

Correlation of protein levels with skin prick test reactions in patients allergic to latex

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Background: Natural rubber latex (NRL) gloves are the major source of proteins that cause latex allergic reactions in sensitized health care workers and patients.

Objective: This study evaluated the effect of manufacturing changes on reducing protein, antigen, and allergen levels of latex medical gloves.

Methods: Three types of NRL gloves were manufactured with a common batch of compounded latex. The NRL gloves were analyzed for total protein by using the American Society for Testing and Materials D5712-95 Lowry method, and specifically for latex proteins by immunoassay. Allergen levels in the extracts were determined by end-point titration skin prick tests (SPTs) on patients allergic to NRL.

Results: Extracts from regular powdered gloves had detectable levels of latex proteins and allergens (62% SPT positive), whereas the powder-free gloves were low in protein content and allergenicity (5% to 8% SPT positive). No significant difference in SPT reactivity was observed between the chlorinated powder-free gloves and the polymer-coated gloves. Although the protein levels determined by the Lowry assay correlated with SPT reactivity ($r = 0.95$), the test was restricted by a high detection limit (9.3 $\mu\text{g/ml}$). Fifty-eight percent of patients allergic to latex reacted at the 50 $\mu\text{g/gm}$ detection limit allowed by the Food and Drug Administration. The ELISA had a good correlation with SPT reactivity ($r = 0.93$), and because of the greater sensitivity, gloves testing below the ELISA reporting limit (0.06 $\mu\text{g/ml}$) have a significantly lower potential for eliciting reactions in patients allergic to latex.

Conclusions: Results of protein assays are acceptable criteria with which to rate the potential allergenicity of gloves; however, the American Society for Testing and Materials D5712-95 assay may lack the sensitivity to provide clinically relevant data. (*J Allergy Clin Immunol* 1996;98:1097-1102.)

Key words: Latex allergy, latex proteins, Lowry assay, immunoassay, skin prick tests

Natural rubber latex (NRL) allergy presents as a clinical spectrum of manifestations including local contact dermatitis, allergic rhinoconjunctivitis, asthma, and life-threatening anaphylaxis.¹ It is generally understood that latex sensitization occurs as a result of repeated contact with NRL-

Abbreviations used

ASTM:	American Society for Testing and Materials
LEAP:	Latex ELISA for antigenic protein
NRL:	Natural rubber latex
SPT:	Skin prick test

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containing products or by inhalation of latex aeroallergens.^{1,2} Individuals in the health care professions (5% to 15%) and children with spina bifida (40% to 68%) have the highest prevalence rates for latex allergy.³⁻⁶ Immediate hypersensitivity reactions to latex occur when individuals have specific IgE antibodies to NRL proteins. Once sensitized, certain patients experience severe sensitivity, and anaphylactic reactions have been re-

ported from NRL inhalation, skin prick testing, and inadvertent contamination in food.⁷⁻⁹

Latex proteins have been demonstrated in most medical devices containing NRL.¹⁰⁻¹⁵ The product most often implicated as causing allergic reactions is NRL gloves, especially powdered gloves. Patients allergic to latex have been reported to react less to extracts of powder-free latex gloves than to extracts of powdered gloves.¹⁶ Use of low-protein and powder-free gloves reduces the consequences of NRL occupational asthma.^{7, 17} Powdered gloves have higher latex antigen and allergen levels than powder-free gloves.^{18, 19} Recently, the Food and Drug Administration approved manufacturers' use of a modified Lowry assay to report latex protein levels on glove packages. However, it is not known what quantitative level of NRL protein is safe to prevent sensitization or allergic reactions.

Our objective in this study was to quantitatively evaluate the effect of processing changes on protein and allergen levels of NRL gloves. We measured protein, antigen, and allergen levels on regular powdered, chlorinated, and chlorinated polymer-coated gloves to correlate the modified Lowry assay and ELISA with skin test reactivity in patients with known latex allergy.

