

Measurement of natural rubber latex allergen levels in medical gloves by allergen-specific IgE-ELISA inhibition, RAST inhibition, and skin prick test

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Exposure to natural rubber latex (NRL) medical gloves poses risks to patients sensitized to NRL and to users of protective gloves. Previous studies have shown that extractable allergen levels of the gloves vary widely. Since most of the available laboratory methods of NRL allergen measurement lack adequate validation, we wanted to evaluate the performance of a recently developed competitive IgE-ELISA-inhibition method in relation to the skin prick test (SPT) and RAST inhibition, as well as to extractable protein quantification and an immunochemical latex antigen assay (LEAP). Twenty samples of surgical ($n=14$) and examination gloves ($n=6$), covering >90% of medical gloves marketed in Finland in 1994–5, were collected by the Finnish National Research and Development Centre for Welfare and Health, coded, extracted, and analyzed by the five methods. The IgE-ELISA inhibition correlated highly significantly with SPT ($r=0.94$) and RAST inhibition ($r=0.96$). Likewise, ELISA inhibition and RAST inhibition showed highly significant correlation ($P=0.96$, $P<0.0001$ in all three instances). Protein quantification by a modified Lowry method also correlated highly significantly with SPT ($r=0.80$), RAST inhibition ($r=0.82$), and ELISA inhibition ($r=0.81$, $P<0.0001$ in all three instances). Clearly weaker correlation, though statistically significant ($r=0.48$, $P=0.03$), was found between SPT and the LEAP assay. An NRL standard preparation was assigned an arbitrary content of 100 000 allergen units (AU) per ml. In relation to this standard, the NRL allergen level was considered low (<10 AU/ml) in 11, moderate (10–100 AU/ml) in two, and high (>100 AU/ml) in seven of the 20 glove brands analyzed. In conclusion, the results of a novel IgE-ELISA-inhibition method of measuring NRL allergen levels in medical gloves correlated highly significantly with those of SPT. The ELISA method was found to be sensitive, reproducible, technically easy, inexpensive, and suitable for the analysis of large numbers of NRL products. The results of extensive market surveys in 1994 and 1995, communicated to the medical community in Finland, appear to have had a clear effect in moving glove purchasing policies toward the use of low-allergen gloves.

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A major group at risk of immediate hypersensitivity reactions to NRL are operating room nurses and surgeons, of whom 5–11% have been shown to be allergic to latex (reviewed by Turjanmaa et al. [1]). In particular, the problem of NRL allergy is associated with the current extensive use of protective gloves made of NRL. Importantly, it has

also been shown that rubber gloves are the major contributor to latex aeroallergen levels in operating rooms (2). Over the past few years, data have accumulated to indicate that there may be considerable differences between the allergen levels of gloves made by different manufacturers, between different glove brands, and even between gloves of

different batches from a single manufacturer (3–7). In most instances, powdered gloves have been shown to contain higher allergen levels than non-powdered ones (6).

Measurement of the "total" allergen content of extracts of manufactured NRL products can be effected *in vivo* by SPT in NRL-allergic subjects or *in vitro* by specific IgE-antibody-inhibition assays. The development of specific *in vitro* assays has been hampered by our limited knowledge of NRL allergens and their clinical significance. Recently, rapid progress has been made in characterizing several NRL allergens at the molecular level (8–17); this, in turn, has allowed development of methods for both demonstrating the presence of and quantifying these allergens in NRL products. Obviously, there is room for additional, currently unrecognized allergens, and systematic studies are needed to clarify to what extent these NRL allergens are present and may be eluted from various manufactured NRL products. This information is of crucial importance when evaluating the performance of novel immunoassays for measurement of NRL allergens.

The primary aim of the present study was to find out whether appropriate *in vitro* tests could replace SPT in assessing allergen levels of NRL gloves. We therefore evaluated two *in vitro* tests, a novel IgE-ELISA-inhibition assay and conventional RAST inhibition, in relation to SPT. In particular, several purified NRL allergens were utilized to characterize in detail the IgE-ELISA-inhibition method. A second aim was to analyze NRL allergen and protein levels in a nationwide market survey covering the vast majority of medical gloves used in Finland in 1994–5, and to find out whether its results, when offered to health authorities and glove-purchasing personnel, would guide future glove-use policies toward low-allergen gloves. The preliminary results of a part of these studies were recently published in abstract form (18).

