

Comparison of skin-prick test and specific serum IgE determination for the diagnosis of latex allergy

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Summary

Background Latex IgE-mediated hypersensitivity has been recognized as an international health problem. However, there is poor information on the efficiency of the diagnostic methods available.

Objective The purpose of this study was to specify the efficiency of several diagnostic methods for latex allergy.

Methods We designed a prospective study involving 50 adult patients with latex allergy, as diagnosed by a suggestive clinical history and a positive skin-prick test (SPT) to a latex extract. One control group of 50 subjects paired for age, sex, total IgE levels and latex exposure, and another control group of 30 subjects with pollen allergy were used. A low-ammoniated natural-latex and several glove-latex extracts were elaborated. SPTs with these extracts, as well as with four different commercial-latex extracts were performed. Latex-specific serum IgE was determined by the CAP and the AlaSTAT methods.

Results Diagnostic sensitivity was 98% for the natural-latex extract SPT, from 90% to 98% for the commercial-latex extract SPT, and from 64% to 96% for the glove-latex extract SPT. Diagnostic specificity of SPT was 100%, and no severe adverse reactions were observed during skin testing. With respect to the latex-specific serum IgE determinations, sensitivity was 86% for the CAP system and 84% for the AlaSTAT assay, and specificity was dependent on the population considered.

Conclusion SPT with natural latex extracts has shown a diagnostic efficiency close to 100%, significantly higher than that of latex-specific serum IgE determination.

Keywords: latex, diagnosis, skin-prick test, specific IgE

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Introduction

In the last few years, latex IgE-mediated allergy has been recognized as a very important medical problem for an increasing number of patients [1–3], due to the apparent increase in frequency [4], the potential severity of the latex-induced reactions [5], and its presentation as an occupational disease in workers using gloves [6–8]. The prevalence of latex allergy in the general population is unknown, but preliminary data suggest that it may be higher than one

might expect [9]. It has also been demonstrated that latex antigens act as aeroallergens, capable of inducing rhinoconjunctivitis and bronchial asthma through inhalation, often being carried on cornstarch powder [10–12]. Moreover, a 'latex–fruit syndrome' has been recently proposed, because as much as 50% of latex allergic patients are at risk of developing anaphylactic reactions to certain fruits, mainly avocado, banana, chestnut and kiwi [13,14]. The existence of this syndrome has been confirmed by other authors [15–17], making the diagnosis of latex hypersensitivity even more important.

The diagnosis of latex allergy is based on clinical history, and supported by laboratory tests [2,3]. Skin tests have been

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performed with latex extracts from different sources, generally showing a good sensitivity and specificity [18–20]. However, skin testing with latex extracts has been associated with anaphylactic reactions [21,22], and the lack of a standardized latex extract for skin testing has been the major disadvantage of this diagnostic method [3]. Recently several commercial latex allergens have become available in Europe, although there is only limited data about their diagnostic efficiency [1].

In the past, *in vitro* diagnosis of latex allergy has been performed with commercially available latex RAST disks (Pharmacia Diagnostics), showing a lower sensitivity than skin tests, detecting only about 50–70% of the latex skin-test-positive latex-allergic patients [18,19,23]. Moreover, the sensitivity and specificity of this method is highly dependent on the population being studied [1]. More recently, the commercial latex CAP (Pharmacia Diagnostics, Sweden) and AlaSTAT (Diagnostics Products Corporation, USA), may have increased the sensitivity of *in vitro* testing for latex allergy [1,3]. These new diagnostic tests for latex allergy require careful evaluation. We have prospectively studied several methods available for the diagnosis of latex allergy.

Methods

Patients and controls

To determine the diagnostic efficiency of latex SPT and specific serum IgE determination, a prospective study was designed in the outpatient service of our Allergy Section. It was approved by our hospital Institutional Review Board, and was described in detail to all participants before obtaining informed consent and admission into the study. It consisted of SPTs with latex extracts and serum extraction for *in vitro* tests. Inclusion criteria were a clinical history of immediate reactions to latex, suggestive of IgE-mediated hypersensitivity, and a positive SPT to a fresh latex extract, obtained by washing the inside of a Triflex-examination latex-glove (Baxter Healthcare Co, Valencia, CA, USA) during 10 min with 10 mL of saline soln., prepared just before performing the test. Fifty adult patients were included in our protocol. They were 47 females and three males with a mean age ($\pm$ SD) of 32.7 ± 8.6 years. None of them had spina bifida or urogenital abnormalities. Twenty-three (46%) of them were atopic, as defined by a clinical history of respiratory allergy and positive skin tests to common aeroallergens. Thirty-two (64%) of our patients were exposed to latex in an occupational setting. The diagnosis was confirmed by a positive glove use test in 27 subjects, and by a positive specific bronchial challenge test in another six patients [3]. The glove use test was contraindicated in 10 cases, due to the severity of the latex-

