

Allergenicity of natural rubber latex gloves

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The concentrations of proteins, allergens and rubber chemicals are essential parameters of the allergenicity of NRL gloves. To date, a standardized method has been given only for analysis of the protein concentration (DIN EN 455-3), and not for the concentrations of allergens and rubber chemicals. In the present study, we investigated 11 brands of surgical gloves currently available on the German market. Additionally 1 glove, not subjected to final leaching procedures, was added for comparison purposes. Protein concentrations were analysed by different methods in different laboratories. Allergen concentrations were assayed by prick tests in NRL-allergic volunteers and by RAST inhibition methods. Rubber chemicals were analysed by HPTLC and GC. The protein concentrations analysed by the Lowry method in the 2 laboratories gave concordant results, but the correlations between protein and allergen concentrations were low. The protein concentration analysed by HPLC correlated with the allergen concentration, and gave better information on the allergenicity of the gloves. The development of standardized methods for allergen analysis in the assessment routine is necessary, due to significant discrepancy between protein and allergen levels of some gloves. Thiurams were not found in any of the gloves, though carbamates were present in all gloves tested. Our data indicate that washing procedures have little or no effect on the concentration of rubber chemicals.

Key words: immediate-type hypersensitivity; natural rubber latex; gloves; rubber chemicals; prevention. © Blackwell Munksgaard, 2002.

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Immediate-type allergy to NRL proteins has gained great importance by its increased prevalence in defined risk groups. As a preventive measure, the use of low-protein, low-allergen, powder-free NRL gloves has been recommended. As yet, the established method for the analysis of the extractable protein level of NRL gloves is the modified Lowry assay, but chemical interactions with this assay can result in the levels measured being too high or too low.

Analysis of the allergen level is not well vali-

dated. Standardization of RAST and ELISA inhibition methods depends upon the pool serum needed for these methods. Using inhibition methods, significant correlation between protein and allergen levels has been demonstrated for gloves on the market in the years 1994/1995 (1). Manufacturing processes of NRL gloves have changed during recent years, protein levels being decreased by leaching procedures (2, 3). In preliminary studies, we found discordance between protein and allergen levels in NRL gloves with low protein concentrations. Mahler et al. (4) reported, in a recently published study, that low-allergen gloves can be identified by inhibition tests, basophil histamine release and skin testing, whereas determination of the total protein content of NRL gloves is less useful.

In January 1999, a study began to analyse the allergenic characteristics of 11 brands of surgical gloves frequently used in Germany. Allergen levels were evaluated by prick tests with glove extracts in NRL-allergic volunteers and by analysis with the RAST inhibition method. The protein concentration of the extracts was analysed by the modified Lowry test and the HPLC method in external

Abbreviations:

NRL: natural rubber latex; HPLC: high performance liquid chromatography; GC: gas chromatography; GC-MS: gas chromatography-mass spectrometry; GC-FID: gas chromatography-flame ionization detector; HPTLC: high performance thin-layer chromatography.

Thiurams: TMTD: tetramethylthiuramdisulfide; TETD: tetraethylthiuramdisulfide; TBTD: tetrabutylthiuramdisulfide; TiBTD: tetra-iso-butylthiuramdisulfide; PTD: pentamethylenethiuramdisulfide; PTT: pentamethylenethiuramtetrasulfide.

Carbamates: ZDMC: Zn-dimethyldithiocarbamate; ZDEC: Zn-diethylthiocarbamate; ZDBC: Zn-dibutylthiocarbamate; ZDiBC: Zn-diisobutylthiocarbamate; ZPC: Zn-pentamethylenedithiocarbamate.

Benzothiazoles: ZMBT: Zn-mercaptobenzothiazole; MBI: mercaptobenzoimidazole.

laboratories. The concentrations of rubber accelerators in the gloves were analysed in acetone extracts by HPTLC and GC. The pH of the gloves was measured on the inner glove surface.

A key question in this study was whether or not the protein concentration, analysed by the modified Lowry test, correlates with the allergen level found in low-protein gloves.

Materials and Methods

Gloves

11 brands of surgical gloves (available on the German market in January 1999) were purchased (Table 1). Glove no. 9, which was taken from production prior to final leaching processes, was also investigated and was supplied directly by the manufacturer.

