
Latex allergen IgE assays in the assessment of Veterans Affairs health care workers

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Background: A previous multicenter study of Veterans Affairs health care workers evaluated hospital participants for latex hypersensitivity. Well-defined groups from that study allowed us to explore the diagnostic utility of newer antil latex allergen IgE immunoassays in the present study.

Objectives: To determine whether an enhanced CAP (ENHCAP) assay or an enzyme-linked immunosorbent assay (ELISA) identifies latex glove symptomatic individuals with antil latex allergen IgE that had not been detected by the CAP assay used in the original study and to determine the specificity of the ENHCAP assay.

Methods: The ELISA measured IgE antibody to Malaysian nonammoniated natural rubber latex extract (MNA), Hev b1, Hev b5, and Hev b6. Four patient groups were tested: confirmed latex glove allergic, latex glove symptomatic, latex glove sensitized/asymptomatic, and latex glove nonallergic.

Results: The ENHCAP assay and the MNA ELISA were highly concordant with the original CAP assay. In the subgroup with latex glove symptoms that were previously negative by the CAP assay, the ENHCAP assay value was elevated in 7 (11%) of 64 samples, only 3 of which were class 2 or higher. The MNA ELISA result was positive in only 4 (6%) of these 64 samples, and 3 of these were fractionally above the cutoff value for this assay.

Conclusions: The ENHCAP assay and the MNA ELISA identified a few additional positive individuals in the group that was latex glove symptomatic and originally CAP assay negative. The ENHCAP assay and the MNA ELISA produced only a modest improvement in diagnostic sensitivity over that of the original CAP assay.

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INTRODUCTION

This study is an outgrowth of a multicenter study of Veterans Affairs (VA) health care workers.¹ The multicenter study evaluated personnel from across the hospital workforce for latex hypersensitivity. The VA health care personnel were evaluated for the presence of latex glove allergy and risk factors by interview, questionnaire, serum antil latex IgE antibody determination, and skin testing to common aeroallergens. Of 1,959 participants, 158 (8.1%) had latex glove-associated allergic symptoms, a positive latex CAP assay result, or both (Table 1). A total of 133 participants (6.8%) reported latex glove allergic symptoms, and 36 (1.8%) were CAP assay positive. Of the 133 individuals with latex glove allergic symptoms, only 11 were CAP assay positive, giving a prevalence of confirmed latex glove allergy of 0.6% (11/1,959).

In the present study, we compare 3 immunoassays for antil latex allergen IgE levels: the original UNICAP 100 assay (CAP), the enhanced CAP (ENHCAP) (both from Pharmacia/Upjohn, Kalamazoo, MI), and enzyme-linked immunosor-

bent assay (ELISA). These immunoassays were applied to 4 groups from the original population of 1,959 participants: (1) confirmed latex glove allergic, (2) latex glove symptomatic, (3) latex glove sensitized/asymptomatic, and (4) latex glove nonallergic (Table 1). The primary goal of the present study was to determine whether the ENHCAP assay or the ELISA had enhanced sensitivity, ie, the ability to identify latex glove symptomatic individuals with antil latex allergen IgE that had not been detected by the original CAP assay. An additional goal was to determine the specificity of the ENHCAP assay.

METHODS

Table 1 delineates the study groups and the number of serum samples selected for analysis. All available serum samples from participants with latex allergy symptoms and all available serum samples from those with no latex allergy symptoms who were originally CAP assay positive were assayed, as were samples from randomly selected individuals with no latex allergy symptoms who were CAP assay negative. The CAP and ENHCAP assays were performed according to the manufacturer's instructions. IgE levels were measured in kU_A/L units (U). A CAP class of 1 (0.35–0.70 U) or higher was considered positive. The ENHCAP was augmented with Hev b5 to improve its sensitivity.²

Nonammoniated latex was obtained from Malaysia. The latex was allowed to clot, and the serum was separated by centrifugation at 10,000g for 15 minutes. The supernatant, designated as Malaysian nonammoniated natural rubber latex

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Table 1. Outcomes and Serum Samples Reanalyzed in Participants by Group

Group	Latex symptoms	CAP assay	Serum samples, No.	Serum samples reanalyzed, No.
Confirmed latex glove allergy	Positive	Positive	11	9
Latex glove symptomatic	Positive	Negative	122	64
Latex glove sensitized/asymptomatic	Negative	Positive	25	17
Latex glove nonallergic	Negative	Negative	1,801	97