induced symptoms, and seven patients denied consent to undergo a specific challenge test. Our patients' average total IgE (as determined by Pharmacia CAP system IgE FEIA) was 414 kU/L (a range of 29–1.662), and it was < 100 kU/L in 10 patients. Fifty volunteers from our outpatient clinic, paired for age, sex, total IgE levels, atopy and latex exposure, were used as a control group. They were 27 non-atopic, 20 dust-mite allergic and three mugwort-pollen allergic controls. An additional control group of 30 subjects with a clinical history of pollinosis and positive SPT to a commercial *Artemisia vulgaris* extract (ALK-Abello S.A., Madrid, Spain) was also included. The same *in vivo* and *in vitro* tests were carried out in patients and controls.

Natural latex extract

A low-ammonia natural-latex (kindly supplied by Guzman Cauchos S.A., Valencia, Spain) extract (Lx-Natu) was prepared by diluting it at 10% w/v ratio in phosphate buffered saline (PBS) at pH 7.4, centrifuging at 10 000 *g* for 30 min, filtering through a 22 μ m Millipore filter (Millipore Co, Bedford, MA, USA), storing at -70°C and freeze-drying. Protein content was assayed according to the method of Bradford [24], providing a 1.2% value. Extract for SPT was prepared by diluting it in PBS at 20 mg/mL w/v, adding glycerine (50% v/v) and storing at 4°C until used.

Commercial latex extracts

Four commercial natural-latex extracts for SPT were used, kindly supplied by their respective manufacturers: ALK-Abello S.A., Madrid, Spain (2 mg/mL w/v) (Lx-Com1); Ifidesa-Aristegui S.A., Bilbao, Spain (10 mg/mL w/v) (Lx-Com2); CBF-Leti S.A., Barcelona, Spain (1 mg/mL w/v) (Lx-Com3); and Stallergenes, Fresnes, France (100 ST-IR units) (Lx-Com4).

Glove latex extracts

Latex-glove allergen-test solutions were made by cutting gloves into small pieces, extracting by magnetizing stirring at 10% w/v in PBS at pH 7.4 during 30 min, filtering the supernatant through a 22 μ m Millipore filter (Millipore Co, Bedford, MA, USA), storing at -70°C and freeze-drying. Extracts for SPT were prepared by diluting them in PBS at 20 mg/mL w/v, adding glycerine (50% v/v) and storing at 4°C until used. Triflex examination gloves (Baxter Healthcare Co, Valencia, CA, USA) (Lx-Glo1), Peha-soft examination gloves (Unitex-Hartmann S.A., Mataró, Spain) (Lx-Glo2), Triflex surgical gloves (Baxter Healthcare Co, Valencia, CA, USA) (Lx-Glo3) and Prolax surgical gloves (Productos del latex S.A., Spain) (Lx-Glo4)

were processed as described. Protein content, as measured by the method of Bradford [24], was 0.1% for Lx-Glo1, 0.08% for Lx-Glo2, 0.03% for Lx-Glo3, and 0.12% for Lx-Glo4.

Skin tests

SPTs with natural, commercial and glove latex extracts were performed according to standard procedure [25], in the volar side of the forearm, by puncture with a Morrow-Brown needle (Bayer S.A., Madrid, Spain), using one sterile device for each test. Histamine phosphate at 10 mg/mL w/v was used as a positive control, and saline solution as negative control. The results were expressed as the mean weal diameter in mm 15 min after puncture. A weal diameter greater than 3 mm, compared with the saline control, was considered a positive response.

Specific serum IgE

Levels of latex-specific IgE were assessed both by the Pharmacia CAP System RAST FEIA (Pharmacia Diagnostics, Uppsala, Sweden), and by the AlaSTAT System (Diagnostics Products Corporation, USA), following the manufacturers' instructions. Samples were processed in duplicate, using the standard curve of the respective manufacturer as the reference for their interpretation, classifying results in classes from 0 to 6, and levels higher than 0.35 kU/L (class 1 and up) were considered a positive result.