Extraction procedure

Glove extracts were produced according to the double glove method given in the DIN EN 455-3 (European Committee for Standardisation, CEN). Briefly, 3 pairs of each brand of glove were used. The cuff of 1 glove of each pair was marked at a point 20 cm from the tip of the middle finger. The 2nd glove was inserted inside the marked glove. 25 ml 0.9% NaCl solution were introduced in the space between the gloves. The inner glove was then filled with 100 ml of a dye solution. After removing air bubbles the gloves were sealed with a clamp on the mark. The gloves were shaken for 120 min at room temperature. After centrifugation (2000 g for 15 min), the liquid phase was filtered through a 0.22 μ m membrane and frozen in portions. The glove segment used for the extraction was cut off and weighed.

Protein analysis

Frozen samples of the extracts were sent coded, in random order, to the *Österreichisches Institut für biomedizinische Werkstofftechnik, Wien* (Dr. O. Seycek) (Lowry 1) and to the Clinic for Dermatology, Erlangen (Dr. U. Koch) (Lowry 2). The protein concentrations were analysed blindly, so that the workers performing the tests and analysing the results did not know which glove extract was being analysed. In both laboratories the protein concentration was analysed by the modified Lowry method according to the DIN EN 455-3, and in Erlangen additionally by the HPLC method (3).

In our laboratory, rocket immunoelectrophoreses were performed, according to the Laurell technique (5), to analyse the protein concentration of the extracts semi-quantitatively. 5 ml of the extracts were dialysed against distilled water subsequent to lyophilization. 5 ml of a sheep anti-NRL antibody (produced at our request by The Binding Site, Birmingham, UK, using fresh high-ammoniated NRL from Malaysia) were mixed with 60 ml 1% agarose and applied to a Gelbond plate (Pharmacia). 18 μ l of the glove sample reconstituted in a volume of 100 μ l were placed in preformed wells. Electrophoresis was carried out over a period of 12 h. After washing the gel 5 $\times$ in 0.9% NaCl solution the gel was stained with Coomassie blue. The sum of the height of the resultant arcs, which are proportional to the amount of antigen in the sample, was calculated.

Allergen analysis

The allergen concentration of the glove extracts were analysed by competitive RAST inhibition assays. A serum pool of 8 adult patients with clin-

Table 1. Glove types investigated in the present study

Glove type	Manufacturer	Lot No.
Micro-Touch powder-free	J&J	E-01977
Nutex powder-free	Ansell	811437903
Manex powder-free	Beiersdorf	83111002
Biogel	Regent Medical	97G0274
PEHA Gel	Hartmann	8776039
Micro-Touch post processed	J&J	333837
Gammex	Ansell	811019006
Sempermed supreme	Rauscher	98D912
Micro-Touch	J&J	prior to post processing procedure
PEHA taft	Hartmann	82411763
Sempermed Derma plus	Rauscher	98C55g
Manex	Beiersdorf	83260002

ically relevant NRL allergy was used as source antibodies (NRL-IgE value 53 kU/l). A reference latex protein extract was prepared by centrifugation of high-ammoniated natural rubber tree latex from Malaysia (protein concentration 1.2 mg/ml). Glove extracts with higher protein concentrations were used as they were, while extracts of low protein gloves were concentrated 4× and 20×.

Each 50 µl serum was preincubated (12 h, 4°C) with 50 µl NRL reference extract in different dilutions, or with 50 µl glove extract. NRL-specific IgE antibodies were analysed in duplicates using the Pharmacia RAST system. A standard inhibition curve was constructed by competition of the reference allergen with the solid-phase CAP-FEIA NRL allergen for specific IgE binding. The inhibition capacity of the glove extracts was compared with that of the NRL reference extract. The NRL concentration of the gloves was expressed as allergen units (U) per g glove.

Rubber chemicals

1 pair of each glove brand was sent to the *Österreichisches Institut für biomedizinische Werkstofftechnik, Wien* (Dr. O. Seycek) for the analysis of the rubber chemical concentration by HPTLC and GC (6). Briefly, 5 cm finger tips were extracted in acetone, vacuum dried and dissolved in chloroform. A qualitative analysis was performed by HPTLC and GC-MS, the concentration of the chemicals being analysed by GC-FID. By these methods, the following chemicals were considered.

