

Evaluation of a Dipstick Test (Allergodip®-Latex) for in vitro Diagnosis of Natural Rubber Latex Allergy

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Key Words

Allergy, natural rubber latex · Skin prick test · IgE, specific · Dipstick test

Abstract

Background: IgE-mediated hypersensitivity to latex has been recognized as an increasing health problem with far-reaching consequences for patients, regarding both their occupational situation and safety in medical care. Therefore, a correct diagnosis of natural rubber latex (NRL) allergy is essential. The purpose of the study was to evaluate sensitivity and specificity of several established diagnostic methods for NRL allergy (in vitro assays and skin prick test) in relation to a new semiquantitative dipstick test (Allergodip®-Latex, Allergopharma) as a screening test for NRL allergy. **Methods:** Data obtained with quantitative assays including Pharmacia CAP System® (FEIA), DPC-AlaSTAT® and Magic Lite® were compared with the dipstick test results in latex-sensitized (n = 151) and nonsensitized persons (n = 232). In addition these in vitro findings were related to clinical symptoms after exposure to latex and skin prick test results with a panel of different latex allergen extracts. **Results:** When comparing sensitivity and specificity of all in vitro assays relative to skin prick test results the Pharmacia CAP System® (FEIA) had the highest sensitivity in the range of 90%. Sensitivity of the other in vitro assays was in the

range of 73.7–74.9%, specificity varied from 85.3 to 89.8%. A diagnostic standard was defined in terms of at least three corresponding test results out of all diagnostic methods (in vitro assays and skin prick test). The sensitivity and specificity of each diagnostic test were determined relative to this diagnostic standard. Hereby the Allergodip test results showed a sensitivity of 91% and a specificity of 93%. **Conclusion:** The dipstick test results are in line with the data of the other in vitro assays. In contrast to other in vitro assays the dipstick test requires no further laboratory equipment and is easy to perform.

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Introduction

During the past decade immediate-type allergy to natural rubber latex (NRL) proteins has emerged as a serious health problem. Frequent, prolonged wearing of NRL gloves, especially among healthcare workers, is a major risk factor for such a sensitization. In response to HIV and viral hepatitis pandemic, there was an exponential rise in the production of NRL gloves and their use amongst healthcare workers in the late 1980s. Many of the gloves were poorly manufactured with high levels of residual protein. The increasing prevalence of NRL allergy in the late 1980s amongst healthcare workers is supposed to be a

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Table 1. Clinical symptoms of the study subgroup (n = 215) following latex exposure

Clinical symptoms	Positive	Negative	No information/ambiguous
Urticaria (local, systemic)	88 (40.9)	120 (55.8)	7 (3.3)
Rhinoconjunctivitis	65 (30.2)	146 (67.9)	4 (1.9)
Asthma	36 (16.7)	175 (81.4)	4 (1.9)

Figures represent number with the percentage in parentheses.

consequence of the greater use as well as the poor manufacturing methods of NRL gloves [1–3]. Apart from healthcare workers, there are several other groups who are exposed to a certain element of risk of developing NRL allergy, namely rubber workers and people who undergo frequent surgical procedures, especially children with spina bifida and urogenital abnormalities [3]. The only common feature among those groups appears to be a high degree of exposure to natural rubber [4].

IgE-mediated allergy to NRL has a number of far-reaching consequences for the patients concerned, regarding both their occupational situation and safety in medical care. Therefore, a correct diagnosis of NRL allergy is essential. However, there is no consensus on the tests that should be used for diagnosing latex hypersensitivity, and there is poor information about the diagnostic efficiency and safety of the methods currently used. A complicating factor in establishing means for an accurate diagnosis has been the multitude of potentially significant NRL allergens discovered [5]. In Europe, for example, the diagnosis of NRL allergy is based on both a positive clinical history and positive skin prick test results with a panel of different latex allergen extracts. Various in vitro tests, demonstrating the presence of specific IgE antibodies to NRL proteins, may provide valuable additional support for the diagnosis, but are not sufficient alone due to a lack in sensitivity [6]. Data for sensitivity obtained from the literature vary from 50 to 98% [4] depending on the population being studied. Because of the risk for anaphylactic reactions due to skin prick testing, at first screening with in vitro techniques for the detection of IgE antibodies is recommended in the USA. In spite of this controversial discussion in vitro techniques for IgE antibodies are routinely used and generally accepted to identify patients at risk of severe allergic reactions.

