value of 95 % (SD = 0.05) the correlation of negatively tested samples reached a mean value of 98 % (SD = 0.03).

Celery, carrots, wheat flour, rye flour, fish and egg showed a correlation of positive results of 94 to 100 %, shrimps, hazelnut, soy and milk of 90 to 93.3 %, meat mix correlated in positive results at 87.5 % and peanut at 86,4 %. Negative correlation between the two test systems was 90 % for egg, 94.7 % for milk and 96.2 % for shrimps. All other allergens revealed a correlation of 100 % regarding negative results.

Reproducibility of test results was confirmed by replicates using the same samples of heparin blood several times within one week post sampling. Capillary blood was freshly drawn for every replicate testing, patients were tested several times during the course of one week. Differences between results were found in less than 2 % of the tests.

To confirm the compatibility of the fast test with different sample preparations, capillary blood, heparin blood and serum from 48 patients were tested. Correlation of results from the different sample materials was confirmed for a sample volume of 100 µl. Smaller sample volumes (50 µl) lead to results that differed from the laboratory test results. Sample volumes of 200 µl or 400 µl did not differ from the results obtained using a sample volume of 100 µl.

Discussion

Type I allergies are widely spread in Europe, but only few patients are diagnosed early and receive appropriate treatment, because allergy expertise and immediate access to reliable diagnostic tools are limited particularly in primary care in Europe.

For an early-on diagnosis in disease development a fast allergy screening test represents a simple and effective tool for determination of type I sensitisation. The qualitative results render the fast screening test a useful tool to confirm anamnesis with regard to the presence of an allergic condition. The simultaneous testing of 12 allergens lower the cost of the test and speed it up. Positive results should be confirmed by quantitative *in vitro* testing, skin test or provocation test if indicated, to ensure an adequate therapy. If negative results occur while the symptoms still persist a visit with an allergologist is strongly recommended.

Correlation of Results of Different Laboratory Systems

Good positive and negative correlations of the results from the fast allergy test with those from the Pharmacia CAP system were confirmed. Pharmacia CAP was used for validation, because it is the most widely used system in Germany. It is generally accepted, that skin tests and *in vitro* tests can only hint to a sensitisation, however, clinical relevance cannot be confirmed by these tests. The provocation testing remains the gold standard in allergy diagnostics [4,9,10]. Discrepancies between our fast allergy test and the Pharmacia CAP are most frequently found in CAP-class 1-2, featuring the range from 0.35-0.7 kU/l within the entire range from 0.35-100 kU/l. Therefore, discrepancies are mainly found in a window representing less than 1 % of the complete range.

Choice of Allergens

Allergens were selected according to prevalence and sensitisation of the northern and central European population and cover about 90% of all occurring allergies. This range is sufficient for a screening test, because the remaining 10 % include a plethora of different allergens.

Use of Capillary Blood Instead of Serum in the Fast Screening Test

This test is intended to be performed without the requirement of accessing laboratory equipment let alone a diagnostic laboratory . It does not require sample prepara-

tion of any kind. The test kit is designed to be used with capillary blood, while heparin blood or serum are equally applicable. Laboratory systems can only handle serum. The fast allergy test was validated to guarantee correct and identical test results using different sample materials like heparin blood, serum and capillary blood from the same patient. The fast allergy test demonstrated good data correlation with the CAP system comparable to the correlation of other manufacturer's laboratory tests with the CAP system [1,8,11,12,14]. The choice of tested allergens was based on the prevalence and sensitisation of patients in northern and central Europe.

In summary, the fast allergy test is an easy to use screening method for type I allergies.

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