Material and methods

Collection of gloves and preparation of glove extracts

The National Research and Development Centre for Welfare and Health (Helsinki, Finland) provided for study 20 brands of medical gloves (14 surgical and six examination glove brands) widely used in Finland and other countries in 1994–5. Five surgical gloves and one examination glove were labeled powder-free. Standard extraction of proteins (1 g glove cut into small pieces in 5 ml of phosphate-buffered saline [PBS], pH 7.2) was carried out at the National Public Health Institute

(Finland). The extracts were placed in random order, coded, divided into batches, and sent to the Department of Dermatology, Tampere University Hospital; the University Hospital for Skin and Allergic Diseases, Helsinki; and the Laboratory of Clinical Immunology of the National Public Health Institute for analysis. All analyses were carried out blind so that the workers performing the tests and evaluating the results did not know which glove extract was being analyzed.

Skin prick test

The extracts made of the 20 glove samples were used in prick tests at Tampere University Central Hospital in 20 volunteers allergic to latex. The study had been approved by the ethics committee of Tampere University Hospital. The method has been described in detail elsewhere (19). Briefly, one drop (approximately 30 μ l) of each of the extracts was applied to the skin of the forearm and then pierced with a 1-mm, one-peaked lancet (ALK, Allergologisk Laboratorium A/S, Denmark) with shoulders to prevent deeper penetration. The drops were wiped off immediately with a paper towel. The test was read after 15 min, and a wheal with a size of half that caused by histamine hydrochloride (10 mg/ml, ALK) or larger was regarded as a positive reaction. The diameter of each wheal was measured and recorded. The results for each glove extract were expressed as median ratios (diameter of the wheal produced by the glove extract : diameter of the wheal produced by histamine [mm]).

Latex-RAST inhibition

Each glove extract was tested for allergen levels by latex-RAST-inhibition assay, as described earlier (20, 21). As a source of IgE antibodies, pooled and optimally diluted sera from 30 latex-allergic patients were used. NRL (see ELISA) was used as a source of solid-phase proteins and a reference. Results were calculated by the parallel-line assay method.

IgE-ELISA inhibition

Immobilization of NRL proteins to microtiter plates. As described earlier, NRL extract was prepared from NRL collected from rubber trees in Malaysia, deep-frozen immediately in the field, and transported in dry ice to the laboratory to be stored at -70°C (22). The NRL preparation was diluted to a protein concentration of 20 $\mu\text{g/ml}$ (standard Lowry assay) in 50-mM carbonate buffer, pH 9.6, applied onto 96-well, polystyrene microtiter plates

Table 1. Characterization of IgE serum pool used in ELISA inhibition

Patient no.	Age (years), sex	Symptoms	Associated condition	Latex RAST	Total IgE	Main IgE immunoblot bands (kDa)	IgE against				
							Prohevein (20 kDa)	Hev. C-dom (14 kDa)	REF (14 kDa)	23-27 kDa	45-46 kDa
1	13 M	GU, FE, R, A	SB	2.2	110	14, 20, 27	++	+	+	++	
2	8 F	CU, E	SB	70	286	14	++		+++		
3	12 F	AR, ES	SB	<0.4	193	14			++	+	
4	29 F	R		36	52 000	Many	++	++			+++
5	21 F	CU		NA	NA	14, 20	+++	++			
6	23 F	CU		31	3090	14, 20	+++	++			

GU: generalized urticaria; FE: facial edema; R: rhinitis; A: asthma; AR: anaphylactic reaction; ES: eye symptoms; CU: contact urticaria; SB: spina bifida; NA: not available; Hev. C-dom.: prohevein C-domain; REF: rubber elongation factor (hevea b 1); +, ++, +++: strength of IgE immunoblot reaction.