- *Thiurams*: tetramethylthiuramdisulfide (TMTD),

tetraethylthiuramdisulfide (TETD), tetrabutylthiuramdisulfide (TBTD), tetra-iso-butylthiuramdisulfide (TiBTD), pentamethylenethiuramdisulfide (PTD), pentamethylenethiuramtetrasulfide (PTT)),

- *Carbamates*: (Zn-dimethyldithiocarbamate (ZDMC), Zn-diethyldithiocarbamate (ZDEC), Zn-dibutyldithiocarbamate (ZDBC), Zn-diisobutyldithiocarbamate (ZDiBC), Zn-pentamethylenedithiocarbamate (ZPC))
- *Benzothiazoles*: Zn-mercaptobenzothiazole (ZMBT), mercaptobenzoimidazole (MBI).

Glove surface pH

The surface pH was measured with a Skin-pH-meter (pH 900 PC, Courage + Khazaca Electronic, Köln, Germany) at 5 sites on the inner surface of 2 gloves of each brand of glove used in this study.

Patients

The study protocol was approved by the local ethics committee of the University of Münster. After providing informed consent, 55 adult, paid volunteers with clinically relevant allergy to NRL entered the study. The diagnosis of NRL allergy had been confirmed earlier by prick testing with a panel of NRL extracts in combination with present NRL specific IgE antibodies. Allergen-specific IgE antibodies were analysed with the CAP-FEIA system according to the manufacturer's instructions. Values of allergen-specific IgE above 0.35 kU/l were considered to be positive.

Table 2. Prick tests with glove extracts with reference to protein and allergen concentrations

Glove type	SPT, mean of wheal diameters (mm)	SPT, number of positive reactions ≥ 3 mm	Allergen (U/g)	Lowry (µg/g)		HPLC (µg/g)	Rocket electrophoresis	
				1	2		(n)	(mm)
histamine	5.23	55						
Powder-free								
1	0.40	2	under	21.0	20.0	24.5	0	0
2	0.98	3	0.2	114.4	82.8	8.2	1	7
3	1.82	13	0.3	16.1	13.0	23.5	1	30
4	2.25	22	1.3	3.0	4.7	11.7	1	13
5	2.48	24	6.1	8.5	8.6	10.3	0	0
Powdered								
6	0.57	3	0.2	0.0	5.1	7.0	0	0
7	1.40	10	0.3	70.3	44.1	10.5	0	0
8	2.20	21	1.9	48.2	37.6	33.3	1	7
9	2.45	21	1.8	31.3	26.2	n.d.	2	25
10	2.96	26	3.6	25.4	15.3	45.9	1	6
11	4.89	42	20.0	181.6	99.7	165.4	2	42
12	4.91	43	31.8	35.3	34.1	36.2	2	75

n.d.: not done due to technical problems.

Table 3. Identification of extractable rubber chemicals ($\mu\text{g/g}$) by HPTLC and GC-MS, quantification by GC

Glove type	ZDiBC	ZDBC	ZDEC	ZPC	ZMBT	pH
Powder-free						
1	<220	—	—	—	65	5.4
2	—	—	1025	10800	—	6.4
3	—	<250	—	—	265	5.3
4	—	—	255	310	<20 ²	5.1
5	—	1390	395	—	—	7.1
Powdered						
6	<240	— ³	—	—	—	7.0
7	—	—	1040	10060	— ¹	7.6
8	—	1055	—	835	—	6.8
9	<220	— ³	—	—	—	7.5
10	—	—	2880	—	—	8.4
11	—	2260	—	1860	—	6.9
12	— ⁴	500	—	—	370	8.3

¹ Mercaptobenzimidazole detected by HPTLC, but quantification by GC was not possible.

² ZMBT identified by HPTLC.

³ ZDBC identified by HPTLC, but not detected by GC-MS.

⁴ Diisobutylamine identified by GC-MS but quantification by GC was not possible.

Prick tests

Prick tests with the 12 glove extracts were carried out on the volar forearm using ALK lancets. The extracts were coded so that the technician performing the tests did not know which glove extract was being used. Wheal diameters were measured 20 min after puncture; the size of the wheal was documented by transfer to a transparent tape. A wheal diameter larger than 3 mm was considered to be positive. Skin tests with 0.1% histamine hydrochloride solution served as positive control and with 0.9% NaCl as negative control.

