

Comparison of VIDAS Stallertest and Pharmacia CAP Assays for Detection of Specific IgE Antibodies in Allergic Children

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Abstract. In vitro determination of specific IgE antibodies in serum is the most frequently used method, besides the skin test, for diagnosing allergies. Standardized and reproducible assays of specific IgE antibodies contribute to the quality of diagnosis and treatment of allergic disease. This study compared the results and performance characteristics of the Pharmacia CAP system and a new specific IgE method using the VIDAS Stallertest (manufactured by bioMérieux). To evaluate their clinical efficiency, the results of the CAP and VIDAS Stallertest assays were compared with skin prick test (SPT) results. After allergic patients completed SPTs, serum samples were collected and CAP and VIDAS Stallertest assays were performed to determine specific IgEs for *Dermatophagoides farinae*, *D. pteronyssinus*, cockroach, and alternaria. For egg and milk, we measured only the correlation between the 2 in vitro assays. When SPT was used as a reference standard, the sensitivity and specificity of the CAP assay was a little higher in respect to all inhalant allergens. There were significant correlations between the results of VIDAS Stallertest and CAP assays for IgE antibodies to inhalant and food allergens. This study indicates that the VIDAS Stallertest and Pharmacia CAP assays are feasible and replicable for measuring allergen-specific IgE. (received 1 April 2005; accepted 16 April 2005)

Keywords: VIDAS Stallertest assay, Pharmacia CAP assay, specific IgE, inhalant allergens, food allergens

Introduction

The diagnosis of allergic diseases requires the patient's medical history, a physical examination, and the use of either an in vivo or an in vitro test for relevant allergens. Skin testing is a useful diagnostic test, but some patients cannot be tested because of dermatographism, severe skin eczema, or medications [1]. For these patients, in vitro techniques for allergy testing have improved since the specific immunoglobulin responsible for immediate allergic hypersensitivity was discovered and characterized as IgE by Ishizaka et al [2].

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The radioallergosorbent test (RAST) was widely used to determine specific IgE and proved to be of good specificity [3]. However, the use of isotopes in this method was a drawback and the reproducibility and linearity of the results were not always satisfactory [4]. Recently numerous in vitro tests for the diagnosis of allergy have been introduced [5-7]. Among these procedures, the Pharmacia CAP system is widely used and offers enhanced clinical efficiency compared to the RAST method [8].

The purpose of this study was to evaluate the results and performance characteristics of the Pharmacia CAP system and a new specific IgE method for the VIDAS Stallertest (manufactured by bioMérieux), in comparison to skin tests in the same group of allergic children.

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Materials and Methods

Subjects. This study was approved by the Ethical Review Committees of Yonsei University College of Medicine and Ajou University School of Medicine. Informed consent was obtained from the parents of all children included in this study. The subjects were 130 patients who were referred for evaluation of probable inhalant or food allergies at the Severance Hospital, Yonsei University College of Medicine. The subjects included 77 males and 61 females (age range, 5 mo to 20 yr; mean $\pm$ SD = 7.08 ± 4.12 yr). The mean serum IgE concentration of the subjects averaged 486 ± 579 IU/ml (range, 11–3,000 IU/ml). Control blood samples were obtained from 47 children who were having routine blood tests before elective surgical procedures. They included 31 males and 16 females (age range, 1 to 15 yr; mean, 6.06 ± 2.81 yr); they had no personal or family history of allergic rhinitis, asthma, or atopic dermatitis. Their serum IgE titers were all <50 IU/ml.

Skin prick test. Skin prick tests (SPT) were performed following established guidelines. The patients took no antihistamines for at least 7 days before the prick tests. Allergen extracts (Torii, Tokyo, Japan), along with appropriate negative (saline) and positive (histamine) controls, were applied on the forearm using the prick technique. The following inhalant allergens were tested: *Dermatophagoides farinae*, *D. pteronyssinus*, cockroach, and alternaria. Other allergens were tested based on each child's history, but were excluded from the results of this study. Allergens that elicited a mean wheal diameter of 3 mm, or greater than the negative control, were considered positive. No side effects were observed during the skin prick tests.

Assay of serum specific IgE. Serum collected during each child's initial visit was aliquoted and frozen at -20°C until use. The sera were simultaneously analyzed blindly by the Pharmacia CAP and the VIDAS Stallertest assays. Tests for specific IgE were assessed in all patients for the following 6 allergens: *Dermatophagoides farinae*, *D. pteronyssinus*, alternaria, cockroach, egg, and milk.