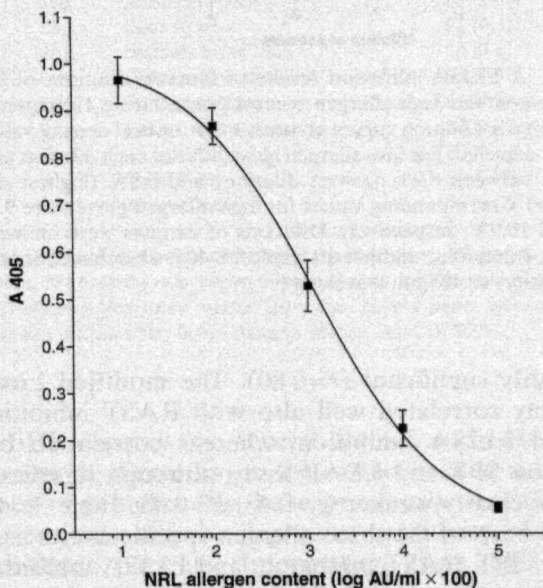


Fig. 1. IgE-ELISA inhibition standard curve (mean of 12 sequential assays, vertical bars denoting $\pm$ SD). Binding of IgE from reference serum pool of six latex-allergic patients to solid-phase NRL extract was inhibited by five 10-fold dilutions of reference NRL solution (standard, assigned NRL allergen content of 100 000 allergen units (AU)/ml). Concentrations of standard were 1000, 100, 10, 1, and 0.1 AU/ml, transformed to logarithmic values on x-axis of plot. A405: absorbance (optical density) at 405-nm wavelength.

(100 μ l/well; Nunc, Roskilde, Denmark), and incubated for 3 h at room temperature and then overnight at 4°C. The wells were then emptied and postcoated for 1 h with 100 μ l of 50-mM carbonate buffer, pH 9.6, containing 1% human serum albumin (HSA) (Finnish Red Cross Blood Transfusion Service, Helsinki, Finland). After postcoating, the wells were rinsed three times with PBS containing 0.05% Tween-20 (PBS-T).

Equal volumes of serial fourfold dilutions of glove extracts in PBS-T containing 0.2% HSA (PBS-T-HSA) and 10-fold dilutions of a standard

NRL preparation (used also to coat microplates; total protein concentration 10 mg/ml), assigned to contain 100 000 allergen units (AU) per ml, were incubated for 1 h at room temperature with an optimally diluted (1 : 10) IgE serum pool comprising six carefully characterized high-titered sera from three NRL-allergic adults and three NRL-allergic children with spina bifida, respectively (Table 1). The mixtures were then incubated for 2 h in the microtiter wells and washed thrice, and then the bound IgE was detected by biotinylated goat antihuman IgE (Vector, Burlingame, CA, USA; diluted 1/1000 with PBS-T-HSA) and streptavidin-conjugated alkaline phosphatase (Bio-Rad, Richmond, CA, USA; diluted 1/3000 with PBS-T-HSA). After three washes, *p*-nitrophenylphosphate (Sigma, St Louis, MO, USA), 1 mg/ml in 50-mM carbonate buffer, pH 9.8, containing 1 mM $MgCl_2$, was added, and the developed color was read at 405 nm by an automated ELISA reader (Titertek Multiskan, Eflab, Espoo, Finland). The readings were taken when the IgE binding of the uninhibited reference serum pool had reached a predetermined OD value of 1.0 (in approximately 45 min). Finally, the OD value obtained for each product was transformed to AU/ml by a third-order equation derived from a standard inhibition curve (Fig. 1). The absorbance value chosen for the calculation of allergen levels in the glove extracts was that of the least diluted extract that fell within the range of the standard curve of the NRL preparation; i.e., within OD values corresponding to NRL concentrations between 0.1 and 1000 AU/ml. Each microplate received its own NRL standards. All values were means of at least two determinations.

Modified Lowry test for total protein determination

Specimens of the 20 gloves were analyzed for total protein content at the Rubber Research Institute of Malaysia, Kuala Lumpur. Cut pieces (7 cm $\times$ 7 cm)

of each test sample were extracted (1:5, w/v) in 10 mM PBS at pH 7.4 at 23°C for 3 h. Proteins were precipitated from the extract with trichloroacetic and phosphotungstic acids. Total protein content was determined by the procedure described in the Malaysian Standard Test Method MS 1392:96P (21, 23).

LEAP assay

Latex ELISA for antigenic proteins (LEAP) assay was carried out with commercial kits purchased from the Guthrie Research Institute, Sayre, PA, USA. The kits were transported overnight from the USA to Helsinki, and used during the following 2–5 days according to the manufacturer's instructions.