Therefore, the development of proper diagnostic 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. The purpose of our study was to examine

the value of a semiquantitative dipstick assay (Allergodip®-Latex, Allergopharma) as a screening test for NRL allergy in a population of latex-exposed persons, namely healthcare workers. Recently in a pilot study the dipstick test was evaluated in patients with spina bifida in relation to the Pharmacia CAP System. Here, Niggemann and Wahn [5] reported a good concordance between these two in vitro assays. In our study the new dipstick assay is compared with three well-established commercial assays and skin prick test results in terms of sensitivity and specificity.

Material and Methods

Patients

The study population consisted of latex-allergic persons, mainly healthcare workers (n = 151) seen in our outpatient's clinic and a nonsensitized control population (n = 232). All subjects of the control group were healthcare workers and therefore belonging to one of the populations at greatest risk of developing latex allergy. The subjects of the control group had no symptoms after exposure to latex and skin prick tests with a panel of different latex allergens were negative. NRL allergy was diagnosed by positive skin test results and clinical symptoms after exposure to NRL. All subjects with NRL allergy suffered from contact urticaria, allergic rhinitis or conjunctivitis after exposure. A subgroup of the study population was defined by including all subjects who had been tested with all four in vitro assays and skin prick test (n = 215) as well. In this defined subgroup 111 persons were latex-sensitized and 104 were nonsensitized individuals. After exposure to latex urticaria (systemic or local) and rhinoconjunctivitis were the most commonly reported IgE-related symptoms, occurring in 88 (40.9%) and 65 (30.2%) of the 215 patients, respectively. Asthmatic symptoms were less common (16.7%, n = 36). Four patients were labelled as 'history ambiguous' due to pruritic sensation while wearing latex gloves (table 1). Atopy was common in this population, 101 patients (47%) reported a history of childhood eczema, allergic rhinitis or asthma. The study of the control group was approved by the local ethics committee and patients gave their written consent.

Test Methods

Allergodip-Latex. This new semiquantitative assay [5] includes ammoniated and nonammoniated latex allergen pads together with positive and negative controls on a plastic strip. For preparing the latex extract used on this strip latex milk from *Hevea brasiliensis*,

clone RRIM 600 (Rubber Research Institute of Malaysia) was collected in ice-chilled containers. The milk was centrifugated (1 h, 20,000 g) resulting in a separation described by Moir [7]. A combination of C serum and B serum proteins was used for preparing the extract. Antibodies specific for Hev b 1 provided by Baur and coworkers [8] were used, and the analysis of the latex extracts of the strip by RAST inhibition, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis was performed in their labs. The monoclonal antibody specific for Hev b 3 was delivered by RRIM. This antibody was prepared as described by Yeang et al. [9]. Relevant latex allergens could be detected (fig. 1). The strip is incubated for 3 h at room temperature in a tube containing 250 µl of serum or plasma. After 2 min washing under cold running tap water, the strip is incubated in a tube with a conjugate (250 µl of monoclonal alkaline phosphatase-conjugated anti-human IgE) for 18–20 h at room temperature. After washing again incubation is performed with the substrate (250 µl of 5-bromo-4-chloride-3-indolyl-phosphate-nitroblue tetrazolium) for 90 min at room temperature. After a short period of drying, the resulting color of the strip is compared with a color chart, allowing classification into five classes (0–4). Results are only accepted if the negative control pad is white to light blue, and the positive control pad is deep blue. The color reaction is stable for at least 6 years at room temperature, which allows storage for documentation.

