

Comparison of a multi-allergen dipstick IgE assay to skin-prick test and RAST

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Summary

A multi-allergen dipstick enzyme immunoassay 'Quidel Allergy Screen' (QAS) has recently been developed commercially for measuring IgE antibodies against nine allergens (house dust 1, house dust 2, *Dermatophagoides pteronyssinus*, *D. farinae*, Japanese cedar, ragweed, cat dander, sweet vernal grass, and egg white) at one time. To assess whether this assay is useful in screening allergen-specific IgE antibody, we compared the titres of IgE antibodies against the nine allergens measured by QAS to those in the skin-prick test and by RAST in 93 atopic asthmatics and 22 normal subjects. We found a good overall agreement between the results of the skin-prick test and the results of QAS (sensitivity = 47.1–81.4%, specificity = 84.5–100%, and agreement = 78.9–88.9%). The sensitivities against house dust 1, *D. pteronyssinus*, and *D. farinae* ranged from 77.2 to 81.4%. However, the sensitivities against house dust 2, Japanese cedar, ragweed, and cat dander were low (47.1–68.8%). We also found a good overall agreement between the results of RAST and the results of QAS, except for egg white (sensitivity = 46.2–94.4%, specificity = 87.4–100%, and agreement = 77.4–96.5%). The sensitivities against house dust 1 and 2, *D. pteronyssinus*, *D. farinae*, and Japanese cedar ranged from 86.0 to 94.4%. The sensitivities against ragweed, cat dander, and sweet vernal grass were low (46.2–52.6%). There were strong correlations between the titres of RAST and the titres of QAS except cat dander and egg white ($r = 0.701$ – 0.924 for the seven allergens). Thus, we conclude that QAS is useful in screening IgE antibodies against multiple allergens at one time. However, because the sensitivities against some allergens tested were low, further improvement of some allergen preparations seems to be necessary in the assay.

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Introduction

Identification of allergens causing atopic diseases is important in diagnosis and therapy of the diseases. To identify causative allergens, the skin-prick test is widely used and has been shown to correlate with clinical symptoms and the results of provocation tests [1]. However, although the skin test can be easily performed in many clinics, the patient has to discontinue anti-allergic drugs prior to the test. The reactivity of skin to specific and non-specific agents also differs from one

patient to another. In addition, to directly measure allergen-specific IgE antibody in serum, the radioallergo-sorbent test (RAST) is most widely used as an *in-vitro* assay [2]. However, since a given allergen is coated on one paper disc in RAST, only the IgE antibody to the given allergen can be measured at one time, and therefore RAST is not necessarily suitable for screening large panels of allergens. To solve these problems, a multi-allergen dipstick enzyme immunoassay (Quidel Allergy Screen; QAS) has recently been developed to measure specific IgE antibodies against nine allergens at one time [3].

The purpose of this study was to assess whether this assay is useful in screening allergen-specific IgE antibody.

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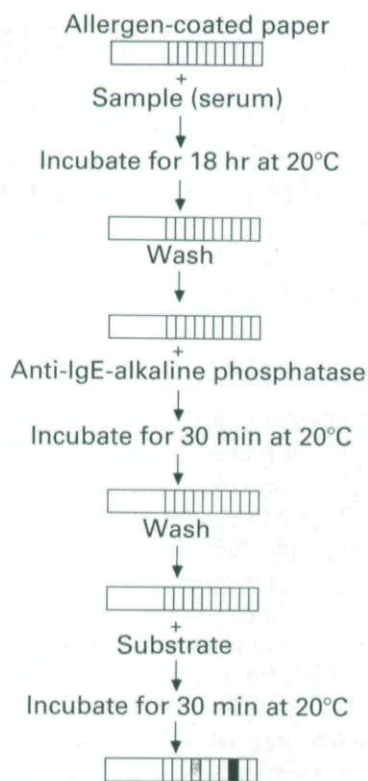


Fig. 1. Method of a multi-allergen dipstick enzyme immunoassay for measuring allergen-specific IgE antibody.

For this purpose, we compared the titres of IgE antibody measured by QAS to those in the skin-prick test and by RAST in atopic asthmatics and normal subjects.