Results

Optimal conditions for the IgE-ELISA-inhibition assay were established in preliminary experiments. Fig. 1 shows a standard curve (mean of 12 sequential assays) obtained when binding of pooled IgE antibodies was inhibited with serial dilutions of the standard NRL preparation. A third-order equation was found to give the best fit to the absorbance values, and the profiles of successive inhibition curves turned out to be quite reproducible (Fig. 1). The reproducibility of the assay was further assessed by recording the OD value for the (uninhibited) reference serum pool in 30 successive tests over a period of 16 months; the mean ($\pm$ SD) OD was 1.052 ($\pm$ 0.048), and the interassay coefficient of variation (CV) was 4.6%. As internal controls, extracts of surgical gloves with low (Ansell Gammex, lot 9294 407, approximately 2 AU/ml) and high (Baxter Triflex, 2D7154 08/92 lot PGS2K332, approximately 1500 AU/ml) allergen levels were included in each assay. Interassay CVs for these samples (based on OD values of undiluted extracts in 30 sequential tests) were 14% for the low-allergen glove and 11% for the high-allergen glove. Intra-assay CVs were 3.2% and 4.4%, for the low-allergen and high-allergen glove extracts, respectively (OD values in 26 parallel wells). The reproducibility of the method was further examined with four fourfold dilutions of the two control gloves in 12 sequential analyses (Fig. 2). CVs in comparable range were observed.

The results of the five assays for the 20 glove extracts are presented in Table 2 and Fig. 3. Highly significant correlations emerged between SPT and ELISA inhibition ($r=0.94$, Pearson's product moment correlation), as well as between SPT and RAST inhibition ($r=0.96$). RAST inhibition and ELISA inhibition also showed strong correlation ($r=0.96$). Correlation between SPT and the modified Lowry assay for total protein was also statistically

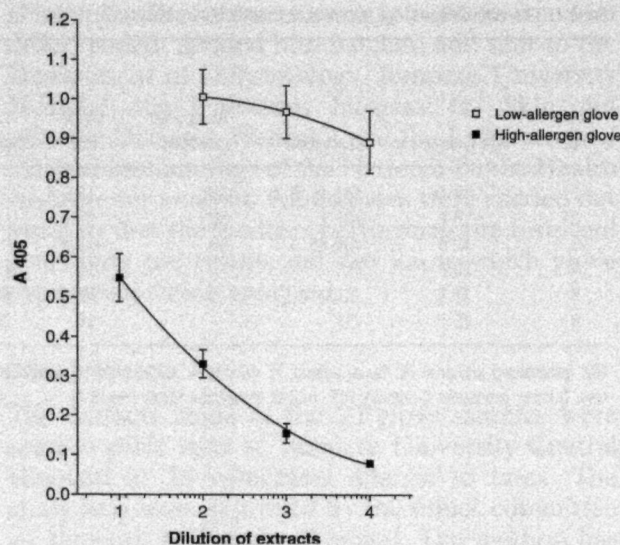


Fig. 2. ELISA-inhibition results of fourfold dilutions of low-allergen and high-allergen control glove during 12 sequential analyses. Dilution curves as mean $\pm$ SD (optical density values) are depicted. For low-allergen glove, CVs at each dilution point fell between 8.9% (lowest dilution) and 6.8% (highest dilution). Corresponding values for high-allergen glove were 9.1% and 10.9%, respectively. Dilutions of extracts were on x-axis, no. 4 denoting undiluted sample. A 405: absorbance (optical density) at 405-nm wavelength.

highly significant ($r=0.80$). The modified Lowry assay correlated well also with RAST inhibition, and ELISA inhibition, whereas correlation between SPT and LEAP assay, although significant, was clearly weaker ($r=0.48$, $P=0.03$; Table 3). On the basis of the three allergen-specific assays used, i.e., SPT, RAST inhibition, and ELISA inhibition, three categories of allergen levels (low, moderate, and high) were established. Accordingly, the NRL allergen level was considered low (<10 AU/ml) in 11, moderate (10–100 AU/ml) in two, and high (>100 AU/ml) in seven of the 20 glove brands (Table 2). All six nonpowdered gloves fell into the category of low-allergen gloves. On the other hand, both surgical and examination gloves were found in low- and high-allergen categories. In 8/11 low-allergen (<10 AU/ml) gloves, total protein content measured by the modified Lowry assay was less than 100 μ g/g; in 6/11, the result was below 50 μ g/g (Table 2).

Discussion

SPT is considered the "gold standard" in diagnosis of type I NRL allergy (1), and it yields relevant information for allergen standardization. SPT also provides the ultimate proof that given proteins or polypeptides can elicit allergic reactions *in vivo*. It has to be kept in mind, however, that the outcome

Table 2. Results of latex-allergen and protein-content analysis of extracts (1:5, w/v) of 20 medical glove brands by SPT, specific allergen measurements, and protein analysis. Gloves were graded into different allergen content categories by ELISA inhibition, RAST inhibition, and SPT results