(MNA), was characterized as described previously and was used in ELISA for detecting specific IgE in serum samples.³⁻⁵ One hundred microliters of MNA (10 mg/mL) in phosphate-buffered saline (PBS) was used to coat the polystyrene microwell plates (Immulon 2; Fisher Scientific, Itasca, IL). The plates were incubated at room temperature for 2 hours, followed by incubation at 4°C overnight. The wells were washed with PBS–Tween 20 and were blocked with 0.5% bovine serum albumin in PBS–Tween 20. The wells were again washed, and 100 mL of a 1:20 dilution of human serum was added and incubated at room temperature for 3 hours. After washing the wells, 100 mL of biotinylated antihuman IgE (1:500 dilution) was added and again incubated. After washing the wells, 100 µL of a 1:1,000 dilution of streptavidin peroxidase (Sigma-Aldrich Corp, St Louis, MO) was added and incubated for 1 hour. Finally, the wells were washed, and peroxidase reactivity was detected by the addition of the substrate orthophenylene diamine.³ The color was developed for 15 minutes in a dark chamber, and the reaction was stopped by the addition of 25 mL of 2N sulfuric acid. The optical density was read in an automated ELISA plate reader using a 490-nm filter (Molecular Devices, Sunnyvale, CA). The ELISAs using recombinant Hev b1, Hev b5, and Hev b6 were similarly performed, and the optical density values of the different groups were compared.

The study data set was analyzed using a statistical program (SPSS 11.5 for Windows; SPSS Inc, Chicago, IL).

RESULTS

Subgroup Analysis

Initially, 187 samples were screened using the ENHCAP assay. These samples had previously been assayed using the

MNA ELISA and the CAP assay. In the confirmed latex glove allergy subgroup, the ENHCAP and the MNA ELISA were concordant with the previous CAP assay, identifying all of the individuals with confirmed latex allergy. All of these were higher than class 1 by the ENHCAP assay. In the subgroup with latex glove symptoms who were previously negative using the CAP assay, the ENHCAP assay result was elevated in 7 (11%) of 64 samples. Only 3 of these 7 samples were higher than class 1. The MNA ELISA result was positive in only 4 (6%) of these 64 samples, and 3 of these 4 positives were only fractionally higher than the cutoff value of 0.1.

In the subgroup of latex glove-sensitized asymptomatic individuals who previously had positive CAP assay results, 88% (15/17) had positive ENHCAP assay results, with 13 of the 15 being higher than class 1. Seventy-six percent (13/17) of the participants were positive by the MNA ELISA. In the latex glove nonallergic subgroup, the ENHCAP assay result was positive in 22 (23%) of 97 samples, with 9 of the 22 being class 1. The MNA ELISA results were positive in 19 (20%) of 97 samples in this subgroup, with 9 of these only fractionally greater than the cutoff value of 0.1. In a further analysis of this latex glove nonallergic subgroup, a random sample of 39 (2%) of 1,801 samples was reanalyzed using the ENHCAP assay, and all 39 results were negative.

Assay Concordance

There were significant associations among the assays (Table 2). The CAP assay, ENHCAP assay, and MNA ELISA were all highly concordant using Pearson correlation analysis. The ENHCAP assay identified more positive samples ($n = 53$) than the CAP assay ($n = 36$). An analysis of the relationship

Table 2. Assay Concordance Using Pearson Correlation Analysis

	ENHCAP	CAP	ELISA			
			MNA	Hev b1	Hev b5	Hev b6
ENHCAP	1	0.806*	0.845*	0.719*	0.367*	0.556*
CAP	0.806*	1	0.912*	0.693*	0.259*	0.709*
ELISA						
MNA	0.845*	0.912*	1	0.598*	0.204*	0.751*
Hev b1	0.719*	0.693*	0.598*	1	0.043	0.277*
Hev b5	0.367*	0.259*	0.204*	0.043	1	0.314*
Hev b6	0.556*	0.709*	0.751*	0.277*	0.314*	1

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ENHCAP, enhanced CAP; MNA, Malaysian nonammoniated natural rubber latex.

* Correlation is significant at $P = .01$ (2-tailed).

between the ENHCAP and CAP assays revealed that most of these additional positives (10 of 17) were class 1 using the ENHCAP assay (Table 3).

