

Original article

Latex allergy: the sum quantity of four major allergens shows the allergenic potential of medical gloves

Background: Assessment of allergenic potential of medical devices made of natural rubber latex (NRL) requires the measurement of concentrations of specific allergenic proteins or polypeptides eluting from rubber.

Methods: Four NRL allergens (Hev b 1, 3, 5, and 6.02) were quantified in all medical glove brands marketed in Finland in 1999, 2001, and 2003 ($n = 208$) by a capture enzyme immunoassay. The results were compared with those obtained from previous nationwide market surveys, using a skin prick test-validated human IgE-based ELISA-inhibition method.

Results: A high overall correlation ($r = 0.87$, 95% CI 0.83–0.90) emerged between the sum values of the four allergens ($\mu\text{g/g}$ glove) and IgE-ELISA inhibition (allergen units, AU/ml, 1 : 5 diluted glove extract). The sum of four allergens when set at 0.15 $\mu\text{g/g}$ discriminated 'low allergenic' (< 10 AU/ml) from 'moderate- to high-allergenic' (≥ 10 AU/ml) gloves at a sensitivity of 0.93 (95% CI 0.85–0.98) and specificity of 0.90 (95% CI 0.83–0.94). When the sum was below the detection limit (0.03 $\mu\text{g/g}$) all gloves belonged to the previously defined low-allergen category.

Conclusions: By comparing the sum concentration of four selected NRL allergens with results obtained in human IgE-ELISA inhibition, it was possible set a cut-off level (0.15 $\mu\text{g/g}$) below which virtually all gloves contain low or insignificant amounts of allergens, and can be considered as low allergenic. At different cut-off-points, one could calculate the likelihood of a given glove to belong to the previously defined low, moderate or high allergen categories.

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Immediate allergic reactions to natural rubber latex (NRL) proteins have been recognized for more than 20 years as an important medical and occupational health problem (1–3). Proteins or peptides eluting from protective NRL gloves are considered as an important source of sensitization (reviewed in Ref. 1). Attempts at minimizing allergen concentration in NRL gloves to prevent sensitization and the development clinical allergy to NRL are acknowledged as a goal of mutual interest for rubber manufacturers and regulatory health authorities. Therefore, for several years, there has been a need for accurate methods for the measurement of the allergenic potential of NRL goods.

Total protein determination has been widely used for the indirect estimation of allergen content in NRL products. Consensus exists now that allergen-specific assays provide much more reliable information. The principle that an ideal test should be based on specific allergen quantification assays was adopted by the

European Committee for Standardisation (CEN) in the recently published new standard, (prEN455-3, Medical gloves for single use – Part 3: Requirements and testing for biological evaluation, Annex B, approved by CEN on October 13, 2006).

In recent years, substantial progress has been made in the characterization and purification of NRL allergens (reviewed in Ref. 4) and in the development of specific and quantitative assays for individual NRL allergen quantification (5, 6). The new assays are usually based on the capture-enzyme immunoassays (EIA)-principle and on the use of monoclonal antibodies and purified or recombinant allergens. These assays are specific; they can be properly standardized and are of sufficient sensitivity and reproducibility.

Consensus exists that proteins or peptides eluting from NRL products are responsible for the sensitization processes in NRL allergy (1, 7). However, a relatively limited number of allergens have so far been unequivocally

demonstrated in manufactured NRL products. The current literature supports the contention that at least Hev b 1, 3, 5, and 6.02, and/or fragments or polymers of them expressing IgE-binding epitopes, can be present in manufactured products (6, 8–11). Other allergens possibly detectable in NRL products include, e.g. Hev b 2 and 13 (12, 13). There is, however, no certainty now whether additional allergens will significantly contribute to the total allergenicity of rubber products.

In the present study, we wanted to analyze how a specific allergen quantification test for four clinically relevant NRL allergens and their sum concentration correlate with human IgE-based ELISA-inhibition, a well-characterized immunological test that was previously standardized and validated by skin prick testing in voluntary NRL-allergic patients.