The Pharmacia CAP assay employs a type of architecture whereby the allergen of interest is covalently bound to a hydrophilic carrier polymer and encased in a capsule, which catches all allergen-specific IgE in the sample. Allergen-specific IgE is detected directly with a combination of polyclonal and monoclonal anti-IgE (Fc) antibodies labeled with beta-galactosidase to generate fluorescence.

The VIDAS Stallertest assay combines a 2-step immunoenzymatic technique with fluorescent detection in the final step. Each single-dose test is comprised of a strip that contains the ready-to-use reagents and a disposable solid phase receptacle (SPR) onto which the allergens are coated. The allergen-coated SPR serves as the solid phase for the capture of the antigen to be detected, as well as the pipettor for the assay. Reagents for the assay are available in sealed reagent strips. Conjugate is a polyclonal antibody linked to alkaline phosphatase. The glass cuvette of the reagent strip contains the substrate (4 methyl-umbelliferyl) and is used for the final reading. At the end of

the assay, results are automatically computed by the VIDAS instrument by reference to a calibration curve stored in memory.

Statistics. We determined the specificity, sensitivity, and efficiency for the inhalant allergen species tested. We calculated sensitivity by dividing the number of positive in vitro assay results among subjects with positive SPT by the total number of subjects with positive SPT. Specificity was calculated by dividing the number of negative in vitro assay results among subjects with negative SPT by the total number of subjects with negative SPT. We calculated efficiency by dividing the number of positive in vitro assay results among subjects with positive SPT plus the number of negative in vitro assay results among subjects with negative SPT by the total number of in vitro assay or SPT results. We calculated the linear correlation coefficient between the results of both in vitro assays. In the case of children tested for food allergen species, we performed the in vitro assays without SPT and calculated only the linear rank correlation coefficients for the results of both of the in vitro assays.

Results

Comparisons of the Pharmacia CAP and VIDAS Stallertest results vs SPT. Sensitivity, specificity, efficiency, and positive- and negative-predictive values of the Pharmacia CAP and VIDAS Stallertest assays in reference to the SPT results are listed in Table 1. The positive-predictive values of Pharmacia CAP assays ranged from 91 to 95% and the negative-predictive values ranged from 93 to 100%.

In comparison, the positive-predictive value of the VIDAS Stallertest assay varied widely, from 93% for *D. farinae* or *D. pteronyssinus* to 69% for cockroach. The negative-predictive value of the VIDAS Stallertest assay also varied widely, from 100% for cockroach to 79% for alternaria.

Correlation of VIDAS Stallertest and Pharmacia CAP assay results. The correlations of results of the VIDAS Stallertest assay with those of the Pharmacia CAP assay for 4 inhalant allergens and 2 food allergens (*D. farinae*, *D. pteronyssinus*, alternaria, cockroach, egg, and milk) are listed in Table 2.

Individual correlation coefficients were *D. farinae* 0.62, *D. pteronyssinus* 0.85, alternaria 0.90, cockroach 0.74, egg 0.89, and milk 0.92. These results indicate that there generally was good agreement between the VIDAS Stallertest and Pharmacia CAP assays.

Table 1. Sensitivity, specificity, efficiency, and predictive indices of the Pharmacia CAP assay and the VIDAS Stallertest assay for allergen-specific IgE antibodies, in comparison to skin prick test results.

Assay and Allergens	Sensitivity (%)	Specificity (%)	Efficiency (%)	PPV (%)	NPV (%)
Pharmacia CAP assay					
<i>Dermatophagoides farinae</i>	96	86	92	93	93
<i>Dermatophagoides pteronyssinus</i>	97	90	94	95	93
Alternaria	100	87	94	93	100
Cockroach	100	83	92	91	100
VIDAS Stallertest assay					
<i>Dermatophagoides farinae</i>	90	85	88	93	79
<i>Dermatophagoides pteronyssinus</i>	96	86	92	93	93
Alternaria	93	82	88	91	86
Cockroach	100	63	76	69	100

PPV, positive-predictive value; NPV, negative-predictive value

Table 2. Correlations of results of the Pharmacia CAP assay and the VIDAS Stallertest assay for allergen-specific IgE antibodies.

