
A blinded, multi-center evaluation of two commercial in vitro tests for latex-specific IgE antibodies

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Purpose: To compare the diagnostic value of two commercial in vitro tests for the detection of latex-specific IgE antibodies.

Methods: Serum samples were collected from latex-allergic and nonlatex-allergic individuals. Persons were classified as latex allergic if they had a positive clinical history and a positive skin prick test with a latex extract. Persons with no latex-related symptoms and negative skin tests were classified as nonlatex allergic. The serum samples were tested in a blinded fashion by a laboratory using the CAP (Pharmacia-Upjohn) and AlaSTAT (Diagnostic Products Company) assays. Values of 0.35 k_A U/L or greater were considered positive in both tests.

Results: The 143 sera studied came from 83-latex allergic and 60 nonallergic persons. The in vitro tests were found to have sensitivities of 79.5% and 73.8%, and specificities of 90.2% and 91.7%, for CAP and AlaSTAT, respectively. The positive predictive values were 91.7% and 92.5%, while the negative predictive values were 76.4% and 71.4% for the CAP and AlaSTAT, respectively.

Conclusion: In individuals classified by the combination of clinical history and skin test results, both the Pharmacia CAP and the DPC AlaSTAT demonstrated acceptable sensitivities, specificities, and predictive values for detection of anti-latex IgE antibodies. These findings suggest that both assays can be useful adjuncts to the diagnosis of latex allergy.

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INTRODUCTION

Latex allergy has become a widely recognized form of allergic disease within the past 15 years. While latex allergy has been most commonly recognized in certain high risk groups, studies have suggested that sensitization to latex may also be found in large, relatively unselected populations in the United States.^{1–6}

The appropriate and accurate diagnosis of latex allergy is critical because

of the potential consequences of the diagnosis or of a missed diagnosis. An issue complicating studies of in vitro tests is the distinction between allergic sensitization, defined as the presence of allergen-specific IgE antibodies, and allergic disease caused by exposure to the allergen. Many studies have shown that sensitized individuals may be asymptomatic.^{7,8} The presence of asymptomatic, sensitized individuals in a population results in falsely low estimates of test specificity.^{7,8}

At the present time only in vitro tests for latex-specific IgE antibody have been cleared by the US Food and Drug Administration for use in the United States. With this limitation, it is important to understand the relationship between the results of in vitro tests and a clinical diagnosis of latex allergy. The purpose of this study was to evaluate two FDA cleared tests for latex-specific IgE antibodies, per-

formed according to the manufacturers' directions, in a group of individuals clinically determined by history and skin testing to either be latex allergic or non-allergic.

MATERIALS AND METHODS

Patients

The sera used in this study had been collected previously by the investigators for other purposes, thus, this study was a retrospective examination of the sera. To be included in the latex-allergic population, a person was to have had a history of two or more symptoms typical of allergic reactions (eg, sneezing and nasal itching, ocular tearing and itching, urticaria, wheezing, or local edema) on at least three occasions following exposure to latex products and have had a positive skin prick test to an extract of natural rubber latex. A positive skin test was defined as a wheal of at least 3 mm in greatest diameter and a sum of perpendicular diameters of at least 5 mm greater than the negative control with a flare larger than the wheal at an extract concentration up to the full strength of the extract. For reasons of safety, skin testing was often started with dilutions of up to 1:100,000 of the full strength latex extracts. The negative controls were saline, with or without glycerin, and positive controls were histamine 1 mg/mL. Persons classified as nonlatex allergic had no history of the symptoms when exposed to latex and negative skin tests to latex extract, although, these persons may have had other allergies.

For this study, the investigators used the skin test results from the latex extract they felt was most reliable. For one of the investigators, the extract

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was of nonammoniated latex while for the other the extract was from ammoniated latex. Using RAST inhibition with the FDA E8 latex extract as a standard (3,890 $\mu\text{g/mL}$, by ninhydrin assay), the nonammoniated extract had a concentration of 255 $\mu\text{g/mL}$ and the ammoniated extract a concentration of 167 $\mu\text{g/mL}$.