Latex-Specific IgE. Blood was obtained by peripheral venipuncture from all subjects. Serum was separated and analyzed for latex-specific IgE by using the Pharmacia CAP System® (FEIA) (n = 380), the DCP-AlaSTAT® (n = 302) and the Magic Lite® (Chiron Diagnostics) (n = 273). The cutoff value for the Pharmacia CAP System® (FEIA) as well as for the DCP-AlaSTAT is <0.35 kU/l, the cutoff value for the Magic Lite (Chiron Diagnostics) is <0.5 kU/l.

Skin Prick Tests. Skin testing was performed in all patients. It was done with a panel of different latex extracts (Alk-Scherax® GmbH, Germany, Allergopharma®, Joachim Ganzer KG, Germany, Staller-gènes®, France, and self-prepared high-ammoniated NRL; as described by Brehler et al. [6]) by the punch technique on the forearm. Histamine phosphate was used as positive control and natrium chloride as negative control. Results were read 15 min after application. They were considered positive if any of the latex skin tests resulted in a wheal response 50% or greater in diameter than that of the histamine control and at least 3 mm larger than that of the negative saline control.

Gold Standard. A gold standard was defined in terms of at least three corresponding test results out of all diagnostic methods (in vitro assays and skin prick test).

Data Analysis

Sensitivity and specificity of each in vitro assay were determined in comparison to skin prick test results because in our collective skin prick test results correlated very well to clinical symptoms. All subjects with a positive skin test result suffered from contact urticaria, allergic rhinitis or conjunctivitis after exposure to NRL. On the basis of this coincidence a validation of each in vitro assay in comparison to skin prick test results was performed. Sensitivity was defined as the proportion of true-positive values detected, specificity as the proportion of true-negative values detected.

Spearman's rank correlation, a measure of the association between rank orders that does not require normality, was used for analysis of the relation between the diagnostic test and the severity of clinical symptoms following latex exposure.

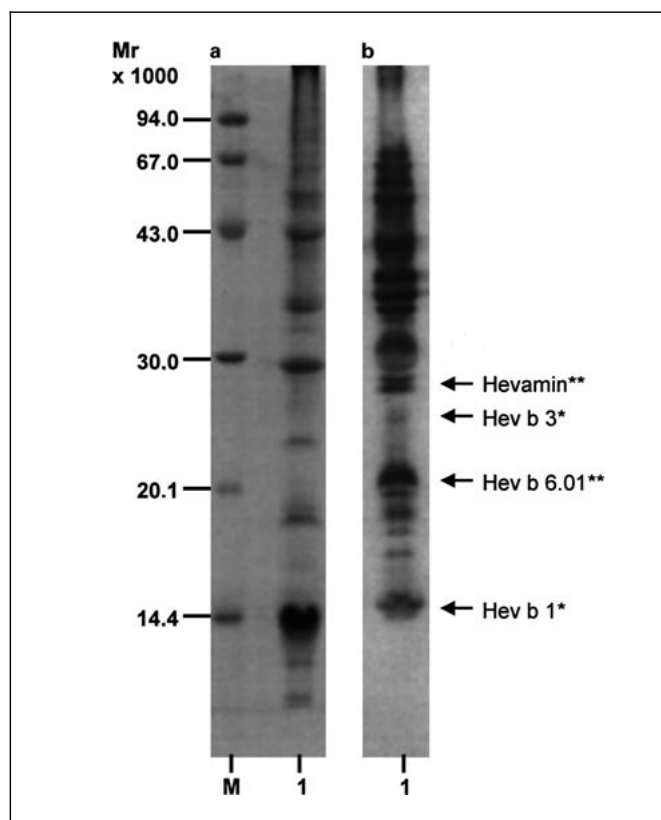


Fig. 1. Characterization of latex extract by SDS-PAGE and immunoblot. Latex extract (60 µg protein) was separated by SDS-PAGE. Separated proteins were either stained by Coomassie (a) or blotted onto PVDF membrane (b). Blotted proteins were incubated with a serum pool of sera of latex-allergic patients (a serum pool of 20 patients allergic to NRL with positive skin prick test was used). Bound IgE was detected using a monoclonal anti-human IgE antibody, alkaline phosphatase-labeled, and visualized by using the chemiluminescence substrate CSPD and x-ray films. M = Molecular weight marker proteins; 1 = latex extract. Allergens are identified by specific monoclonal antibodies (*) or by N-terminal amino acid sequencing after purification (**).