METHODS

Patient population

Thirty-nine otherwise healthy patient volunteers, diagnosed as having latex allergy, with a positive history and positive NRL skin prick test (SPT) results were recruited for this study. NRL SPTs were previously performed on these patients with a standard latex skin test reagent (Bencard, Mississauga, Ontario, Canada), and wheals 4 mm greater than the negative control were considered positive. The control group consisted of 31 volunteers not allergic to latex who were first seen consecutively in the allergy clinic (17 with allergic rhinoconjunctivitis, 5 with asthma, 4 with sinusitis, 3 with urticaria, 1 with migraine headache, and 1 with a drug reaction). The protocol was approved by the Ethical Review Committee of the Ontario Allergy Society.

Skin testing

SPTs were done on the volar aspect of the forearm with a drop of test solution, and the epidermis beneath the drop was pricked with a lancet (Miles, Warwickshire, U.K.). SPT sites were wiped clean after 15 minutes, and the wheal and flare reactions were carefully measured. Positive wheal and flare reactions (≥ 4 mm of the negative wheal) were outlined, transferred onto transparent tape, and recorded as a permanent record. Testing with the latex components was started at a 1:1,000,000 dilution and continued with 10-fold lower

dilutions (higher concentration of extracts) until a positive reaction was obtained.

Test solutions

Allergenic components used for SPTs included a standard latex skin test reagent (Bencard), a polyvalent ammoniated latex protein preparation,²⁰ four extracts prepared from commercially available latex medical gloves, and extracts of three specifically prepared test gloves. Histamine (1 mg/ml) and diluent (human serum albumin, 0.3 mg/ml in 0.9% saline solution) were used as positive and negative control reagents for the skin tests.

Test solutions were prepared by extracting gloves for 2 hours at 37° C under sterile conditions. To obtain a representative extract for skin testing, five gloves from each lot were cut into small pieces and extracted in a polypropylene container with 5 ml/gm sterile saline solution.^{20, 21} The samples were centrifuged (2000 g) to remove glove powder and particulates. To eliminate the lot-to-lot variability²² and directly observe any differences resulting from processing changes, the three test gloves were specially manufactured, starting with a common batch of compounded latex; and the same dip machine was used within an 8-hour period. The three different test gloves were processed differently in order to produce: (1) regular powdered gloves, (2) chlorinated powder-free gloves, and (3) chlorinated polymer-coated gloves (patent pending). The test gloves manufactured for this study were used within 1 month of manufacture.

Protein analysis

Total protein levels. Protein levels were determined according to the American Society for Testing and Materials (ASTM) standard test method D5712-95, which incorporates a precipitation step to reduce the interfering substances before analysis by the Lowry assay. In brief, the precipitation step was performed on 0.5 ml of each sample by first adding 50 μ l of 0.15% (wt/vol) sodium deoxycholate and incubating the sample at room temperature for 10 minutes. Next, 50 μ l of 72% (wt/vol) trichloroacetic acid and 50 μ l of 72% (wt/vol) phosphotungstic acid were added, and the samples were incubated at room temperature for an additional 20 minutes. The samples were centrifuged for 15 minutes at 6000 g, the supernatant was discarded, and the pellet was dissolved in 125 μ l of 0.1 mol/L NaOH (yielding a fourfold concentration of each sample). Protein was determined by using the Detergent Compatible protein assay (Bio-Rad Laboratories, Hercules, Calif.). Each sample was tested in duplicate by using four twofold serial dilutions. The optical density was read at 700 nm, and the concentration of protein was determined by comparing the optical density of unknowns with the ovalbumin protein standard. The detection limit was defined as three times the standard deviation at zero, and the reporting limit was defined as 10 times the standard deviation at zero.²³ The standard deviation at

zero was determined by extrapolating the standard deviations of known concentrations of ovalbumin (6 replicates of 5 protein concentrations) according to the method of Taylor.²³ The limit of detection for the modified Lowry assay was found to be 2.5 µg/ml, and the reporting limit for the method was calculated as 9.3 µg/ml (46.5 µg/gm with a 1:5 weight-to-volume extraction ratio).