Materials and methods

Glove extracts

The National Agency for Medicines (Helsinki, Finland) provided for the study 208 brands of medical gloves available in 1999, 2001, and 2003 on the Finnish market comprising all medical glove brands offered for sale at those time periods. The extraction of proteins from the gloves was performed as earlier described (14). Briefly, gloves were cut to small pieces which were mixed carefully. Subsequently, 1 g of the mixture was weighed and extracted in 5 ml of phosphate-buffered saline, pH 7.2. The extracts were placed in random order, coded, and divided into batches and stored at -20°C until analyzed. All analyses were carried out blind so that the individuals performing the tests and evaluating the results did not know which glove extract was being analyzed. Tests were carried out on three occasions, in the respective years of collection.

Capture enzyme immunoassay (EIA) for quantification of four allergens

Capture EIA kits (FITkitTM), purchased from FIT Biotech Ltd, Tampere, Finland, were used to quantify four NRL allergens (Hev b 1, 3, 5, and 6.02) according to the manufacturer's instructions. The method is based on the use of specific monoclonal antibody pairs against the four allergens, and either purified allergens or proteins produced by recombinant DNA technology as standards. Briefly, the microtiter wells are coated in each test with one specific monoclonal antibody that binds the desired allergen from the sample. After incubation, unbound material is removed by washing the wells. In the second incubation, horseradish peroxidase (HRP)-labelled allergen-specific monoclonal antibody binds to allergen molecules bound on the microtiter plate in the first incubation. After washing, substrate for HRP is added. The absorbance at 414 nm is measured after stopping the reaction. The intensity of the color produced is directly proportional to the allergen concentration of the sample. According to the manufacturer's information, the limit of detection for the four allergens ranges from 0.03 $\mu\text{g/g}$ (Hev b 6.02 and 5) to 0.05 $\mu\text{g/g}$ (Hev b 1 and 3). Intra-assay variation (repeatability) ranges between 2.8% and 5.8%, and inter-assay variation (reproducibility) from 2.6% to 7.6%. Detailed information on the assay procedure is available at <http://www.quattromed.ee>.

The results of IgE-ELISA-inhibition test, performed as described earlier in detail (14) were available for the present study. The source material (liquid latex from the rubber tree) was assigned to contain 100 000 allergen units (AU) per ml. The results from allergen-measurement in glove extracts (1 : 5, w/v) were reported as 'very low' ($< 1\text{ AU/ml}$), 'low' (1.0–9.9 AU/ml), 'moderate' 10–99 AU/ml, and 'high' ($> 100\text{ AU/ml}$).

Statistical methods

Receiver operating characteristic (ROC) curves were constructed to determine the highest accuracy (sum of sensitivity and specificity) cut-off points of the four allergen sum values that corresponded to results obtained with allergen determination by human IgE-based ELISA inhibition with bias corrected accelerated bootstrap CIs. Sensitivity, specificity, positive, and negative predictive values, likelihood ratio and their 95% CI values were calculated for each cut-off point value. Correlations were estimated by Spearman's correlation coefficient method.

Results

A high correlation ($r = 0.87$; 95% CI 0.83–0.90) emerged between human IgE-based ELISA-inhibition test results (AU/ml in 1 : 5 diluted glove extract) and the sum of the four allergens ($\mu\text{g/g}$ glove) in 208 glove samples (Fig. 1).