Pharmacia CAP system inhibition

CAP inhibition was carried out in a pool of sera from our latex allergic patients showing levels of specific serum IgE to latex higher than 3.50 kU/L (CAP class 3 and up). Duplicate samples of 20 µL of undiluted sera were premixed with 30 µL of five progressive 1:10 latex extract dilutions, and incubated in the microplate at room temperature during 1 h. Latex commercial ImmunoCAPs (k82) were then added and specific IgE levels assessed as indicated in the CAP system manual. PBS pH 7.4 containing 0.03% w/v albumin was used for dilutions and also as a control for maximum uptake (0% inhibition level). The 100% inhibition level was defined as that obtained with the most potent extract available. A *D. pteronyssinus* commercial extract (ALK-Abello S.A., Madrid, Spain) was used as a negative-inhibition control. Inhibition-dilution relationships were calculated by plotting the mean inhibition as a function of the log allergen dilution, and the assay was validated if the linear correlation coefficients were greater than 0.95.

Another pool of sera was prepared from our controls with positive latex-specific IgE, being preincubated with our natural latex extract and a *Artemisia vulgaris* commercial

extract (ALK-Abello S.A., Madrid, Spain) during 1 h, before measuring the latex-specific IgE antibodies through the CAP assay.

Statistics

Results are given as means $\pm$ SD, and comparison of the mean values was performed by using the paired and unpaired Student's *t*-test. For test analysis, the sensitivity (true positive test results/all patients), specificity (true negative/all controls) and overall accuracy or efficiency (true positive + true negative/all tests) were calculated. Comparison of the rates of positive test results was performed by means of Fisher's exact test. Statistical significance was accepted if $P < 0.05$. Linear regression analysis on log latex-specific IgE values was also performed.

Results

All patients and controls completed the study. Table 1 summarizes the results of the SPTs with the different latex extracts. SPT mean weal diameter was higher for the Lx-Com2 ($P < 0.05$) and lower for the Lx-Com4 ($P < 0.01$) than for the other commercial extracts. Among glove extracts, SPT mean weal diameter was higher for the Lx-Glo2 ($P < 0.01$) and lower for the Lx-Glo3 extract ($P < 0.05$). The diagnostic sensitivity of the SPTs with the five natural-latex extracts (including Lx-Natu and the four commercial extracts) ranged from 90% to 98%, and with the glove-latex extracts from 64% to 96%. None of the controls showed positive SPTs to the latex extracts, and therefore the diagnostic specificity of the SPTs was 100%. The diagnostic efficiency for the five natural-latex extracts ranged from 95% to 99%, with no significant differences among them, but it was lower for the glove-latex extracts, with the exception of the Lx-Glo2 extract, that was 98%. Only one mild reaction, consisting of rhinoconjunctivitis,

Table 1. Results of the SPT with the latex extracts

Extract	SPT (mm) mean $\pm$ SD	Sensitivity (%)	Specificity (%)	Efficiency (%)
Lx-Natu	9.0 $\pm$ 3.7	98	100	99
Lx-Com1	6.5 $\pm$ 2.5	90	100	95
Lx-Com2	9.4 $\pm$ 3.7	98	100	99
Lx-Com3	7.9 $\pm$ 4.7	94	100	97
Lx-Com4	4.5 $\pm$ 2.2	90	100	95
Lx-Glo1	5.0 $\pm$ 2.4	84	100	92
Lx-Glo2	6.8 $\pm$ 3.5	96	100	98
Lx-Glo3	3.3 $\pm$ 2.3	64	100	82
Lx-Glo4	5.2 $\pm$ 2.8	82	100	91

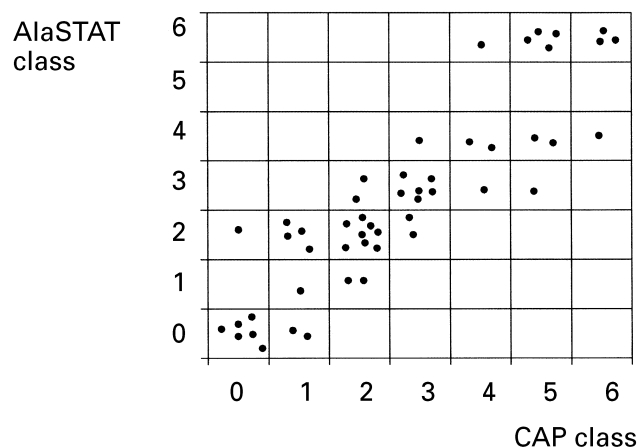


Fig. 1. Comparison of classes of latex-specific IgE antibodies as measured by CAP and AlaSTAT methods.

was observed in relation to skin testing with the latex extracts.