Antigen levels. Antigen levels in the glove extracts were quantitated by an indirect ELISA with the Latex ELISA for Antigenic Protein (LEAP) as previously described.^{12, 20-22} Latex protein antigens from the standard polyvalent ammoniated latex protein preparation or the glove extracts were adsorbed to the wells of a 96-well ELISA plate (Easy Wash; Corning Glass Works, Corning, N.Y.) by incubating eight duplicate twofold serial dilutions of the extracts (carbonate buffer, pH 9.6) in the wells for 4 hours at 37° C. Nonspecific binding sites were blocked by using 2% bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, Mo.). A rabbit anti-latex antiserum (1:5000 dilution) was allowed to react with the plastic-bound latex proteins overnight. The specifically bound rabbit anti-latex antibodies were then reacted for 1 hour with a goat anti-rabbit IgG (1:1000 dilution) conjugated with horseradish peroxidase (Sigma). Finally, a colored reaction product was produced by incubation in 10 mg/ml o-phenylenediamine (Sigma) containing 0.001% H₂O₂.

The LEAP uses a pooled rabbit anti-serum specific for latex protein, which has been shown to have an immunoblot profile similar to that of pooled IgE from 20 patients allergic to latex.²⁴ The LEAP has a good linear correlation, with range of sensitivity and specificity similar to those of a human IgE inhibition assay.²¹ The detection limit for the ELISA was determined as described above by extrapolating the standard deviations of known concentrations of latex protein to zero.²³ The assay has a detection limit of 0.03 µg/ml and a reporting limit of 0.06 µg/ml (0.3 µg/g with a 1:5 extraction ratio). Intra-lot variability for the test gloves was determined by using four or more individual glove samples for each test condition. Samples below the detection limit were assigned a value of one half the reporting limit (0.03 µg/ml).

Statistical analysis

The proportions of patients allergic to latex reacting to glove extracts were compared by using the Mantel-Haenszel chi square test or Fisher's exact test, as appropriate.

RESULTS

Protein analysis

Several gloves for each test condition were analyzed with the modified Lowry assay (ASTM 5712-95) and the ELISA to determine intra-lot variability. The Lowry assay detected 29 ± 8.8 µg/ml (mean $\pm$ SD, $n = 5$) for the regular powdered

gloves, whereas all of the samples tested for both the chlorinated ($n = 6$) and the polymer-coated gloves ($n = 7$) were below the detection limit of the Lowry assay (9.3 µg/ml). With the LEAP, extracts of the regular powdered gloves had 28.1 ± 8.2 µg/ml (mean $\pm$ SD, $n = 4$), whereas the chlorinated gloves had 0.045 ± 0.023 µg/ml ($n = 6$) and the polymer-coated gloves had 0.067 ± 0.082 µg/ml ($n = 7$). Three of six chlorinated gloves and five of seven polymer-coated gloves were below the detection limit (0.06 µg/ml) of the ELISA. These data indicate that the processing changes to produce the chlorinated and polymer-coated gloves consistently lower latex protein levels.

Skin testing results

Control. Thirty-one volunteer control patients, who were not allergic to latex, were skin prick tested with all test reagents; and full-strength testing solutions were used. All control patients reacted positively to the histamine control; however, no reactions to the glove extracts were observed. It was concluded that the glove extracts did not contain substances that would cause irritation reactions.

Patients allergic to latex. The population of 39 patients with positive responses to latex was found to be highly allergic to latex. As shown in Table I, 56% of these patients reacted to a 1:10,000 or greater dilution of the Bencard standard skin test reagent. The extracts were ranked according to the percent of patients allergic to latex with positive skin test responses, and the data are summarized in Table II. Sixty-two percent (24 of 39) of the patients reacted to the extract from the regular powdered gloves. The proportion of SPT reactivity for the test powdered gloves is significantly greater than that for test powder-free gloves (2 of 39, $p = 0.0000005$) and that for test polymer-coated glove extract (3 of 39, $p = 0.0000019$). The proportion of SPT reactivity was not different between the test powder-free and the polymer-coated gloves ($p = 1.0$, two-tailed Fisher's exact test). This level of reactivity was similar to that observed for another powder-free, polymer-coated glove (4 of 39, 10%) also tested in this study. In general, the rank order of reactivity of the extracts matched with the protein levels by both the Lowry assay and the ELISA.