Statistical analysis

SPSS software was used to produce scatter diagrams and to calculate Pearson correlation coefficients. The Mann-Whitney *U*-test was used for analysing differences in allergen and protein concentrations and pH between powder-free and powdered gloves.

Results

The results of the prick tests, the protein and allergen assays, the rubber chemical analysis and the surface pH values are presented in Tables 2, 3. The protein pH values of the 12 brands of gloves ranged between 0 and 181.1 $\mu\text{g/g}$ glove by the Lowry method and between 7 and 165.4 $\mu\text{g/g}$ glove by the HPLC method. The protein concentration analysed in 2 external laboratories by the modified Lowry assay correlated to a high degree of signifi-

cance (Fig. 1), indicating close consistency of this method between both laboratories. Correlation between the protein concentrations measured by the Lowry and the HPLC methods was not as high (Fig. 2). Some glove extracts showed high protein concentrations in the Lowry assay but very low levels in the HPLC, indicating interactions between 1 of the methods with rubber chemicals. The number of positive prick test reactions ranged between 2 and 43 reactions for the different glove types, the mean wheal diameters ranging between 0.4 mm and 4.91 mm (Table 2). The correlation between the number of positive prick test reactions and the

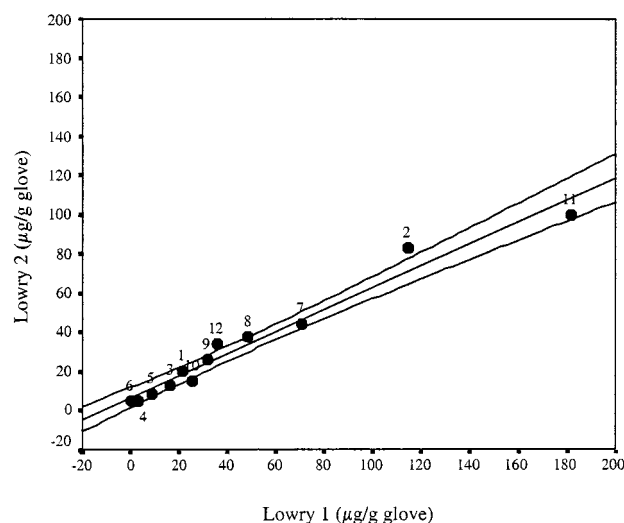


Fig. 1. Correlation between protein concentration of the 12 NRL gloves analysed by the Lowry method in 2 laboratories; $r=0.982$, $p<0.0001$.

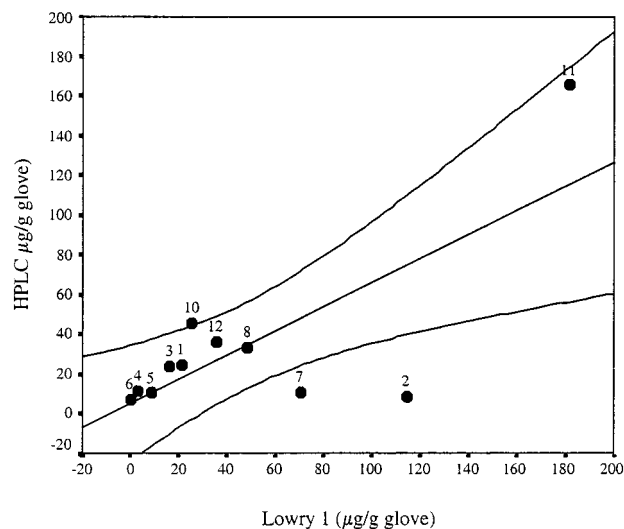


Fig. 2. Correlation between protein concentration of the 11 NRL gloves analysed by HPLC and by the Lowry method (Lowry 1); $r=0.744$, $p=0.009$.