The diagnostic sensitivity of the measurement of latex-specific IgE was 86% for the CAP and 84% for the AlaSTAT. The agreement between the results of the CAP and the AlaSTAT was 94%: in two patients, a positive CAP combined with a negative AlaSTAT, and in one patient the reverse was observed. The comparison of classes of latex-specific IgE antibodies as measured by the CAP and AlaSTAT methods revealed that the class concordance was 52% (Fig. 1). In 13 patients, AlaSTAT result was at least 1 class higher than CAP, and in 11 cases the reverse was observed. A very good correlation coefficient ($r = 0.91$) was demonstrated between log CAP values and log AlaSTAT values. Only one false positive latex-specific IgE result occurred in the paired control group, in a mugwort-allergic control, and no false positive results were observed among nonatopic or dust-mite allergic controls. A new group of 30 mugwort-allergic controls was also tested for latex-specific IgE, finding a positive rate of 40% for CAP and 30% for AlaSTAT. Among these pollinic controls, positive latex-CAP results were class 1 in four sera, class 2 in six, and class 3 in two; meanwhile positive latex-AlaSTAT were class 1 in five sera and class 2 in another five cases. The agreement between latex CAP and AlaSTAT results was 80% in this control group: in four subjects, a positive CAP combined with a negative AlaSTAT, and in two subjects the opposite was observed. None of the pollinic controls with positive latex-specific IgE gave a clinical history suggestive of latex allergy, and all of them showed negative latex-SPT. Therefore, the specificity of the latex IgE determination was highly dependent on the controls considered, ranging from 100% in non-atopic or dust-mite allergic controls, to less than 70% in our pollinic controls. In any case, the diagnostic

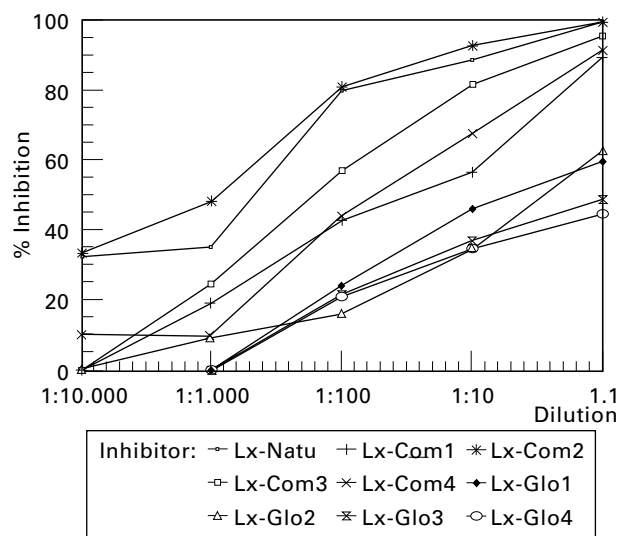


Fig. 2. CAP-inhibition curves using commercial latex ImmunoCAP in solid phase.

efficiency of the measurement of latex-specific IgE was $\approx 85\%$ for both *in vitro* methods tested, being significantly lower than that of the five natural-latex extracts ($P < 0.05$).

On incubating the pool of sera of our latex-allergic patients with the different latex extracts, a progressive CAP inhibition was obtained, reaching the maximum inhibitions with the different natural-latex extracts, showing parallel curves suggesting a similar antigenic composition (Fig. 2). No inhibition was obtained on incubating with the *D. pteronyssinus* extract. If you compare the allergen conc. necessary to induce a 50% inhibition, extrapolating the data from the graphic, Lx-Com2 was the most potent extract, followed by Lx-Natu, and glove-extracts were the least potent extracts. Using the pool of sera from our pollinic controls with positive latex-specific IgE, CAP to latex was completely inhibited by both the latex and the mugwort extracts, at conc. higher than 0.1 mg/mL w/v.