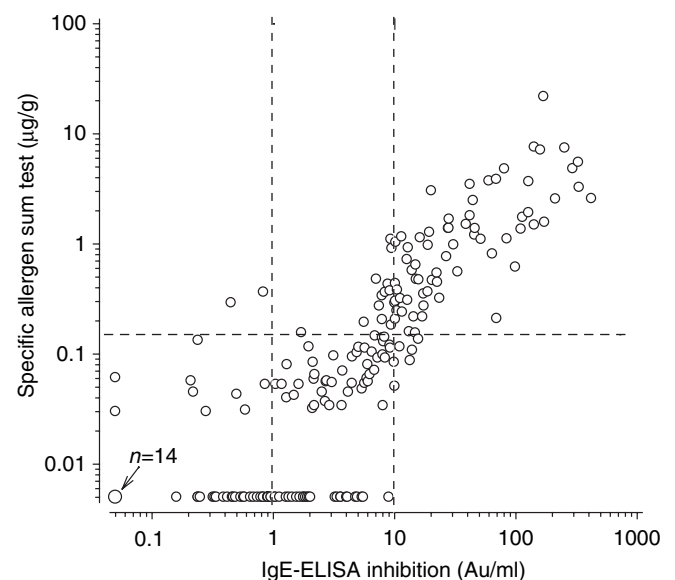


Figure 1. Relationship between the results from the four natural rubber latex (NRL) allergen sum test ($\mu\text{g/g}$) and human IgE ELISA-inhibition test (AU/ml) in 208 medical gloves. In the human IgE-based assay values below 1.0 AU/ml are considered as 'very low' allergenic, values from 1.0 to 9.9 AU/ml as 'low' allergenic, 10–99 AU/ml as 'moderately' allergenic and, values $> 100\text{ AU/ml}$ as 'highly' allergenic. The dotted vertical lines denote limits for the very low (1 AU/ml) and low (10 AU/ml) limits. The horizontal dotted line marks the 0.15 $\mu\text{g/g}$ limit in the four allergen sum test.

Table 1. Estimated cut-off points of four NRL allergen sum test ($\mu\text{g/g}$ glove) corresponding to human IgE ELISA-inhibition test (allergen units or AU/ml in 1 : 5 diluted glove extract) in assessing the allergenic potential of medical gloves

IgE-ELISA inhibition	Specific allergen sum test					
	Cut-off point ($\mu\text{g/g}$)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)
< 1.0 AU/ml	< 0.03	0.85 (0.78–0.90)	0.80 (0.67–0.90)	0.92 (0.86–0.96)	0.66 (0.53–0.77)	4.25 (2.61–7.38)
	< 0.15	0.52 (0.43–0.60)	0.96 (0.87–1.00)	0.98 (0.91–1.00)	0.42 (0.33–0.51)	14.20 (4.15–51.84)
	< 0.30	0.42 (0.34–0.50)	0.98 (0.90–1.00)	0.98 (0.92–1.00)	0.38 (0.30–0.46)	23.00 (4.31 to >100)
	< 1.15	0.20 (0.14–0.27)	1.00 (0.94–1.00)	1.00 (0.88–1.00)	0.31 (0.24–0.38)	NA
	< 0.03	1.00 (0.95–1.00)	0.49 (0.41–0.58)	0.51 (0.43–0.60)	1.00 (0.95–1.00)	1.97 (1.68–2.34)
< 10 AU/ml	< 0.15	0.93 (0.85–0.98)	0.90 (0.83–0.94)	0.83 (0.73–0.90)	0.96 (0.91–0.99)	9.04 (5.60–14.98)
	< 0.30	0.40 (0.29–0.52)	0.94 (0.89–0.97)	0.78 (0.62–0.90)	0.75 (0.68–0.81)	6.85 (3.38–14.06)
	< 1.15	0.42 (0.30–0.54)	1.00 (0.97–1.00)	1.00 (0.88–1.00)	0.76 (0.69–0.82)	NA

In the human IgE-based assay values below 1.0 AU/ml are considered as 'very low' allergenic, and values from 1.0 to .9.9 AU/ml as 'low' allergenic.

NRL, natural rubber latex; NA, not assessed; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio for positive result (ratio of the chance for a positive result if the glove contains allergens to the chance of a positive result if the glove does not contain allergens).

To define biologically meaningful and practicable cut-off points for the four allergen sum value corresponding to the human IgE-based results characteristics of tests were calculated (Table 1) and ROC curves were established (Fig. 2). The sum of the four allergens, when set at 0.15 $\mu\text{g/g}$, discriminated 'moderate- to high-allergenic' gloves (i.e. those containing more than 10 AU/ml) from the 'low-allergenic' gloves (< 10 AU/ml) at a sensitivity of 0.90, specificity of 0.93 and a likelihood ratio for a positive result of 9.04 (Table 1). When inspected at a sum value of 0.30 $\mu\text{g/g}$, the discrimination was poorer. Although moderate- to high-allergenic gloves were detected at a high specificity (0.94) the sensitivity of the assay was low (0.40).