No.	Type	Glove Name	Allergen content assessed by:				Protein concentration		Allergen content category
			SPT median (range) (mm)	SPT histamine ratio (mm)	ELISA-I AU/ml	RAST-I AU/ml	Mod. Lowry µg/g	LEAP µg/ml	
1	Surg.	Ansell Medical No Powder	0.5 (0-3)	0.00	0.2	0.72	23	1.3	Low
2	Surg.	Ansell Medical Gammex	1.8 (0-4)	0.27	0.7	2	99	15	Low
3	Surg.	Digitact Latex sans poudre	1.1 (0-3)	0.18	0.8	0.68	7	0.87	Low
4	Surg.	Ansell Medical Nutex Powder Free	1.7 (0-3)	0.20	1.0	3	35	0.60	Low
5	Exam.	Ansell Medical Conform nonsterile	1.5 (0-3)	0.20	2	3	169	0.46	Low
6	Exam.	Ansell Medical Conform sterile	3.8 (0-7)	0.78	3	6	478	0.04	Low
7	Exam.	Ansell Medical Ex No Powder	1.7 (0-4)	0.31	3	3	117	0.97	Low
8	Surg.	Biogel Regent	3.8 (1-8)	0.63	3	6	10	0.16	Low
9	Surg.	Sempermed Ultra No Powder	2.6 (0-5)	0.40	5	4	16	0.97	Low
10	Surg.	Baxter Ultraderm	1.7 (0-5)	0.18	6	2	65	1.3	Low
11	Exam.	Glads procedure glove	2.9 (0-6)	0.46	8	7	45	1.3	Low
12	Surg.	Micro-Touch (J&J)	5.7 (2-10)	1.00	20	33	112	15	Moderate
13	Surg.	Digitil HP latex	6.7 (4-11)	1.08	29	61	922	0.70	Moderate
14	Surg.	Neutrolon brown (J&J)	4.5 (0-8)	0.80	31	21	121	45	Moderate
15	Exam.	Sempermed nonsterile	8.4 (4-15)	1.49	245	72	813	84	High
16	Surg.	Sempermed Classic	8 (4-13)	1.34	349	75	1038	2.0	High
17	Surg.	Prolax surgeons gloves	6.7 (4-11)	1.18	388	67	967	2.2	High
18	Exam.	Lic examination	8.1 (5-13)	1.39	657	459	620	42	High
19	Surg.	Baxter Triflex	9.3 (5-15)	1.60	782	258	739	26	High
20	Surg.	Bextmold latex	7.7 (4-13)	1.34	993	217	1640	1.7	High

Surg.: surgical glove; Exam.: examination or procedure glove; SPT median: skin prick test, median wheal diameter (mm) produced by glove extract in 20 latex-allergic patients; SPT histamine ratio: median diameter of wheal (mm) produced by glove extract : diameter of wheal produced by histamine; ELISA-I: IgE-ELISA inhibition; RAST-I: RAST inhibition; Mod. Lowry: modified Lowry assay for total protein, performed at Rubber Research Institute, Kuala Lumpur, Malaysia; LEAP: latex ELISA antigenic protein assay (kits purchased from Guthrie Research Institute, Sayre, PA, USA).

of SPT depends on several factors, such as the amount of allergen(s) in the extract related to the types of proteins that have previously sensitized the patients and the degree of sensitivity of the individual patients. Information from SPT is extremely valuable for validating *in vitro* assays, but, for several obvious reasons, prick tests cannot be recommended for routine testing of allergen levels of manufactured rubber products. Therefore, there is an explicit need for specific and sensitive *in vitro* assays capable of quantifying NRL allergens.

In the present study, an ELISA-inhibition method was developed, and its performance in measuring NRL allergens was evaluated in the first place in relation with SPT. The ELISA-inhibition method showed a highly significant correlation with SPT and also RAST inhibition, a method that has been previously standardized for assessing allergen activity (24) and used for latex allergen determination (6, 20, 21). The lower level of detection of NRL allergens in the present ELISA-inhibition assay was found to be as low as 0.1 AU/ml, corresponding to a total protein concentration of approximately 10 ng/ml. The reproducibility of the ELISA assay at this level of sensitivity, assessed on the basis of the lowest standard in the reference curve, was remarkably

good (day-to-day CV 5.1%, Fig. 1), but since glove extracts of very low-allergen gloves were not analyzed repeatedly, caution should be exercised when interpreting the lowest obtained values. Extracts of several nonlatex gloves tested later gave results beyond the level of detection; i.e., below 0.1 AU/ml (data not shown).

