

Prevention of Latex Allergy Among Health Care Workers: Evaluation of the Extractable Latex Protein Content in Different Types of Medical Gloves

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Background Since the 1980s, there has been increased use of latex gloves by health care workers and a concomitant increase of irritant and allergic reactions. The total protein content and the latex allergenic protein content in different types of medical gloves commonly used in our hospital were evaluated to acquire information useful for preventing latex allergy in our hospital personnel.

Methods The total protein content and the allergic latex protein contents were evaluated with Lowry modified method and RAST inhibition assay in samples and extracts of 29 different types of medical gloves.

Results The highest concentrations of total proteins and allergenic latex proteins were found in examination powdered latex gloves and in surgical powdered latex gloves; a significant amount of latex proteins was found in some brands of nitrile gloves.

Conclusions The clear association between the total protein levels and the allergenic latex protein levels suggests that the gloves with highest total protein content have the greatest allergenic potential. Therefore, it is recommended that manufacturing companies should provide package inserts including the total protein contents and possibly allergenic latex protein levels. They should declare whether they have added latex to their nitrile glove formulation. RAST-inhibition assays directly on glove samples instead of glove extract seems to be a good reliable and faster alternative for the evaluation of the allergenic potential of latex gloves. Am. J. Ind. Med. 44:24–31, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: latex allergy; latex gloves; health care workers; prevention

INTRODUCTION

Since the beginning of the 1980s there has been an increasingly widespread use of latex gloves by health-care

workers, with a concomitant increase of glove-related irritant and allergic reactions. Very relevant, from a clinical point of view, are the IgE-mediated allergic reactions to latex. They are characterized both by skin symptoms (urticaria) and ocular, respiratory, and systemic symptoms [Levy et al., 1992; Liss et al., 1997; Sinha and Harrison, 1998; Kujala, 1999].

Recent epidemiological studies have revealed a frequency of latex allergy in health care workers between about 3% and 14%. [Liss et al., 1997; Holness and Mace, 2001; Larese Filon et al., 2001; Ohlson et al., 2001; Shahar et al., 2001]. The highest percentage were found among dental personnel and operating theater nurses [Kujala, 1999].

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Accepted 19 February 2003

DOI 10.1002/ajim.10232. Published online in Wiley InterScience (www.interscience.wiley.com)

Therefore, allergic reactions to latex are an important problem and indicate the need to adopt effective preventive measures aimed at reducing the number of new sensitizations and at preventing relapses in already sensitized subjects, preserving when possible, their job fitness.

Sensitization can be prevented by reducing the extent of exposure to latex through the use of gloves with high biocompatibility, i.e., powder-free gloves with a low content of extractable latex proteins. Moreover, in case of no risk of frequent and/or prolonged contact with potentially infectious material, the use of gloves made by synthetic polymers (e.g., vinyl, nitril, butyl) is advisable. However, found that some patients with latex allergy could use vinyl gloves without any problems, but with a possibility to develop urticaria at the site of contact after using nitril gloves. This observation led us to hypothesize that latex may occasionally be present, even if in small amounts, in gloves made of synthetic polymers.

In this study we assessed the total protein content and the latex allergenic protein content in different types of latex powdered and powder-free surgical gloves, latex powdered examination gloves, nitrile and vinyl gloves which are commonly used in our hospital. The correlation between these two parameters and the correctness of the data provided by the glove manufacturers were also verified. The main purpose of this study was to acquire useful information to prevent latex allergy in hospital personnel.

MATERIALS AND METHODS

In this study, 29 types (commercial brands) of medical gloves were examined: seven brands of latex powdered surgical gloves, five brands of latex powder-free surgical gloves, seven brands of latex powdered examination gloves, five brands of nitrile gloves, and five brands of vinyl gloves. The glove commercial names are reported in Table I. In all these gloves, except for the vinyl ones, the total latex protein content was measured by Lowry-assay, modified in accordance with European Union regulation EN 455-3/1996. For every brand, the total protein content was measured in two different gloves chosen from the same lot. For the gloves currently used in our hospital, two samples from two different lots were also chosen. In all the gloves, an evaluation of the latex extractable protein content by RAST-inhibition assay was performed. The results were expressed as average inhibition percentage of the four tests (repeated three times) that were carried out on each glove. Both the Lowry-assay and the RAST-inhibition assay were performed directly on glove samples and on latex extracts obtained from the same gloves. The following procedures were performed.