When the role of Hev b 6.02 (the most abundant major glove allergen) was looked separately (data not shown), it could be seen that when the amount of Hev b 6.02 was below the detection limit, all gloves in this series fell to the low-allergen category (< 10 AU/ml). If small amounts of Hev b 6.02 (5–15 $\mu\text{g/l}$ in 1 : 5 diluted glove extract) were detectable, 81% of such gloves contained < 10 AU/ml. Hev b 5 behaved somewhat differently. When no Hev b 5 was detectable, 87% of gloves belonged to low-allergen category, but even small amounts (5–15 $\mu\text{g/l}$) brought more than half of the gloves to moderate- to high-allergen category.

In Fig. 1, some outliers can be seen in the upper left corner of the graph, representing cases where the two tests associated poorly (the sum of the four allergens was more than 0.15 $\mu\text{g/g}$ while the AU/ml value was below 10 AU/ml). A more detailed examination of six such gloves (located most distantly from the hypothetical correlation line) revealed that the only measurable allergen in the six glove extracts was Hev b 3, in quantities ranging from 11.2 to 72.0 $\mu\text{g/l}$ (mean 24.9 $\mu\text{g/l}$, data not shown). It may also be noted that another group of five gloves, located in ≥ 10 AU/ml area, but having the four allergen sum value below 0.15 $\mu\text{g/g}$, contained all readily measurable amounts of Hev b 6.02.

The explanatory role of the individual allergens in this set up was 74%, 24%, 11%, and 0.3% for Hev b 6.02, 5, 3, and 1, respectively (Fig. 3).

Discussion

The present study revealed the interesting observation that quantification of only four specific allergens provided highly useful information on the allergenic properties of NRL gloves. High correlation emerged between the allergen levels measured by a skin prick test (SPT)-validated IgE ELISA inhibition (14) and the present capture EIA allergen sum test. As the spectrum of IgE antibodies in the ELISA inhibition assay (14, Palosuo T, unpublished) covers a wide spectrum of NRL antibody specificities (recognizing at least Hev b 1,2,3,5,6,7, and 13), it is likely that these four proteins represent important allergens that are highly dominant in determining the allergenicity of NRL gloves. On the other hand, it has not yet been thoroughly assessed whether additional allergens would add to the performance of this type of assay. Interestingly, the present study as well as that by Tomazic et al. (15) revealed a very high correlation between human IgE-based assays and the capture EIA sum of the four allergens.

An ideal method of measuring the allergenicity of NRL products will, in theory, be SPT in voluntary latex-allergic subjects. For obvious ethical reasons such tests are now not possible. Human IgE-based semi quantitative *in vitro* methods, such as radioallergosorbent assay-inhibition and ELISA-inhibition, have been used for many years in research laboratories, but these methods are difficult to standardize, and they suffer from the paucity of human sera containing clinically-relevant latex-specific IgE antibodies (14, 15). In particular, human sera containing sufficient amounts of IgE to the hydrophobic allergens Hev b1 and 3 are becoming rare, as pediatric patients with spina bifida and other congenital abnormalities are