In the present ELISA-inhibition assay, the NRL allergen levels were calculated with the first inhibition value (least diluted sample) falling within the range of values of the standards. One reason for this practice was that today the allergen content of most medical gloves sold, at least in Scandinavian countries, is low, and 50% inhibition may be impossible to reach without concentrating the extracted samples. On the other hand, the glove extracts may contain chemicals and/or other additives that may influence the immunologic competition reaction, and the slopes and the shapes of the dilution curves may vary. This is exemplified in Fig. 4, which shows dilution curves for six gloves representing low-, moderate-, and high-allergen gloves in the present series of 20 gloves. Usually, however, no major differences came up when higher dilutions were used for calculation. It can also be argued that different affinities and avidities of antibodies involved in the immune reactions influence the end points. This is

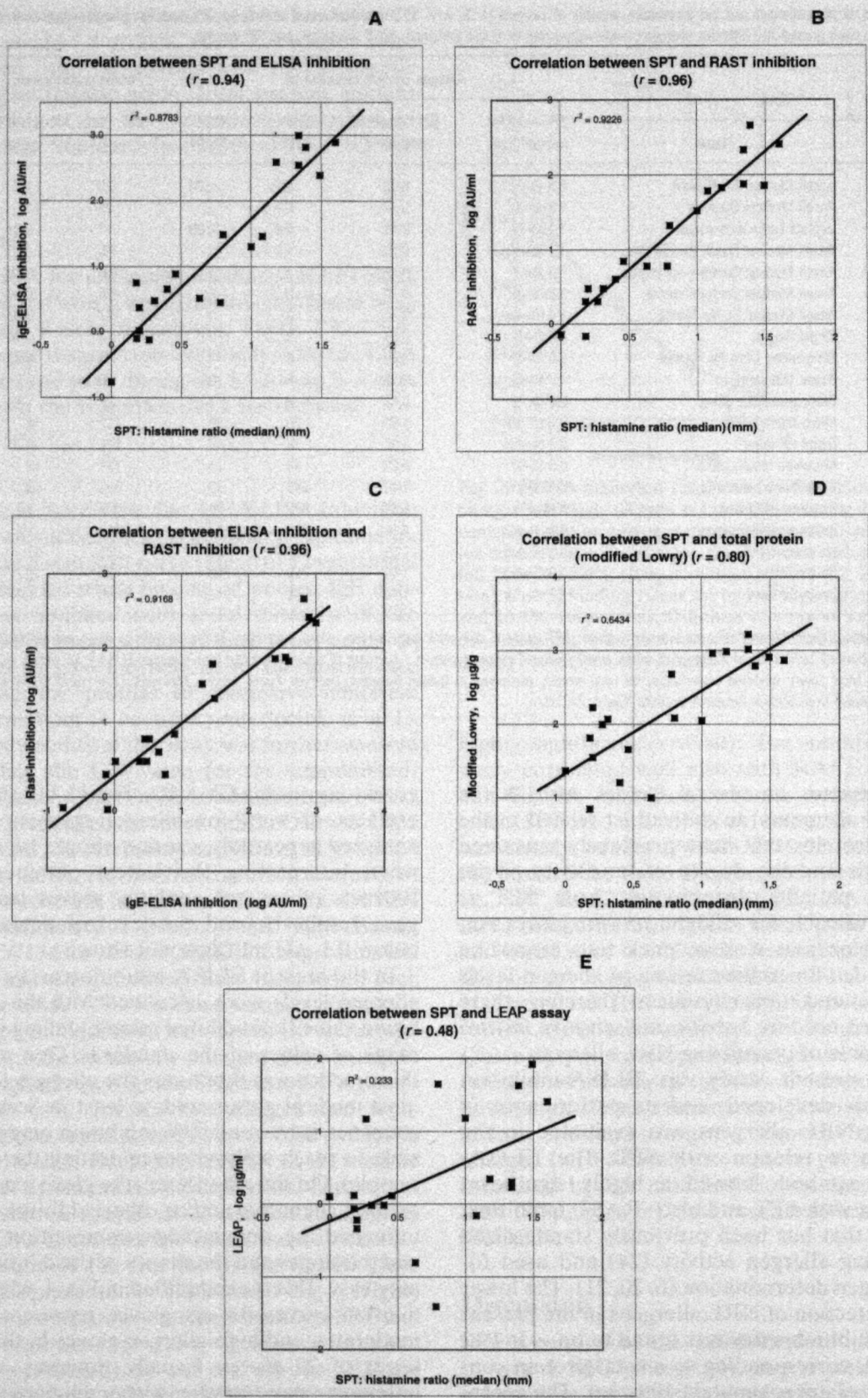


Fig. 3. Results of analyses of NRL allergen and protein contents in extracts of 20 NRL glove samples. Correlations between results of SPT and IgE-ELISA inhibition (A), SPT and RAST inhibition (B), ELISA inhibition and RAST inhibition (C), SPT and modified Lowry assay (D), and SPT and latex ELISA antigenic protein (LEAP) assay (E).