Correlation of protein assays to SPT reactivity

The percent of patients reacting to the different latex extracts was plotted against the protein concentration (log) in an attempt to correlate these

TABLE I. Sensitivity of the latex-allergic population

Reagent dilution	Bencard SPT reagent	Cumulative reactivity (%)	AL reference protein	Cumulative reactivity (%)
1,000,000	7/39 (18%)	18	2/39 (5%)	5
100,000	9/39 (23%)	41	4/39 (10%)	15
10,000	6/39 (15%)	56	9/39 (23%)	38
1,000	8/39 (21%)	77	8/39 (21%)	59
100	2/39 (5%)	82	4/39 (10%)	69
10	1/39 (2.6%)	85	6/39 (15%)	84
1	6/39 (15%)	100	3/39 (8%)	92
No reaction	0/39 (0%)		3/39 (8%)	

AL, Ammoniated latex.

TABLE II. Comparison of protein level to SPT reactivity in patients allergic to latex

Test solution ^a	Percent reacting	Lowry (μg/ml)	ELISA (μg/ml)
Saline control	0	bd	bd
Test powder-free	5	bd	0.08
Test polymer-coated	8	bd	0.16
Powder-free glove	10	bd	0.08
Powdered glove 3	15	bd	0.94
Test powdered	61	29	28
Powdered glove 2	74	36	15
Powdered glove 1	85	140	18
AL reference protein	92	591	1844
Bencard reagent	100	1850	13000

bd, Below detection limit of 9.3 μg/ml for the Lowry assay and 0.06 μg/ml for the LEAP; AL, ammoniated latex.

assays. Samples below the detection limit were not included in the analysis. As shown in Fig. 1, there was a good correlation between Lowry protein levels and SPT reactivity ($r = 0.95$). The equation for the regression line comparing Lowry protein levels with SPT reactivity was ($y = 18.8 \log[x] + 40$), where x is micrograms per milliliter of latex protein determined by the protein assay. This equation allows one to predict the percent (y) of patients allergic to latex that will react to a glove extract. Likewise, there was a good linear correlation ($r = 0.94$) between the ELISA and SPT reactivity (Fig. 1). The equation for the regression line was ($y = 19.2 \log[x] + 34$).

The major difference found between the protein assays was a reduced sensitivity of the Lowry assay. Although the Lowry assay correlated well with skin test reactivity, it has a reporting limit of 9.3 μg/ml or 47 μg/gm (with a 5:1 extraction ratio). If the regression line is used to predict reactivity at the reporting limits, 58% of this population would

react at the Lowry limit, whereas only 11% would react at the reporting limit of the ELISA. Thus the ELISA allows one to predict SPT reactivity over a broader protein range and at a lower protein level than does the Lowry assay.

DISCUSSION

Latex allergy is problematic for health care workers who require barrier protection and who are working in a hospital environment laden with airborne NRL allergen.^{2, 7, 25} A simple solution to reducing airborne latex is the use of powder-free gloves. In this study we compared regular powdered gloves with powder-free gloves manufactured by using chlorination and/or polymer coating processes. We attempted to determine the relationship between residual protein levels and skin test reactivity. Three brands of gloves were specially produced from a common batch of compounded latex to eliminate the variability caused by differences in the starting materials.²² Residual protein in extracts was analyzed for total protein by using the ASTM 5712-95 method, and specifically for latex antigens by using ELISA. Relative allergen levels in the extracts were determined by end-point titration skin prick testing.

It has been reported that powder-free gloves have lower protein and allergen levels than regular powdered gloves.¹⁷⁻¹⁹ The chlorination step appears to dramatically reduce latex allergen levels.²⁶ Coating latex with a polymer is another process used to eliminate the need for donning powder. We found dramatic differences between the powdered and powder-free gloves in residual protein, antigen, and SPT reactivity. We were unable to determine a significant difference in protein or SPT reactivity between the chlorinated and the polymer-coated gloves.