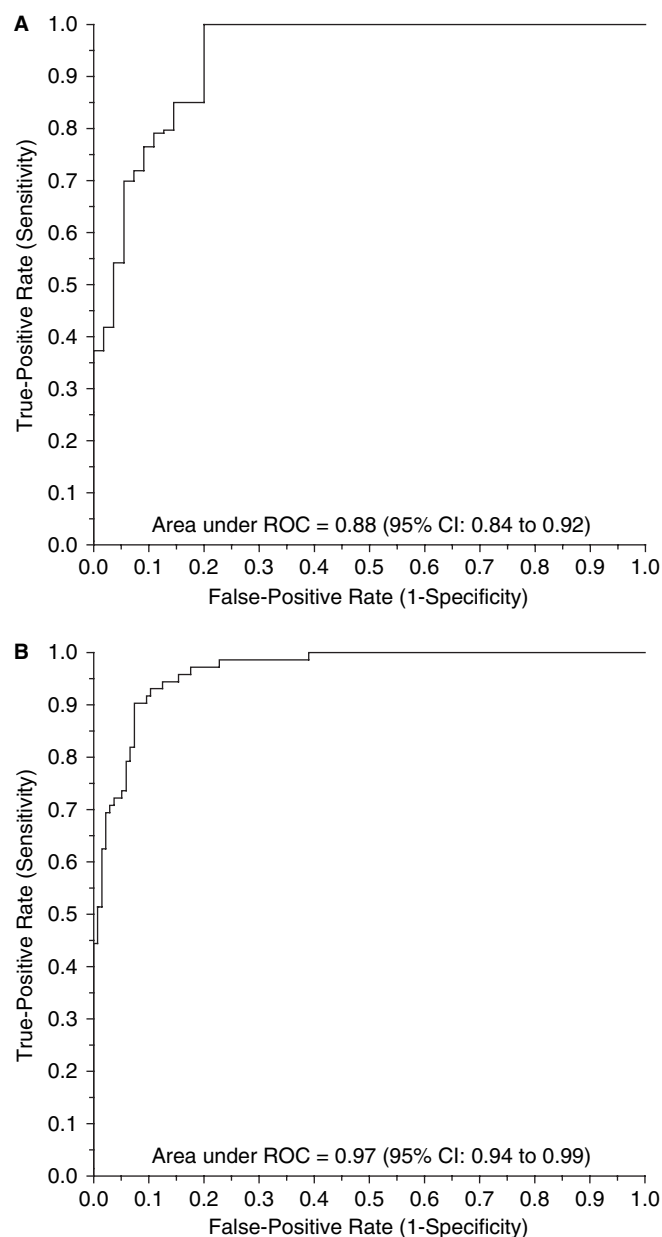


Figure 2. Receiver operating characteristic curves of four NRL allergen sum test as used to define the presence of natural rubber latex (NRL) allergens in glove extracts by human IgE ELISA inhibition using cut-off point of 1.0 AU/ml for human IgE ELISA inhibition (A) and 10 AU/ml (B).

currently treated in western countries almost exclusively in a latex-free environment.

It can be argued that reliable determination of allergenicity of NRL products requires the quantification of all clinically-relevant allergens. The task is, however, difficult because of the fact that, for the time being, little is known about the spectrum and molecular characteristics of allergenic proteins that resist the harsh rubber manufacturing process, and remain detectable in immunologically

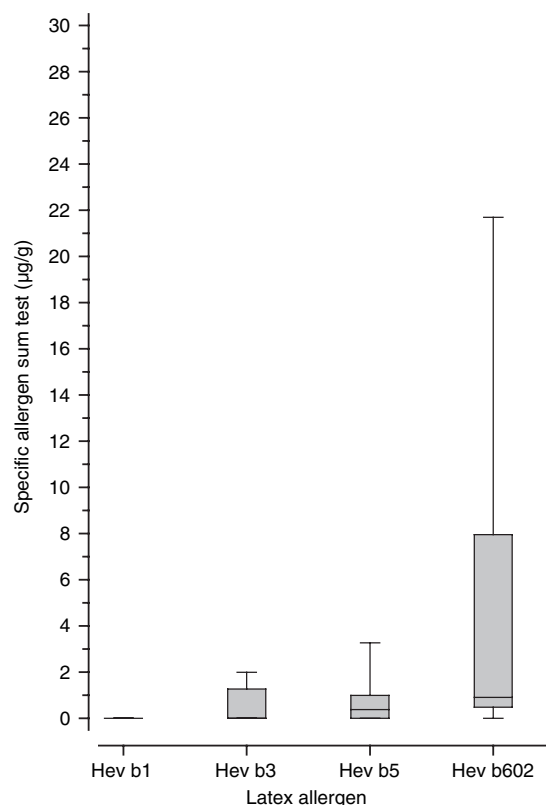


Figure 3. Box-and-whisker plot for explanatory roles of the four natural rubber latex (NRL) allergens in determining total allergenic potential by the four allergen sum assay. The box shows the distance between the quartiles, with the median marked as a line, and the whiskers show the 10th and 90th percentiles.

active form in the manufactured product. Therefore, it has been difficult to reach a consensus on which of the numerous allergens present in the NRL source material should be measured to obtain sufficient, and clinically useful, information about the allergenic potential of a given NRL product. The possibility should also be kept in mind that new allergenic proteins or IgE-binding epitopes may develop during the industrial processes.