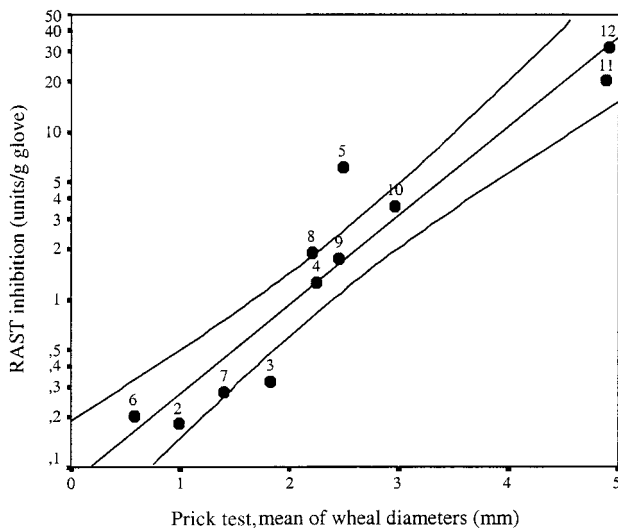


Fig. 3. Correlation between allergen concentration of the 11 NRL gloves analysed by the RAST inhibition method and prick test results; $r=0.886$, $p<0.0001$ (allergen concentration of glove no. 1 below detection limit).

calculated mean wheal diameters was highly significant ($r=0.988$, $p<0.0001$). For further calculations, the mean wheal diameters are used as a measure of the allergen concentration of the gloves.

The allergen concentration of the glove extracts analysed by the RAST inhibition method ranged up to 31.8 U/g glove. A highly significant correlation was found between these allergen levels and the mean wheal diameters ($r=0.889$, $p<0.0001$) (Fig. 3). Comparing the protein levels analysed by the modified Lowry method and the allergen levels measured with the prick test and the inhibition tests we did not find a statistically significant correlation (prick test – Lowry 1, $r=0.379$, $p=0.225$ / prick test – Lowry 2, $r=0.334$, $p=0.289$; RAST inhibition – Lowry 1, $r=0.209$, $p=0.337$ / RAST inhibition – Lowry 2 $r=0.329$, $p=0.324$). A large number of positive prick test results in contrast to low protein concentrations were found, especially with extracts of gloves with gel coatings (gloves no. 4 and 5). Comparing the data for allergen and protein concentrations a significant correlation was found only between the prick test and the HPLC result ($r=0.685$, $p=0.020$) (Fig. 4).

By rocket electrophoresis up to 2 rockets were found for the glove extracts, the height of the sum of the rockets varying between 0 and 75 mm. These results correlated closely with the allergen concentration analysed by RAST inhibition method ($r=0.897$, $p<0.001$) (Fig. 5) and with the mean prick test wheal diameters ($r=0.828$, $p=0.001$), but not with the protein concentration of the extracts.

The glove surface pH varied between pH 5.1 and

pH 8.4. Analysing the extractable rubber chemicals of the gloves, thiurams were not found in any of the gloves. ZMBT was found more frequently in the powder-free gloves, while dithiocarbamates were found in all gloves (Table 3).

Comparing pH, protein and allergen levels of the powder-free and powdered gloves, we found higher protein and allergen concentrations in the extracts of the powdered gloves than those of the powder-free gloves, but the only statistically significant difference was in the higher pH of the powdered gloves (Table 4).

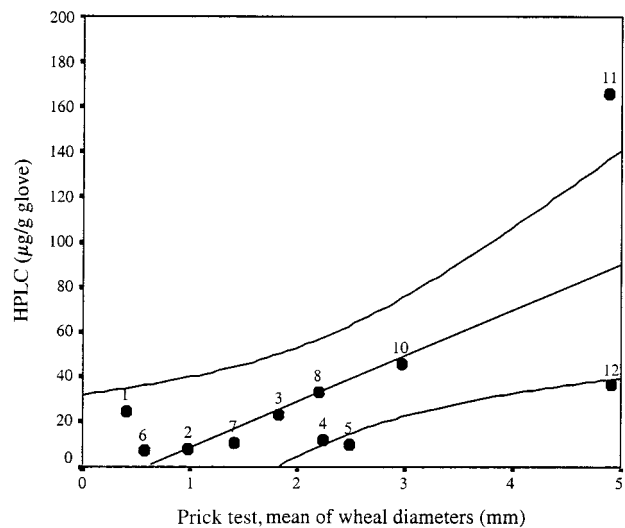


Fig. 4. Correlation between protein concentration of the 12 NRL gloves analysed by HPLC method and prick test results; $r=0.685$, $p=0.020$.