undoubtedly true, and it is likely that the varying populations of different antibody specificities bind to their corresponding antigenic targets heterogeneously. Importantly, however, it was observed that highly significant correlations emerged between the ELISA inhibition and SPT (as well as RAST-inhibition results), suggesting that levels of NRL allergens measured by the present allergen-specific immunoassays closely paralleled their *in vivo* ability to induce immediate type I reactions.

Under the present test circumstances, both of these *in vitro* IgE-inhibition assays, i.e., ELISA inhibition and RAST inhibition, seemed to measure highly similar activities in the glove extracts. The ELISA method, however, offers several well-known advantages over radioimmune assays, such as markedly lower equipment and reagent expenses and freedom from the hazards of radioisotopes. A drawback of both assays is that they depend on the properties of the IgE antisera used and on the composition of the reference NRL preparation. Therefore, it appears to be important that the antiserum contain sufficient amounts of IgE antibodies to the significant NRL allergens. To fulfil this requirement, one must study the candidate sera in detail with purified allergens. In the present study, the serum pool for ELISA inhibition consisted of sera from three latex-allergic children with spina bifida and three adults (primarily with dermal symptoms), shown to contain specific IgE antibodies to at least prohevein, hevein, and prohevein C-domain (major NRL allergens designated Hev b 6), to Hev b 1 (rubber elongation factor; REF), to Hev b 2 (endoglucosidase), and to Hev b 3 (hydrophobic REF-like protein of 23–27 kDa), and IgE antibodies to clinically significant NRL allergens with apparent molecular weights of 45–46 kDa and 75 kDa.

It is of interest that although different IgE serum pools were used in ELISA and RAST inhibitions, the results of the two assays correlated with each other and with SPT in a highly significant manner. The IgE serum pool used in RAST-inhibition analyses was from 30 adult latex-allergic patients, presenting, as a rule, cutaneous symptoms and clear-cut positive latex-RAST results. The high correlation may reflect the fact that SPTs were carried out in adult latex-allergic subjects including no spina bifida patients. Unfortunately, this RAST-inhibition serum pool has subsequently been used up, and we have not been able to analyze its allergen-binding profile; e.g., by immunoblotting with purified allergens.

Until now, relatively little was known of the exact molecular specificities and forms of allergens eluting from NRL gloves or other manufactured products. A recent study showed that hevein (Hev b 6.1,

Table 3. Correlation between results of SPT, NRL allergen levels, total protein, and latex antigenic protein measurement in 20 glove samples

	SPT	ELISA-I	RAST-I	Mod. Lowry	LEAP
SPT	1				
ELISA-I	0.94***	1			
RAST-I	0.96***	0.96***	1		
Mod. Lowry	0.80***	0.80***	0.81****	1	
LEAP	0.48 ¹	0.51 ²	0.51 ³	0.33 ⁴	1

SPT: skin prick test; ELISA-I: IgE-ELISA inhibition; RAST-I: RAST inhibition; Mod. L: modified Lowry assay for total protein; LEAP: latex ELISA antigenic protein assay.

*** $P < 0.0001$ (Pearson's correlation coefficient); ¹ $P = 0.03$; ² $P = 0.02$; ³ $P = 0.02$; ⁴ $P = 0.15$.

molecular weight 4.7 kDa) constituted up to 70% of the total allergen activity of a high-allergen glove (10). Preliminary results suggest that, in quantitative terms, hevein is indeed the most important NRL allergen eluting from at least high-allergen medical gloves (25). In addition, the presence of Hev b 1 (12, 25), Hev b 3 (14, 26), and Hev b 5 (17) in some latex gloves has been documented, but additional studies are needed to elucidate to what extent these and other proteins or peptides are responsible for the total allergenic potential of various manufactured products.