Many allergens (i.e. some 50–60 proteins or polypeptides) capable of binding to IgE, have been detected in the source material, the liquid latex of the rubber tree (16, 17), which, in practice, precludes all-inclusive determinations. Another problem is the uneven distribution and great variation in the amount of allergens in various products (14, 15). As a compromise, one should try to define a selection of allergens that could enable sufficiently accurate but still practicable assessment of allergenicity. In line with this view, Yeang et al. (13) recently suggested that certain proteins (in their experience Hev b 5 and 13) could represent useful marker proteins reflecting the allergenicity of NRL products. This contention and the individual roles of Hev b 5 and 13 need, however, to be confirmed, as it is proved difficult to prepare native

Hev b 13 in sufficient purity (Palosuo et al. 2006, submitted for publication).

Studies so far conducted support the view that the four NRL allergens used in the present study (Hev b 1, 3, 5, and 6.02), contribute significantly to the allergenicity of NRL gloves. Other allergens, namely, Hev b 2 and 13, have also been reported occurring in NRL gloves (13, 18), but their role remains still to be assessed. It has been convincingly shown that the most abundant allergens in gloves are usually Hev b 6.02 and 5 (6, 11, 19, the present study). As has been shown earlier (6) and in the present study, the amount of Hev b 6.02 may be exceedingly high in comparison with other allergens. This naturally poses remarkable technical demands to all kinds of immunological and other assays.

The hydrophobic proteins Hev b 3 and 1 are continuously considered as important NRL allergens. Hev b 3 is frequently found, sometimes in high concentrations, in medical gloves marketed worldwide. These days, these allergens may be difficult to find in NRL products by using assays that are based on human IgE. In the present study, it was also observed that there was a group of gloves in which the human IgE-associated allergenicity index was very low, although the gloves contained readily measurable amounts of Hev b 3. This could be explained by the assumption that the human IgE serum pool did not contain sufficient amounts of IgE class anti-Hev b 3 for optimal detection of low amounts of Hev b 3. Likewise, there were a few additional gloves showing an allergen sum value over 0.30 µg/g, while the human IgE-based results were slightly below 10 AU/ml. In these instances, again, a likely explanation is that the human serum pool did not measure optimally one or several of the four allergens. Hev b 1 could be found only in a few gloves in the present study series, but this hydrophobic protein has been found in several gloves in other glove series recently analysed (20). Moreover, it has been reported that Hev b 1 may be enriched in glove powders (19) and, in our judgment, measuring its concentration could play an important role in the evaluation of the allergenicity of medical gloves. However, Hev b 1 had only a minimal impact on the results of the present glove series.

The present study provided data from which it was possible to calculate statistical parameters, such as

specificity and sensitivity, denoting how the four allergen sum test can differentiate between low-allergen gloves and gloves with increased allergen contents. From the results it can be further calculated (data not shown) that among the 208 glove brands tested, 122 had allergen levels below 10 AU/ml, and, at the same time, the four allergen sum levels below 0.15 µg/g. These glove brands might be considered as products that would be unlikely to sensitize people or to cause reactions in already sensitized individuals. At the other end, 30 gloves had sum values exceeding 1.15 µg/g (15 of the 30 having conventional IgE-based allergen levels exceeding 100 AU/ml). On the basis of the sum test, such glove brands could be considered as highly allergenic. It has to be emphasized, however, that it is still not possible to make claims of safety. Yet, it is likely that the sum test will be a very useful practical guide to personnel responsible for glove-purchasing in hospitals and health care centers and to conventional glove-users to support their glove-selection policies.

The latex allergy epidemic may have passed its peak in many western industrialized countries (reviewed in Refs 4, 21), but to ensure continuing good development, NRL devices used in medical work and in household and industry should contain as low allergen levels as practicably possible. Therefore, efforts to further develop methods that can reliably indicate 'true' allergenicity of NRL products are warranted.

In summary, the present study showed that it is possible to assess allergenic potential of medical gloves with high specificity and sensitivity by using the sum of four clinically relevant allergens as denominators of allergenicity. The high correlation emerging between the sum value and results from human IgE based *in vitro* ELISA inhibition, earlier validated by SPTs, support the contention that the results from the sum test provide a reliable and useful aid for decision making when gloves are selected for medical work.

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