Results

Comparing sensitivity and specificity of all in vitro assays relative to skin prick test results the Pharmacia CAP System® (FEIA) had the highest sensitivity in the range of 90%; the specificity of 85.3% was in line with the other in vitro assays. Sensitivity of the DCP-AlaSTAT was 73.7%, specificity 87.7%. The Magic Lite system reached a sensitivity of 74.5% and a specificity of 89.8%, the Allergodip achieved a sensitivity of 73.9% and a specificity of 85.5%. Of the 380 individuals tested with the Pharmacia CAP System® (FEIA) there were 229 latex-allergic and 151 nonallergic persons. The DCP-AlaSTAT

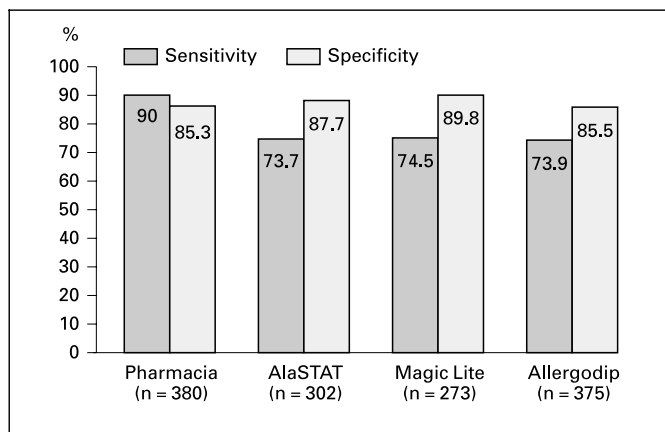


Fig. 2. Validation of in vitro assays in comparison to skin prick tests.

Table 2. Correlation of latex diagnostic test with the number of clinical symptoms (Spearman's correlation coefficient; n = 215)

Diagnostic test	Spearman's correlation coefficient (rho value) severity of clinical symptoms
Pharmacia CAP	0.622*
AlaSTAT	0.586*
Magic Lite	0.537*
Allergodip	0.509*
Skin prick test	0.684*

* p value (significance) <0.0001.

test was performed in 302 individuals, 133 were allergic to latex and 169 were nonallergic subjects. Data were obtained in 273 subjects analyzed with Chiron Diagnostics Magic Lite; here the study population consisted of 134 allergic and 139 nonallergic individuals. The Allergodip was performed in 375 individuals, 230 were latex-allergic subjects, 145 nonallergic individuals (fig. 2).

In order to analyze sensitivity and specificity for all in vitro assays we selected a subgroup including all subjects who had been tested with all four in vitro assays and skin prick test (n = 215). This subgroup of 215 subjects consists of 100 latex-allergic persons and 115 control persons. We defined as described above a diagnostic standard in terms of at least three corresponding test results out of all diagnostic methods (in vitro assays and skin prick test). The sensitivity and specificity of each diagnostic were determined relative to this diagnostic standard.

In the defined subgroup, the Allergodip-Latex dipstick test identifies correctly 91 of 100 (91%) latex-sensitized

persons and 107 out of 115 (93.0%) nonsensitized persons. These data are in line with the other in vitro assays.

The severity of symptoms after exposure to latex and the results of each in vitro assay and the skin prick test results were related. Most patients report contact urticaria as their first clinical symptom of NRL allergy. Especially healthcare workers appear to have a progression of symptoms from cutaneous reactions to rhinoconjunctivitis and to bronchospasm and anaphylaxis [4]. In our study population the initial symptom of NRL allergy was local urticaria followed by rhinoconjunctivitis and asthma. Therefore, severity was defined in terms of a patient suffering from a total number of three clinical symptoms (e.g. urticaria, rhinoconjunctivitis and asthma) after exposure to latex. Anaphylaxis as the severest systemic reaction following latex exposure was not present in our study population. A higher class or titer of each in vitro assay was strongly associated with the severity of clinical symptoms after exposure to latex (table 2).