Discussion

Nowadays, there is no doubt about the great importance of latex IgE-mediated allergy [1–4]. However, there is no consensus on the tests that should be used for diagnosing latex hypersensitivity, and there is poor information on the diagnostic efficiency and safety of the methods currently being used. Therefore, the development of proper diagnostics methods for latex allergy is a very important challenge, and new tests should be evaluated with respect to sensitivity and specificity, in order to prevent inappropriate diagnosis and treatment. However, evaluation of diagnostic test results is not easy to perform, because a definite gold standard method for diagnosing latex allergy is not

available. We have studied the diagnostic efficiency of several tests by selecting patients with a suggestive clinical history and a positive SPT with a fresh latex extract, obtained from a very allergenic brand of gloves. In our experience, this simple SPT method has proved useful and with no systemic reactions, but it is time consuming and the allergenic potency of the extract is variable from day to day. To avoid this problems, conveniently processed glycerinated extracts conserve the allergenic potency for several months, making possible to compare *in vivo* or *in vitro* different extracts in order to achieve the desired allergenic potency. At this time, there is no FDA-approved latex extract for skin testing, but recently several commercial latex allergens have become available in Europe [1], and the *in vivo* and *in vitro* standardization of one natural rubber latex extract has been reported [26].

In the present study, we have analysed the diagnostic efficiency of some newly commercialized natural-latex extracts for SPT, together with a natural-latex extract and some glove-latex extracts prepared in our laboratory. We have clearly demonstrated that all the natural-latex extracts for SPT evaluated, showed excellent sensitivity and specificity, reaching diagnostic efficiencies higher than 95%, with no significative differences among them. Although our inclusion criteria, based on positive SPT, could influence the sensitivity of the diagnostic tests evaluated, the confirmation of latex allergy by a positive challenge test in the majority of our patients, reinforce the reliability of our results. Furthermore, our data are in agreement with the sensitivity and specificity reported for the natural rubber latex extract that has recently been standardized [26]. In spite of this, the commercial extracts differed significantly in their allergenic potency, both *in vivo* and *in vitro*, and for this reason an international standardization of latex extracts should be achieved. Glove extracts generally showed lower overall accuracies, probably due to lower protein concentration, being better for examination-glove extracts than for sterile-glove extracts. In consequence, SPT with a natural-latex extract should be considered the method of choice for diagnosing latex allergy, at least in adult patients without spina bifida. The possibility of antigen neoformation during latex processing could make it advisable to perform simultaneously a SPT with a examination-glove extract, but in our study it did not significantly increase the diagnostic efficiency. Although we have observed only one mild adverse reaction in relation to the SPTs, it is a matter of concern that skin testing with latex extracts has been associated with systemic anaphylactic reactions [21,22]. This fact makes it advisable to limit this diagnostic procedure to settings with appropriate experience and the means for treating systemic allergic reactions, until the safety of standardized latex extracts is completely demonstrated.

In our study, the two methods evaluated for detecting

latex-specific IgE showed sensitivities higher than 80%, with no significative differences between them. However, both of them are limited by an unexpected high rate of false positive results in pollinic patients, between 30% and 40%, resulting in low specificities. This fact could be explained on the basis of crossreacting allergens between latex and certain pollens, as we have demonstrated by CAP-inhibition between mugwort and latex. It should be emphasized the discrepancy between positive SPTs demonstrating a true cross-sensitization, as for example in the latex–fruit syndrome [13], and positive *in vitro* tests showing only a cross-reactivity, which with respect to latex and mugwort does not seem to have any clinical implications. As profilin has been identified as an IgE-binding component in latex, it could be responsible for this *in vitro* crossreactivity [27]. Moreover, the existence of crossreacting allergenic proteins among latex and certain pollens could also explain the unexpectedly high prevalence of latex sensitization, reported by some authors, who have screened populations by latex-specific IgE determination [28–30]. Probably most of these asymptomatic subjects showing positive IgE to latex represent in fact false positive results in pollinic patients, with no clinical implications concerning latex allergy. Nevertheless, it is obvious that CAP and AlaSTAT assays for measuring latex-specific IgE offer a better diagnostic sensitivity than RAST, and they can be very useful to complement latex diagnosis, specially in settings where SPT with a latex extract is not available. Previous studies comparing CAP and AlaSTAT systems for detecting specific IgE to aeroallergens (31) or food allergens (32), have showed good concordances between them, although with some variations depending on the allergen considered. In the same way, we have demonstrated a very good concordance between CAP and AlaSTAT latex-specific IgE results.

In conclusion, our study strongly suggests that the SPT with a natural-latex extract should be considered the method of choice for diagnosing latex allergy, although extracts should be appropriately standardized. Latex-specific IgE determination by the CAP and AlaSTAT methods has shown a good sensitivity, but specificity and therefore interpretation was dependent on the population considered.

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