Subjects and methods

Subjects

Ninety-three atopic asthmatics in our allergy clinic and 22 normal subjects were conducted in this study. The asthmatic subjects (54 males and 39 females aged 18–55) were diagnosed asthmatic by their clinical histories and by evidence that their symptoms were reversible with a β -adrenergic agonist [4]. All of them gave positive skin-prick tests or positive RAST (0.7 PRU/ml or more) for at least one of 30 inhalant allergens used in our clinic. None of them were receiving specific immunotherapy. Twenty-two normal subjects (12 males and 10 females aged 22–47), most of whom were medical staff or students in our university, had no history of wheezing or other symptoms of atopic diseases, had no positive skin-prick tests for the following six allergens used in this study and less than 0.35 PRU/ml of RAST against the following nine allergens, and had less than 100 IU/ml of serum IgE levels determined with PRIST (Pharmacia, Uppsala, Sweden).

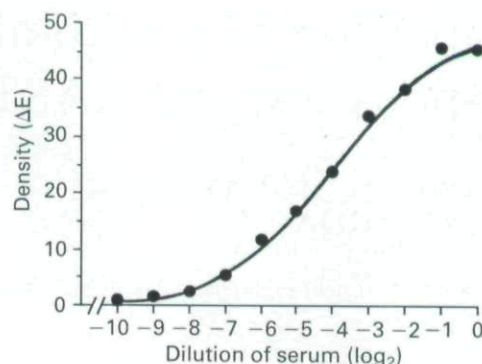


Fig. 2. Dose-dependent relationship between the amount of specific IgE antibody and the density by QAS. A serum containing *D. pteronyssinus*-specific IgE antibody was serially diluted with pooled human serum and the 1- to 1024-fold diluted sera were measured by QAS.

Quidel Allergy Screen

The multi-allergen dipstick enzyme immunoassay QAS for allergen-specific IgE antibody determination was performed using QAS kit (Quidel Inc., La Jolla, U.S.A.) (Fig. 1) [3]. The dipstick was attached with 10 paper pads which were individually coated with allergens (house dust 1; Greer Lab., house dust 2; Hollister-Stier Lab., *D. pteronyssinus*, *D. farinae*, Japanese cedar, ragweed, cat dander, sweet vernal grass, egg white, and a negative control). Briefly, the multi-allergen coated dipstick was first incubated with 1 ml of the serum to be tested for 18 hr at 20°C. The dipstick was then washed and incubated with an alkaline phosphatase-labelled mouse monoclonal anti-human IgE antibody (5 μ g/ml) for 30 min at 20°C. After the dipstick was washed, it was incubated with the substrate 5-bromo-4-chloro-3-indlyl-phosphate (4×10^{-3} M) for 30 min at 20°C. The development of blue colour was measured by a densitometer (Minolta CR-221, Tokyo, Japan) and the titre of allergen-specific IgE antibody was expressed as a density ΔE .

Skin-prick test

None of the subjects had taken any medication for at least 24 hr prior to the skin-prick test. The test was performed on the volar surface of forearms of the subjects using six allergens (house dust, *D. farinae*, Japanese cedar, ragweed, cat dander, and egg white) (Torii Pharmaceutical Co., Tokyo, Japan). We only used *D. farinae* as a mite allergen in the skin-prick test because *D. pteronyssinus* was not available for the test in Japan. Sweet vernal grass was also not available. The weal was measured with a planimeter after 20 min and a weal greater than 5 mm in diameter was considered to be positive [5].

Table 1. Relationship between the results of the skin-prick test and the results of Quidel Allergy Screen (QAS)

Allergen	S(+)Q(+)	S(+)Q(-)	S(-)Q(+)	S(-)Q(-)	Sensitivity (%)	Specificity (%)	Agreement (%)
House dust 1*	44	13	1	32	77.2	97.0	84.4
House dust 2*	38	19	0	33	66.7	100	78.9
<i>D. pteronyssinus</i> **	48	11	1	30	81.4	96.8	86.7
<i>D. farinae</i> **	48	11	1	30	81.4	96.8	86.7
Japanese cedar	22	10	9	49	68.8	84.5	78.9
Ragweed	9	7	3	71	56.3	95.9	88.9
Cat dander	8	9	4	69	47.1	94.5	85.6
Egg white	—	—	—	—	—	—	—

S(+) or S(-) = Positive or negative in the skin-prick test.