The sera selected for study were coded and shipped to a laboratory distant from the site of collection to be assayed. The laboratory was blinded as to the allergic classification of the patients until after completion of the study.

Three sites were initially selected to provide sera for analysis, however, auditing of one site demonstrated multiple violations of the study protocol and, therefore, none of the samples from this site were included in the analysis.

Assays

The two assays evaluated in this study were the Pharmacia ImmunoCAP System (Pharmacia & Upjohn, Diagnostics Division, Kalamazoo, MI) and the AlaSTAT Microplate Allergen-Specific IgE (Diagnostic Products Corporation, Los Angeles, CA). The source of allergen for both tests is nonammoniated Malaysian latex. Both tests were performed in duplicate within 24 hours of each other once a serum specimen was thawed in the laboratory. All tests were performed in accordance with the manufactures' directions and the conventional scoring systems were used with both assays. Per manufactures' guidelines for conventional scoring, samples with values equal to or greater than 0.35 kUA/L were considered positive in both assays.

If the concentration of antilatelx IgE permitted, samples testing positive in one in vitro system and negative in the other were evaluated in the positive system for latex-specific inhibition. To estimate latex inhibition, serum samples were incubated with either an equal volume of phosphate buffered saline containing 2% human serum albumin or the FDA-E8 latex standard extract at dilutions of 1/28, 1/280, and 1/2800 in the same buffer. After an overnight incubation at 4°C, the samples were assayed for residual antilatelx IgE in duplicate. Inhibition of 70% or more was considered significant if a dose-response pattern was evident.

Six serum pools ranging from negative to strongly positive for antilatelx IgE were run in triplicate in each assay run. All replicates of the negative pools were consistently negative in all assay runs. The coefficients of variation for the positive pools were calculated and averaged for all runs to produce an average coefficient of variation. The average coefficients of variation were 6.8% for the CAP and 5.5% for the AlaSTAT.

Data analysis

The results of the in vitro analyses were compared with the clinical classification using the Chi-square statistic. Sensitivity, specificity, positive and negative predictive values of the tests were calculated by standard methods using the combination of the clinical classification and the skin test results as the standard. The correlation between the two assays was calculated by least-squares regression.

RESULTS

Table 1 shows the characteristics of the 143 individuals evaluated for this study. They represented a broad age range and there were more than two females for every male. The majority were currently employed as health care workers, while most of the children included in the study had spina bifida.

Comparisons between the presence or absence of latex allergy, defined by the combination of history and skin testing to latex, and the results of the two in vitro tests are shown in Table 2. Both tests performed well when compared with the clinical diagnoses.

The values of commonly used diagnostic indices are shown in Table 3. When the entire population is considered, the CAP had a sensitivity of 79.5%, a specificity of 90.2%, and a positive predictive value of 91.7%; while the AlaSTAT had a sensitivity of 73.8%, a specificity of 91.7%, and a positive predictive value of 92.5%. The sensitivities and specificities of these two tests are not significantly different ($P < .50$). If the cutoff for positive results is increased to class 2 (0.70 kUA/L), the specificity of the CAP increases to 96.7% and the AlaSTAT to 95.0%.

Figure 1 shows the correlation between the two tests. Only samples with values of 0.35 kUA/L or greater are

Table 1. Description of the Study Populations

Variable	Site 1	Site 2	Total
Males/females	13/34	30/66	43/100
Mean age, yr (range)	36.5 (13–55)	24.2 (1–58)	28.3 (1–58)
Health care worker	46 (98%)	38 (40%)	84 (59%)
Spina bifida	1 (2%)	36 (38%)	37 (26%)
No risk factors*	0	21 (22%)	21 (15%)

* No risk factors = persons who are not health care workers, do not have spina bifida, do not wear latex gloves as part of an occupation, and do not have a history of more than two surgeries.