It is generally believed that the use of low-

protein gloves will reduce the potential for latex reactivity and sensitization. However, several important points need to be addressed including: (1) How is the residual protein or allergen level measured? and (2) What amount of protein is acceptable? Previously, total protein levels were not found to correlate with clinical reactivity.^{10, 27, 28} This was presumed to occur because not all proteins are allergens, and measuring total protein would obscure the measurement of only those proteins that are allergens. However, numerous investigations have demonstrated that multiple latex proteins are allergens^{24, 28-31}; thus, measuring total protein would be expected to correlate with allergen levels. A probable explanation for lack of correlation in previous studies is that accelerator chemicals in latex extracts strongly interfere in the protein assay, resulting in elevated protein readings.¹² To overcome this problem the ASTM D5712-95 protocol introduces a precipitation step, which serves to concentrate the protein and to remove the chemical compounds that interfere in the assay. Using this method, we now report a good correlation between the Lowry total protein levels and SPT reactivity. The ELISA, which measures antigenic protein, also demonstrated a good correlation with SPT reactivity. The rank order of SPT reactivity of the nine NRL-containing extracts matched the protein levels measured by both protein assays. The major exception observed was the test powdered glove that measured higher protein levels than expected on the basis of SPT reactivity. This glove was produced by using a calcium carbonate powder rather than the more common cornstarch powder. This processing change may have altered the allergenic potential of the residual latex proteins, or this glove may have had increased amounts of proteins that were less allergenic in this patient population. However, given the heterogeneity of reactivity to specific latex proteins,²⁸⁻³¹ our data support the conclusion that protein levels can be useful in assessing the relative allergenicity of NRL gloves.

An equally important question relates to the relevance of protein levels. The ASTM D5712-95 test has limited sensitivity, resulting in a high limit of detection. Recently, the Food and Drug Administration has allowed manufacturers to make "low protein" claims on glove packages by using the ASTM D5712-95 protocol. Because of the limitations of the assay, documenting actual protein levels below the 50 µg/g level was not allowed. Our data demonstrate that at the detection limit of the Lowry method, 58% of patients allergic to latex

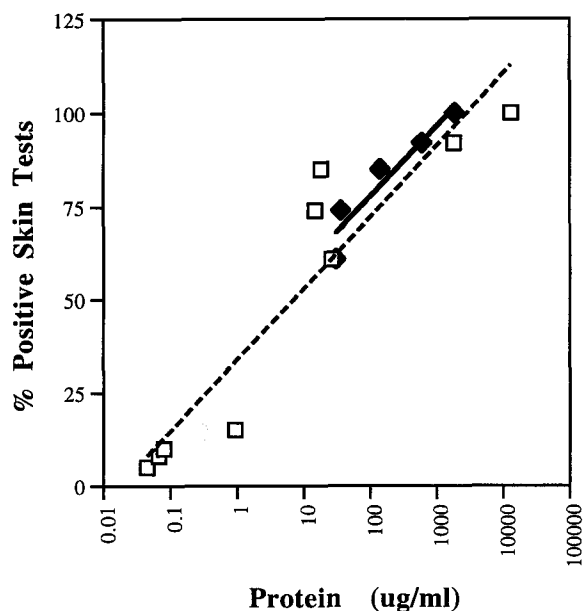


FIG. 1. Correlation of protein levels to SPT reactivity in patients allergic to latex. Patients were skin tested by end-point titration with nine NRL-containing samples. Protein levels in extracts were determined by the ASTM D5712-95 method (solid diamonds) or the LEAP method (open squares). Total percent of patients with skin test reactivity (to any dilution of extract) was plotted against protein levels, and curve fitting was performed by logarithmic regression analysis.

will still react to lower protein levels. The ASTM D5712-95 may lack the sensitivity to provide clinically relevant data.

Immunologic assays are physiologically more relevant assays in which antibodies are used to specifically measure reactive protein. The LEAP was found to be 150 times more sensitive than the ASTM D5712-95 method, and it correlates well with the RAST inhibition assay.²¹ The good correlation of the ELISA method with the SPT results and the expanded range of detection allows for a method that can more easily discriminate low protein levels. Glove extracts testing below the minimum level of detection of 0.06 µg/ml (0.2 µg/g) would have a significantly lower potential (11%) for eliciting type I reactions in latex-sensitized individuals. However, caution must be exercised in attempting to extrapolate these results to the actual clinical situation. The results of SPT reactivity may be different than the actual in-use situation. It could be speculated that hydrophobic proteins, which might not be extracted in saline solution, may be released by the sweat on a gloved hand or by direct skin contact with latex. Latex-sensitive

individuals must still be assumed to be highly allergic to any NRL-containing glove and cautioned to only use non-latex products.

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