Discussion

A strong clinical history of latex allergy with a positive skin prick test response to latex is sufficient to label an individual as having a latex allergy. However, the severity of latex allergy can vary among those affected. Therefore, a clinical history alone is inadequate in establishing the diagnosis of type I latex allergy. In a recent study by Hamilton and Adkinson [10], a careful clinical history incorrectly diagnosed latex allergy in 15% of the subjects. These patients had a very high clinical score indicative of latex allergy, but were completely negative on latex skin testing, serological tests, and glove provocation tests. Especially symptoms such as those of rhinoconjunctivitis and asthma are commonly a result of other environmental allergies and can be a source of diagnostic bias [11].

Skin prick testing performed with different extracts has been demonstrated to be quite sensitive and specific for establishing the diagnosis of latex allergy. In their large study of more than 900 healthcare workers, Moneret-Vautrin and Laxenaire [12] obtained a sensitivity of 100% and a specificity of 99%. Blanco et al. [13] compared different skin prick test extracts and specific serum IgE determination for the diagnosis of latex allergy. They reported the highest sensitivity for the natural latex extract skin prick test (98%), followed by a sensitivity ranging from 90 to 98% for a commercial latex extract skin prick test and a sensitivity from 64 to 96% for the latex glove extract skin prick test. Diagnostic specificity was 100%.

Unfortunately, anaphylaxis can occur during skin testing. Anaphylactic events associated with skin testing have occurred without regard to risk group or history of anaphylaxis [4]. In contrast to others, we did not observe any side effects while performing skin testing with different latex extracts in our study population (n = 380).

In order to predict the severity of latex allergy several studies were done to examine the value of in vitro assays or skin tests. In a retrospective study Hadjiliadis et al. [14] showed a strong correlation between the wheal diameter of commercially available latex extracts used for skin prick testing and the severity of latex-induced symptoms. Recently Kim and Safadi [15] investigated the relation between latex-specific IgE titer and symptoms in patients allergic to latex. According to their data higher classes of either AlaSTAT or CAP assay to latex were strongly associated with latex-related asthma and urticaria and marginally associated with latex-related conjunctivitis. Based on our data a higher class or titer of each in vitro assay was also strongly associated with the severity of clinical symptoms after exposure to latex.

Ebo et al. [16] reported in their collective a very low specificity of 33% for the AlaSTAT assay when interpreted according to the threshold value of 0.35 kU/l, whereas the sensitivity remained high. According to this threshold the ImmunoCAP assay achieved a good sensitivity (97%) and specificity (85%) in the same collective. Therefore, they propose to adopt a specific positive cutoff for each allergen and abandon the usually arbitrarily chosen positive threshold of at least some in vitro IgE tests. In contrast to Ebo et al. [16] we observed a specificity of

87.7% for the AlaSTAT, whereas the sensitivity was in the range of the other in vitro assays except the Pharmacia CAP System® (FEIA). Based on our data the Pharmacia CAP System® (FEIA) had the highest sensitivity of all in vitro assays (90%); the specificity of 85.3% was in line with the other in vitro assays. In the defined subgroup the Pharmacia CAP System® (FEIA) even reached a sensitivity of 99%, whereas the sensitivity of AlaSTAT, Magic Lite, Allergodip and skin prick test is in the range of 89–93%. Our findings suggest that the newly developed Allergodip latex test is a viable supplement in the diagnosis of NRL allergy. In contrast to other in vitro assays the Allergodip requires no further laboratory equipment. The test is very easy to perform and the hands-on time is only 5–10 min. Although the results of each semiquantitative test depend on subjective assessment, interpersonal variability is low in this test.

In summary, the Allergodip fulfils the requirements for the semiquantitative screening test of NRL allergy and might be a useful supplement in identifying subjects at risk of severe allergic reactions to NRL, especially in the absence of laboratory equipment. In agreement with others [5] we would like to point out that in situations of doubtful test reactions or discrepancies with patients' history further diagnostic methods have to be undertaken.

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