Q(+) or Q(-) = Positive or negative by QAS.

Sensitivity = $S(+)/[S(+) + S(-)]$.

Specificity = $S(-)/[S(-) + S(+)]$.

Agreement = $[S(+)Q(+) + S(-)Q(-)]/\text{total number}$.

*House dust 1 or 2 (QAS) vs. house dust (skin-prick test).

***D. pteronyssinus* or *D. farinae* (QAS) vs. *D. farinae* (skin-prick test).

RAST

We also measured allergen-specific IgE antibodies in serum using the Phadebas RAST kit (Pharmacia) against the nine allergens as described elsewhere [2,6]. Briefly, the allergen-coated disc was incubated with 100 μ l of the serum to be tested for 3 hr at 20°C. Then the disc was washed and incubated with 50 μ l of ^{125} I-labelled rabbit anti-human IgE for 18 hr at 4°C. After the disc was washed, the radioactivity was measured in a γ -counter. The titre of allergen-specific IgE antibody was expressed as Phadebas RAST units (PRU) using reference sera supplied with the kit and 0.7 PRU/ml was considered to be positive.

Data analysis

The correlation coefficient was obtained by linear regression analysis.

Results

Linearity and reproducibility of Quidel Allergy Screen

To assess the linearity of this multi-allergen dipstick enzyme immunoassay QAS for allergen-specific IgE antibody determination, we measured a 1- to 1024-fold diluted serum containing *D. pteronyssinus*-specific IgE antibody (372 PRU/ml) by QAS (Fig. 2). The titre of the IgE antibody by QAS (density ΔE) increased dose-dependently in 1024-fold ($\Delta E=0.79$) to twofold ($\Delta E=45.9$) diluted serum. To assess the reproducibility of

this assay, we examined the coefficient of variation of ΔE in QAS by measuring two sera containing *D. pteronyssinus*-specific IgE antibodies (13.8 and 15.9 PRU/ml) 10 times each. The coefficients of variation in the two determinations were 9.0% and 6.1%, respectively.

To determine the cut-off point of ΔE in QAS between the negative and positive reactions, we measured ΔE in QAS against the nine allergens and a negative control in 22 normal subjects. The ΔE was 0.61 ± 0.28 (mean $\pm$ s.d., $n=220$) and therefore a ΔE greater than 1.5 (mean + 3 s.d.) was considered to be positive in this assay.

Skin-prick test and Quidel Allergy Screen

There was a good overall agreement between the results of the skin-prick test and the results of QAS (Table 1). The sensitivities against house dust 1, *D. pteronyssinus*, and *D. farinae* ranged from 77.2 to 81.4%. The sensitivities against house dust 2, Japanese cedar, ragweed, and cat dander were low (47.1–68.8%). No data were recorded for egg white because too few egg white-sensitive subjects were studied. The specificities against the remaining seven allergens ranged from 84.5 to 100% and the agreements ranged from 78.9 to 88.9%.

RAST and Quidel Allergy Screen

There was a good overall agreement between the results of RAST against the eight allergens, except for egg white, and the results of QAS against them (Table 2). The sensitivities against house dust 1 and 2, *D. pteronyssinus*,

Table 2. Relationship between the results of RAST and the results of QAS

Allergen	R(+)Q(+)	R(+)Q(-)	R(-)Q(+)	R(-)Q(-)	Sensitivity (%)	Specificity (%)	Agreement (%)
House dust 1	63	7	4	41	90.0	91.1	90.4
House dust 2	49	8	7	51	86.0	87.9	87.0
<i>D. pteronyssinus</i>	68	7	0	40	90.7	100	93.9
<i>D. farinae</i>	68	4	0	43	94.4	100	96.5
Japanese cedar	43	6	5	61	87.8	92.4	90.4
Ragweed	10	9	3	93	52.6	96.9	89.6
Cat dander	13	15	11	76	46.4	87.4	77.4
Sweet vernal grass	6	7	1	101	46.2	99.0	93.0
Egg white	0	7	4	104	0	96.3	90.4

R(+) or R(-)=Positive or negative by RAST.

Q(+) or Q(-)=Positive or negative by QAS.

Sensitivity = $R(+)/[R(+) + R(-)]$.