Allergen	Correlation coefficient	p value
<i>Dermatophagoides farinae</i>	0.62	<0.01
<i>Dermatophagoides pteronyssinus</i>	0.85	<0.01
Alternaria	0.90	<0.01
Cockroach	0.74	<0.01
Egg white	0.89	<0.01
Cow milk	0.92	<0.01

Each correlation was assessed using Spearman's rank correlation coefficient.

Discussion

In vitro tests have grown in popularity for evaluation of patients suffering allergies. They offer a painless and noninvasive method for identifying the presence of specific IgE-mediated allergies in a sample of blood. They also provide a quantitative assessment of the amount of specific IgE present so that the physician can tailor a safe starting dose of immunotherapy according to the individual patient's level of sensitivity [9]. Furthermore, the evaluation of IgE sensitization is relevant to monitoring changes in a patient's response to allergen avoidance, or as a predictive value in evaluating the response to a food challenge, or for development of allergic respiratory sensitization.

For the last 2 decades the RAST (radioallergo-sorbent test) assay was the method of choice to determine specific IgE levels [3]. Recently, the Pharmacia CAP assay has emerged as a standard method to which newly developed methods are compared [10]. The VIDAS Stallertest assay is a new screening test. The method is an immunoenzymatic reaction that contains a cartridge and a cone covered with the allergen's mixture and is analyzed on the automated VIDAS system. Felden et al [11] reported that the VIDAS Stallertest assay is a reliable primary method for the general practitioner dealing with a putative inhalant allergy and an excellent means to check a questionable or negative skin test result.

The objective of this study was to evaluate the new VIDAS Stallertest assay for common allergens in comparison to the established Pharmacia CAP assay. The results obtained by the VIDAS Stallertest and Pharmacia CAP assays correlate very well with the allergic history and the results of skin prick tests. The VIDAS Stallertest assay provides slightly lower sensitivity and specificity than the Pharmacia CAP assay, but the differences were small in the present study.

Many studies have examined the correlations between results of skin test and in vitro assays. Overall, there is an excellent correlation for well-characterized and standardized allergens, as well as for well-characterized patients, although in vitro assays are usually less sensitive than skin tests [12,13].

Failure of the various specific IgE assay results to correlate precisely with each other and with the results of *in vivo* tests has been documented. This lack of correlation is attributable to many factors, including variability of *in vivo* responses, inherent technical limitations of each method, lack of standardization of allergen extracts, and human errors. Despite these limitations, the *in vivo* and *in vitro* assays appear to be roughly equivalent in efficacy and are relatively easy to use [14]

Skin testing and *in vitro* tests should not be considered as diagnostic tests for clinical sensitivity to a given allergen. They are merely assays for specific IgE. Regardless of the method, the physician's experience and clinical judgment remain essential for assessing the significance and relevance of positive and negative test results. Clinically, *in vitro* assays for specific IgE are most useful in situations when skin testing is unreliable or impracticable, or when skin tests are negative in a patient with a positive history [15]. This order of testing is appropriate since no other assay has yet been developed that provides the sensitivity and specificity of a properly performed skin test.

For diagnosis of food hypersensitivity, the double-blind, placebo-controlled food challenge (DBPCFC) has been the "gold standard" since its introduction in 1976 by May [16]. DBPCFC is time-consuming and not without risk. To reduce the need for DBPCFC, Sampson [17] showed the utility of food-specific IgE concentrations for milk, egg, peanut, fish, soybean, and wheat using the Pharmacia CAP assay. Roehr et al [18] reported a sensitivity of 78% for SPT and 84% for specific IgE in subjects with milk allergies and a sensitivity of 89% for SPT, 96% for specific IgE, and specificity of 57% for SPT, 36% for specific IgE in children with egg allergies. In the present study, since most food allergy-suspected patients were children <4 yr old, we compared the VIDAS Stallertest and the Pharmacia CAP assays for food allergens without the performance of SPT or DBPCFC. Our findings showed that the VIDAS Stallertest results correlated well with the specific IgE levels obtained by the Pharmacia CAP assay.

In summary, we conclude that the VIDAS Stallertest assay gives results that are virtually

equivalent to those of the Pharmacia CAP assay for the evaluation of respiratory and food allergies. These results suggest that both tests are generally feasible and replicable for measuring allergen-specific IgE in allergic children.

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