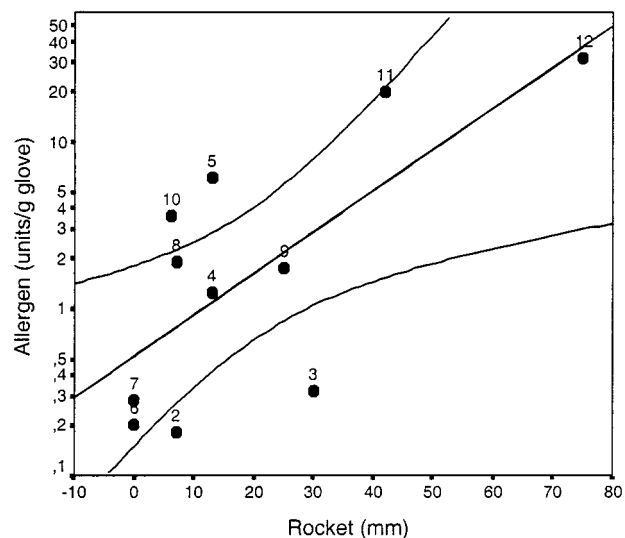


Fig. 5. Correlation between allergen concentration of the 11 NRL gloves analysed by the RAST inhibition method and rocket electrophoresis results; $r=0.897$, $p<0.0001$.

Table 4. Differences between powdered and powder-free gloves

	Powdered		Powder-free		Mann-Whitney <i>U</i> -test <i>p</i>
	mean	SD	mean	SD	
glove pH	7.52	0.67	5.88	0.88	0.019
HPLC ($\mu\text{g/g}$)	49.70	58.69	15.62	7.76	0.201
Lowry Erlangen ($\mu\text{g/g}$)	37.45	30.54	25.82	32.34	0.223
Lowry Wien ($\mu\text{g/g}$)	56.03	59.38	32.63	46.31	0.223
prick test, mean wheal diameters (mm)	2.77	1.64	1.59	0.88	0.223
allergens (RAST inhibition) (U/g)	8.50	12.42	1.97	2.82	0.273
rocket electrophoresis (mm)	22.14	27.89	12.60	11.10	1.000

Discussion

The concentrations of leachable proteins in the 12 brands of NRL gloves were up to 181.6 $\mu\text{g/g}$ latex and therefore lower than the protein levels of gloves analysed in previous studies (1–3). In several countries, the use of gloves with low protein concentrations has been recommended to quell increasing problem of immediate-type sensitization to NRL (2, 7). The reported lower protein concentration of powder-free NRL gloves compared to powdered gloves (1–4, 8) is one of the reasons why the use of powder-free gloves is now recommended. The lower pH and the lower protein and allergen concentration of glove no. 6 compared to glove no. 9 are evidence that the protein and allergen concentration of all NRL gloves can be minimized by leaching procedures, which also decrease the pH of the glove. Glove no. 6, available on the German market, is the post-processed glove no. 9. To our knowledge, the decrease in the pH and the protein concentration of NRL gloves during washing procedures has not been systematically analysed.

Nowadays, the modified Lowry test is a well-standardized assay for the protein analysis of NRL gloves, but rubber chemicals can interfere with this test (2, 3). The HPLC method is supposed to be a more reliable method for analysing the protein concentration of NRL gloves (2, 3). In our study, lower protein levels were found in some gloves and slightly higher levels in others, compared to the Lowry method, but overall the correlation between both methods was high.

The concentration of leachable allergens in NRL gloves can be assayed by prick tests with glove extracts in NRL-sensitized volunteers or by RAST or ELISA inhibition tests (1, 4, 8, 9). Both methods are not well validated for the analysis of the allergen concentration of gloves. The results of RAST and ELISA inhibition methods depend on the concentrations of IgE antibodies to the different NRL allergens present in the serum pool. Therefore, test results from different laboratories are not comparable. Having defined an in-labora-

tory standard, the result of RAST and ELISA inhibition methods correlate well with prick test results (1, 4).