Table 2. Comparison of the Diagnosis Based on History and Skin Tests with the Result of the in Vitro Tests Using ≥ 0.35 kUA/L as Positive

		Latex Allergy Diagnosis	
		(History and Skin Test Results)	
		Positive	Negative
CAP			
Site 1	Positive	20	0
	Negative	6	22
Site 2	Positive	46	6
	Negative	11	32
AlaSTAT			
Site 1	Positive	18	1
	Negative	8	21
Site 2	Positive	44	4
	Negative	13	34

Table 3. Diagnostic Indices (as percentages) for CAP and AlaSTAT Assuming the Validity of the Combined History and Skin Test Results Using 0.35 kU_A/L Versus 0.70 kU_A/L as Criteria for Positive Tests

Test	Sensitivity	Specificity	Positive PV*	Negative PV*
Considering 0.35 kU _A /L as positive				
CAP	79.5†	90.0†	91.7	76.1
AlaSTAT	74.7†	91.7†	92.5	72.4
Considering 0.70 kU _A /L as positive				
CAP	69.9†	96.7†	96.7	69.9
AlaSTAT	68.7†	95.0†	95.0	68.7

* PV = predictive value.

† The sensitivities and specificities of the two tests are not significantly different using either 0.35 kU_A/L or 0.70 kU_A/L as criteria for positive tests, $P > .50$.

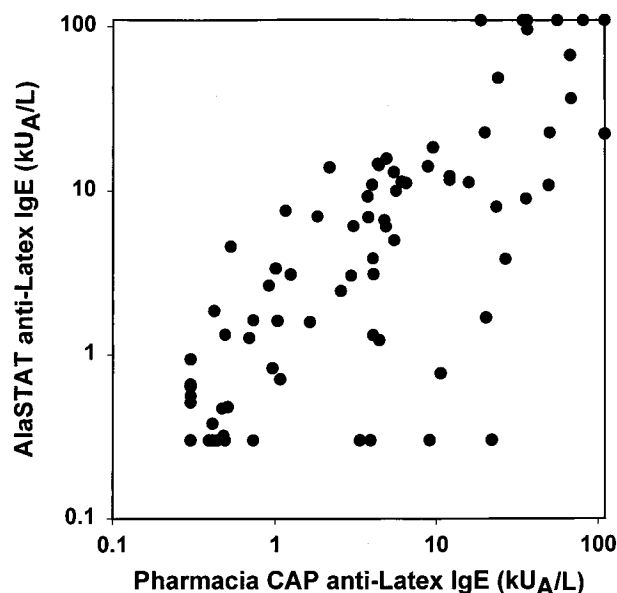


Figure 1. Correlation between antilatex IgE antibody concentrations measured by Pharmacia CAP and Diagnostic Products AlaSTAT in 143 sera ($r = 0.73$, $P < .001$).

plotted since there is no meaning to correlating results for samples with no detectable specific IgE. The regression coefficient is 0.73 ($P < .001$).

Discrepant results between the two assays were also examined. There were 15 sera that were positive in one assay and negative in the other: ten in which the CAP was positive and the AlaSTAT negative and five in which the reverse was true. In the 10 cases where the CAP was positive and AlaSTAT negative, the patient had been classified as allergic in six and nonallergic in four cases. In the five sera positive in the AlaSTAT and negative in the CAP, two were classified

as allergic and three had been classified as nonallergic. Five of the sera producing discrepant results (all were positive in the CAP and negative in the AlaSTAT) had latex-specific IgE levels high enough to allow dose-response inhibitions and all five of these sera could be inhibited. The low levels of specific IgE in the other sera did not produce clear inhibition results.