Receiver operating characteristic (ROC) curves were used to compare the ENHCAP assay and the MNA ELISA with the CAP assay (Table 4). The CAP assay, ENHCAP assay, and MNA ELISA were highly concordant, with areas under the ROC curve of 0.95 and higher. Setting the optimal cutoff value for a positive ENHCAP assay result at 0.49 kUA/L (97% concordance with CAP-positive samples and only 6% discordant on CAP-negative samples) would have eliminated 8 of 10 class 1 ENHCAP assay values that were classified by CAP as negative.

The Hev b1 and Hev b6 ELISA results were moderately concordant with the CAP assay, ENHCAP assay, and MNA ELISA results by correlation analysis, whereas the Hev b5 ELISA demonstrated low correlations with the other assays (Table 2). The Hev b5 ELISA did not identify any additional latex IgE-positive individuals than the CAP assay, ENHCAP assay, and MNA ELISA and had low sensitivity for identifying positive samples compared with the other assays.

DISCUSSION

In the previous survey,¹ we identified a few VA health care workers who had confirmed latex glove allergy using the CAP assay (Table 1). In the present study, the ENHCAP assay and MNA ELISA results were positive in these individuals, confirming the previous CAP assay results.

In the important subgroup of participants with latex glove allergic symptoms previously negative by the CAP assay, the MNA ELISA and the ENHCAP assay identified a few additional participants, 4 (6%) of 64 and 7 (11%) of 64, who had IgE antibody to latex allergens. Only 3 of the 7 samples positive by the ENHCAP assay were higher than CAP class 1, and 4 of the 7 were borderline positive for this assay. Therefore, these additional assays did not add significantly to the sensitivity of the original CAP assay. Other investigators have reported only a modest improvement in diagnostic sensitivity using the ENHCAP assay, with essentially unchanged diagnostic specificity.⁶

In the subgroup of individuals who previously had positive CAP assay results without latex glove allergic symptoms, these additional assays were highly concordant with the previous CAP assay results and confirmed that these participants

Table 4. ROC Comparisons of the CAP Assay, the ENHCAP Assay, and the MNA ELISA

CAP as comparison standard	Area under the ROC curve (95% confidence interval)	P value
ENHCAP	0.949(0.902–0.996)	<.001
MNA ELISA	0.955(0.927–0.983)	<.001

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ENH-CAP, enhanced CAP; MNA, Malaysian nonammoniated natural rubber latex; ROC, receiver operating characteristic.

were latex allergen sensitized but asymptomatic. The ENH-CAP assay and the MNA ELISA were highly concordant with the previous CAP assay results, with correlation values that were highly significant ($P < .01$) (Table 2). Where the ENHCAP assay levels were higher than the CAP assay levels (18 samples), 10 of these were class 1, ie, positive by the ENHCAP assay at low levels.

The ROC curve analysis and modeling revealed that raising the cutoff value for the ENHCAP assay from 0.35 U to just 0.47 U eliminated 80% of the ENHCAP assay class 1 positive results that had been identified as negative by the CAP assay. Others have noted intra-assay differences near the cutoff values for these assays.⁷ In addition, the ROC curve analysis revealed how highly concordant the CAP assay, ENHCAP assay, and MNA ELISA were, with areas under the ROC curve of 0.95 and higher (Table 4).

In the initial analysis of 187 samples, the ENHCAP assay identified 23% (22/97) of the samples from the latex glove nonallergic subgroup as positive, 9 of these being positive at low levels (class 1). Nineteen of these 97 samples had been positive by MNA ELISA and were, therefore, a highly selected group from the total subset of 1,801 individuals. A random selection of 2% of the samples from the total subset of 1,801 was reanalyzed using the ENHCAP assay, with none of these identified as positive by the ENHCAP assay.

In summary, the ENHCAP assay and the MNA ELISA identified a few additional positive individuals in the group that was latex glove symptomatic and originally CAP assay negative. Therefore, not many participants were missed by the original CAP assay, and they represent a group that reports latex glove allergic symptoms in the absence of identifiable antilatel allergen IgE.¹ The ENHCAP assay and the MNA ELISA produced only a modest improvement in diagnostic sensitivity over that of the original CAP assay.

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Table 3. CAP and Enhanced CAP Results by Class*

CAP class	Enhanced CAP class					Total
	0	1	2	3	4	
0	133	10	7	1	0	151
1	1	5	10	3	0	19
2	0	0	8	2	1	11
3	0	0	0	4	2	6
Total	134	15	25	10	3	187

* Data are given as numbers.

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