We used prick tests in 55 NRL-allergic volunteers and a RAST inhibition assay to analyse the allergen concentrations of the 12 brands of gloves. The number of positive reactions obtained with extracts of the different brands of gloves correlated to a high degree of significance with the prick test mean wheal diameters ($r=0.988$, $p<0.0001$). RAST inhibition tests were performed using a serum pool from 8 well-characterized NRL-allergic patients. The correlation between the results of both methods was highly statistically significant.

Between the protein concentrations, analysed by the modified Lowry test, and the allergen levels of the gloves we found a poor and statistically not significant correlation. In contrast, Alenius et al. (9) and Palosuo et al. (1) reported significant correlations between protein and allergen levels of NRL gloves. In a recent study, Mahler et al. (4) demonstrated variable amounts of proteins not binding IgE antibodies in glove extracts, resulting in discrepancies between allergen and protein concentrations of gloves. It was concluded that determination of the total protein concentration of a glove is not sufficient to identify such a glove as low-allergen. NRL gloves with very high protein levels were included in the studies reporting a high correlation between the Lowry test and allergen measurement by ELISA and RAST inhibition (1, 9). The protein levels in the Finnish study (1) ranged between 7 and 1640 $\mu\text{g/g}$ glove and were therefore much higher than the protein levels of the gloves investigated in this study or in the study of Mahler et al. (4). Analysing the data from Palosuo et al. only of gloves with protein levels $<200 \mu\text{g/g}$, correlation between the Lowry test and prick test results is poor, and we consider that the good correlation between protein and allergen measurement in this study is a result of the overall higher protein levels of the gloves investigated.

For 2 gel-coated gloves (nos. 4, 5) and for 1 powdered glove (no. 12) low protein levels were found

with the Lowry and the HPLC method, but in contrast the mean prick test wheal diameter and allergen concentration measured by the inhibition assay were not conclusively low. Alenius et al. (9) reported a protein concentration of 15 µg/g for glove no. 4, though no positive prick test reaction was observed in 10 prick-tested NRL-allergic subjects. In the study of Palosuo et al. a protein concentration of 10 µg/g was reported for this glove, but in contrast the prick test median was found to be 3.8 mm and to be much higher compared to other gloves with low protein levels (1).

To find out whether chemicals in the inner gel coating might be responsible for positive prick test reactions, we extracted only the outer surface of the 2 gel-coated gloves. Performing prick tests with these extracts in 17 of the NRL-allergic volunteers, no significant differences were found in comparison with the original extract of the inner and outer glove side. The protein concentrations in these extracts were in the same range as the original extracts. At present, we do not have an adequate explanation for the contrast between allergen concentrations analysed by different methods of some types of gloves.

Our data indicate that the HPLC method may be the method of choice for analysis of the protein concentration of NRL gloves with low protein concentrations. Chemicals may interfere with the modified Lowry test, especially in the case of low protein levels. Therefore, we recommend the HPLC method for the protein analysis of low-protein NRL gloves. In contrast, we found that the correlation between allergen and protein concentrations of NRL gloves is low in some gloves. Therefore, the development of standardized methods for allergen analysis in the assessment routine must be recommended. Rocket electrophoresis is a relative simple method of analysing protein concentrations in a semi-quantitative manner, if standardized antibodies are available. In this study, we used a goat anti-NRL antibody and found a highly significant correlation between the allergen concentration of the gloves and the results of rocket electrophoreses, indicating that this cheap method could be developed to become a useful test, comparable with the LEAP method, until standardized methods for allergen analysis are available.

To reduce sensitization to rubber chemicals, efforts have been made to reduce the amount of residual chemicals in NRL gloves (10). The use of thiurams in glove production has declined during recent years, according to the manufacturers. In 1992, thiurams were detected in all 4 sterile surgical NRL gloves investigated (11). In 2000, Knudsen et al. found such chemicals in only 1 of 19

single-use gloves (6). In our study, none of the gloves contained thiurams. ZDEC and ZDBC were the most common accelerators in the study of Knudsen et al. (6), and were also present at variable concentrations in most gloves analysed in our study. The accelerator concentrations were not lower in powder-free than in powdered gloves. These chemicals have low water-solubility and our results indicate that the accelerator concentration in the gloves will not be reduced by washing procedures.

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