DISCUSSION

This study is consistent with most other studies that have evaluated newer technologies for the in vitro measurement of allergen-specific IgE antibodies. The diagnostic indices show that

both the Pharmacia CAP and the DPC AlaSTAT performed well compared with a clinical diagnosis of latex allergy, based on history and skin test results, in this diverse group of individuals. Other studies have suggested that when studied in this manner, these in vitro tests should have sensitivities and specificities of 80% to 90% for latex and other allergens; our results are consistent with those expectations.⁷⁻¹⁰

In an effort to produce as clear an evaluation as possible, the individuals included in this study were classified as either latex allergic or nonallergic based on both positive histories and skin tests or negative histories and skin tests. In contrast to most other studies, individuals in this study with negative histories were also required to have negative skin tests to reduce the possibility of inapparent sensitization. Those with ambiguous histories were excluded whether they had positive or negative skin tests. Excluding individuals with ambiguous histories from the study population produces a population differing from those encountered in clinical practice but allows a clearer comparison of the performance of the two in vitro tests.

In the absence of a widely accepted gold standard for diagnosis of latex allergy, our calculations of test sensitivity and specificity are subject to potential errors because we do not know how many persons were misclassified as allergic or nonallergic. As in other areas of allergy, a diagnosis of latex allergy depends upon a compatible history and demonstration that the persons symptoms result from latex-specific IgE antibodies. Even though we tried to use clear criteria for judging a person's history, the decision as to whether the history is positive or negative is still subjective and may have been influenced by many other factors. Since many of the persons investigated were health care workers, it is possible that histories were enhanced or embellished for a variety of reasons. Similarly, skin tests were performed using latex extracts that had not been extensively characterized.¹¹ Even though

skin tests are typically accepted as evidence of specific IgE antibodies, skin tests may be falsely negative or positive for a variety of reasons. Errors in interpreting either the history or skin test would result in misclassification and hence in errors in assessing the diagnostic value of the in vitro tests. Furthermore, some individuals may have positive in vitro tests and negative histories. These positive tests may indicate preclinical sensitization. Accurate prediction of preclinical sensitization would be valuable knowledge, especially if a person was occupationally exposed to latex, because it might allow institution of preventive measures and prevention of overt disease.

The sensitivities and specificities of the two tests were consistent between samples from two different investigators in two different areas of the country. Unfortunately, the number of samples was not large enough to allow meaningful analyses of subpopulations, such as persons with spina bifida.¹² While both tests produce results reported in actual mass units of IgE per serum volume, the correlation between the results of the two tests is not as high as would be expected if they measuring the same populations of antibodies.⁸ There are many factors that may influence the degree of correlation beyond the differences in the technologies employed by the two tests. The two manufactures are using different sources of latex allergen, different methods for attaching the allergens to the solid phases, different methods and antibodies for detecting IgE, and different standard curves. All of these differences are likely to contribute to discrepancies in the quantity of IgE detected in the assays.⁸

Our results for the in vitro tests vary from those recently reported by Kim et al.¹³ These differences may be due to differences in the criteria for positive skin tests, in the composition of the skin test reagents, and by our exclusion of persons with ambiguous histories

which Kim et al appeared to include in the latex-allergic group. Kim et al stated that they used three batches of "unmodified raw ammoniated latex" among their skin test reagents. Raw ammoniated latex has rarely been used by others because of the nonspecific irritating properties of the ammonia. Furthermore, Kim et al stated that they used highly concentrated extracts increasing the risk that some of the "positive" skin test responses reported were none specific and thus false positives.¹¹ This factor coupled with the number of tests performed and the criteria for positive tests may have resulted in an overestimation of patients classified as latex allergic. Overestimating the number of persons with latex allergy results in underestimations of the true sensitivity and specificity of diagnostic tests. Differences in laboratory performance of the tests is not likely to have grossly affected the results reported by Kim et al, since the same laboratory methods used by Kim and co-workers were also used in this study.¹³ We have found that the Pharmacia CAP System and the Diagnostic Products Company AlaSTAT perform well for the detection of latex-specific IgE antibodies. As with all tests for specific IgE antibodies, the results of these tests must be interpreted by a knowledgeable physician in light of the patient's history and physical findings.

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