Specificity = $Q(-)/[Q(+) + Q(-)]$.

Agreement = $[R(+)Q(+) + R(-)Q(-)]/\text{total number}$.

Table 3. Correlation between the titers (PRU/ml) of RAST and the titers (density) of QAS

Allergen	<i>r</i> value	<i>P</i> value
House dust 1	0.884	<0.01
House dust 2	0.819	<0.01
<i>D. pteronyssinus</i>	0.912	<0.01
<i>D. farinae</i>	0.924	<0.01
Japanese cedar	0.895	<0.01
Ragweed	0.714	<0.01
Cat dander	0.464	<0.01
Sweet vernal grass	0.701	<0.01
Egg white	-0.014	>0.5

D. farinae, and Japanese cedar ranged from 86.0 to 94.4%. The sensitivities against ragweed, cat dander, and sweet vernal grass were low (52.6%, 46.4%, and 46.2%, respectively) and the sensitivity against egg white was 0%. The specificities ranged from 87.4 to 100% and the agreements ranged from 77.4 to 96.5%.

There were also strong correlations between the titres of IgE antibodies by RAST and those by QAS, except for cat dander and egg white (Table 3). The correlation coefficients (*r*) against the 7 allergens were 0.701–0.924 ($P < 0.01$). However, there was a relatively weak correlation between RAST and QAS against cat dander ($r = 0.464$, $P < 0.01$) and there was no correlation between RAST and QAS against egg white ($r = -0.014$, $P > 0.5$).

Discussion

We compared the results of a multi-allergen dipstick enzyme immunoassay QAS to those of the skin-prick tests and RAST because the two latter tests are widely-used in *in-vivo* and *in-vitro* allergen-specific IgE assays, respectively, and because we have not had any 'gold' standard for IgE antibody determination yet. In this study, we showed that allergen-specific IgE antibodies measured by QAS correlate with those in the skin-prick test and by RAST. We found that there was a good overall agreement between the results of the skin-prick test and the results of QAS (Table 1). The sensitivity, specificity, and agreement between the skin-prick test and QAS against all the eight allergens except egg white were 74%, 94%, and 84%, respectively (Table 1). The sensitivities, specificities, and agreements between the skin-prick test and QAS were similar to or greater than those between the skin-prick test and RAST [7–10; and our unpublished data]. In a representative study, Berg and Johansson [8] reported 57% sensitivity, 90% specificity, and 75% agreement between skin-prick tests and RAST against nine inhalant allergens including house dust and cat dander. We also found that there was a good overall agreement between the results of RAST and the results of QAS (Table 2) and that there were strong correlations between the tiers of RAST and the tiers of QAS (Table 3). The sensitivity, specificity, and agreement between RAST and QAS against all the eight allergens except egg white were 84%, 94%, and 90%, respectively (Table 2).

Among the nine allergens tested, the specificities and agreements against the allergens were high between the skin-prick test and QAS and also between RAST and QAS (Tables 1 and 2). However, although the sensitivities of QAS against most of the allergens tested were high, the sensitivities of QAS against some allergens, i.e. ragweed, cat dander, sweet vernal grass, and egg white, were low (Tables 1 and 2). The correlations between RAST and QAS against cat dander and egg white were also weak (Table 3). One reason for low sensitivities of QAS against these allergens might be due to relatively small amounts of these allergens coated on paper pads of the dipstick.

Another possibility for low sensitivities and weak correlations in these allergens might be due to the difference in allergenicity of these allergens used in the three methods, as the allergen preparations used in the skin-prick test, in RAST, and in QAS were different. Therefore, the study using the same preparations of these allergens in the skin test, RAST, and QAS needs to be done in the future.

In addition, there is a problem in selecting the nine allergens used in the dipstick. As there are crossreactivities between house dust 1 and 2, between *D. pteronyssinus* and *D. farinae*, and also between house dust and mite allergens [11–13], it is not helpful to use these four similar allergens for screening allergens. Consequently, it would be sensible if one or two house dust mite allergens were replaced with other unrelated allergens.

In summary, we have shown that a multi-allergen dipstick enzyme immunoassay QAS is useful in screening IgE antibodies against multiple allergens at one time. However, further improvement of some allergen preparations seems to be necessary in the assay.

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