Wide variation in the "total" allergen levels of NRL gloves has been observed in earlier studies by Turjanmaa et al. (3) using SPT and by Yunginger et al. (6) using RAST inhibition. In general terms, results of the present study seem to agree fully with these observations. However, it should be emphasized that if the *in vitro* assay parameters discussed above are not given, it may be difficult to determine how efficiently various NRL allergens in the extracts are detected. Thus, in the RAST-inhibition study of Yunginger et al. (6), in which the solid-phase allergen was an extract prepared from a single high-allergen glove, it was left open that some relevant allergens may not have been present in the reference extract. Although we used different serum pools in RAST-inhibition and ELISA inhibition and found good correlation between them, it is possible that the IgE antibody source in the study of Yunginger et al. (6) (comprising plasmas from five latex-allergic health-care workers) was not adequate to cover the full range of NRL-specific IgE antibodies. This possibility may be pertinent, since several studies have shown that sera from adult NRL-allergic patients do not usually contain detectable levels of IgE antibodies to the hydrophobic Hev b 1 (26–29) and Hev b 3 (13, 14) molecules. Consequently, the contribution of these allergens to the "total" allergen activity of NRL glove extracts could have been underestimated.

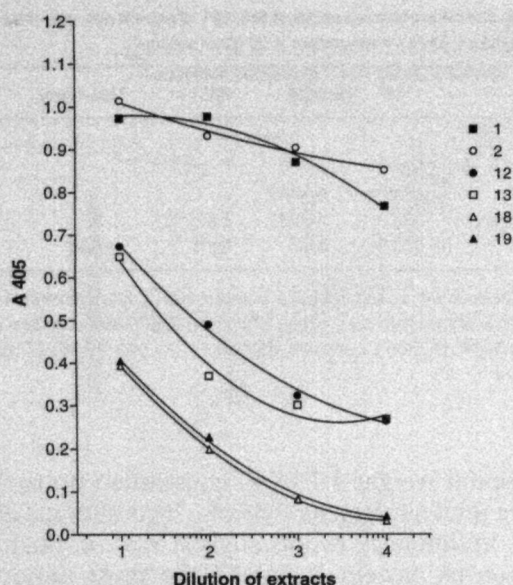


Fig. 4. ELISA-inhibition analysis: comparison of dilution curves for fourfold dilutions of six glove extracts. Numbers of symbols refer to numbers of gloves in Table 2. Dilutions of extracts are on x-axis, no. 4 denoting undiluted sample. A 405: absorbance (optical density) at 405-nm wavelength.

Since specific assays for measuring NRL allergens are not yet generally available, health authorities in both the USA (Food and Drug Administration [FDA]) and Europe (European Standardization Committee [CEN]) have recently taken steps to develop and define methods and standards for the measurement of the amount of total protein eluting from NRL products. Along these lines, the FDA has recently accepted that manufacturers can report extractable protein levels of NRL gloves obtained by the modified Lowry assay; however, due to methodological limitations, values less than 50 µg/g cannot be reported.

Significant correlations between total protein content and allergen levels of extracts of manufactured NRL products, based on both *in vivo* and *in vitro* assays, have recently been reported (6, 7, 30, this study), but it is widely agreed that, overall, the total protein content and IgE-binding capacity of certain allergen preparations may show no correlation. Furthermore, at present, it is not possible to define levels of "safety", denoting concentrations of NRL proteins that will not sensitize exposed persons or induce allergic reactions in already sensitized patients. It may also be noted that correlation coefficients between NRL proteins and NRL allergens (especially when measured by SPT), although statistically significant, have not been impressive enough to warrant the use of extractable protein levels as a safe way to present quantitative data on allergens in the extracts. The results

of the present study are in line with the above considerations. Yet, total protein quantification, being technically easy and possible to standardize, today offers a reasonable interim compromise while specific methods of quantification of NRL allergens are being developed. In particular, measuring total protein levels during various phases of rubber manufacture could be very helpful in development of less allergenic products.

With regard to the LEAP assay, the present results suggest that the rabbit antiserum used in this assay detects also nonallergenic proteins which may have little relevance to induction of symptoms in latex-allergic patients. These observations agree with those of Wrangsjö & Lundberg (31), who did not find a good correlation between the LEAP assay and a latex-specific IgE antibody inhibition assay (based on the Pharmacia CAP System) when analyzing randomly sampled medical and surgical gloves used in Sweden in 1994–5.

The results of the present study were communicated in early 1995 to the medical community in Finland. It was assumed that these data would affect the glove market and would be helpful in glove purchasing. An extended market survey of medical gloves sold in 1995–6 was then undertaken by the Finnish health authorities. A brief description of the results of this second-phase survey has been published (32). It is concluded that such nationwide market analyses, arranged by national health authorities, now offer an effective means of directing glove purchasing and use toward low-allergen gloves.

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