Preparation of Latex Extract

The latex serum (the aqueous fraction obtained after centrifugation of non-ammoniated latex), kindly provided by

TABLE I. List of the Gloves Examined in a Study of Latex Allergy Among Health Care Workers

Identifying letter	Gloves model
Surgical latex gloves	
A	Braun Perry (powdered)
B–B1	Baxter Triflex (powdered * (2 different lots))
C	Ethicon Microtouch (powdered)
D	Foromed Medigloves Surgigloves (powdered)
E	Clinilab Profeel Orthopaedic (powdered)
F	Ansell Gamex (powdered)
G	Santex Kemweel Prod. (powdered)
H	Agsa Profex (powder free)
I	Braun Perry Encore (powder free)
L–L1	Foromed Medigloves Surgiglove (powder free * (2 different lots))
M	Prime Medical Prolax (powder free)
N	Ethicon Microtouch (powder free)
Examination latex gloves	
O	Mediberg/Foromed Reference (powdered)
P	Medea Planet (powdered)
Q	Monlycke Glads (powdered)
R	CEA/Biocommerciale Safeskin (powdered)
S	Artsana (powdered)
T–T1	Clinilab Comfit (powdered (2 different lots))
U	Pharmagic (powdered)
Nitrile gloves	
V	Biocommerciale Safeskin (nitril)
Z	Chemil Safegloves (nitril)
J–J1	ICO Syntho (nitril * (2 different lots))
Y	Braun (nitril)
K	Amplimedical Nitratrex (nitril)
Vinyl gloves	
1	Artsana (vinyl)
2	VIN Vinyl 2000 (vinyl)
3	Vinyl Plus 2000 (vinyl)
4	Glads Vinyl (vinyl)
5	TRU Touch (vinyl)

*Gloves used in our hospital in the period 2000–2001.

Professor Yip (Rubber Research Inst. of Malaysia, Kuala Lumpur, Malaysia), was dialyzed with phosphate buffer (PBS) containing 0.05% NaN₃ before to be centrifuged at 20,000 rpm for 1 hr at 4°C. This latex extract was used in this study as the coating on sensitized solid polystyrene beads.

Preparation of Latex Coated Solid Phases (Beads)

One thousand polystyrene beads (Precision Plastic Balls, Chicago, IL) were activated by treatment with a solution of glutaraldehyde (2% v/v) in PBS. The activated beads were washed three times and then coated with latex

extract (diluted 1:250 in PBS) by overnight incubation at room temperature. Coated beads were washed again before 1 hr treatment with NaBH_4 , 0.01 M, in order to stabilize the linkage between latex proteins and activated beads. Finally, latex coated beads were saturated with bovin serum albumin (BSA) solution (1% p/v in PBS-Tween 20, 0.05%). After further washings such beads were kept at 4°C until use.

Preparation of Latex IgE Positive-Human Pool Serum

Sera from 10 latex allergic subjects was checked by RAST assay to establish their positiveness class: 50 µl of each serum specimen were added into tubes containing latex-coated beads. After 6 hr of incubation and subsequent washings with PBS-BSA, 50 µl of a solution of I125 labelled goat anti-human IgE serum (Sferikit, Lofarma, Milano, Italy) was added and incubated overnight. At the end of incubation, beads were washed and residual radioactivity (expressed as counts per minute, cpm) was measured by gamma-counter and compared to a positive standard curve. Sera showing positiveness class 3 or 4 were pooled and used for the subsequent experiments.

Preparation of Glove Extracts

For the preparation of extract, gloves were cut into small piece (less than 1 cm²) and aqueous soluble proteins extracted with PBS (20% p/v). After overnight incubation under stirring, such material was centrifuged at 20,000 rpm for 30 min at 4°C and supernatant collected, dialyzed against distilled water, and concentrated by lyophilization. Subsequently proteins from glove extract were precipitated as described by Baur et al. [1998]. To 1 ml of each extract 0.1 ml of 0.15% (w/v) sodium deoxycholate was added. After vortexing and standing for 10 min at room temperature, 0.1 ml of 72% (w/v) trichloroacetic acid and 0.1 ml of 72% (w/v) phosphotungstic acid were added. After 20 min the extract mixture was centrifuged at 20,000 rpm for 1 hr; the supernatant was discarded while protein pellet was completely redissolved in 0.1 N NaOH and protein content determined by Lowry modified assay (European Guideline, EN455-3, 1996). For each different kind of glove, two different extracts deriving from the same or when possible from different, lots were prepared.

RAST-Inhibition Assay

Villanta's modification [Villanta et al., 2000] of traditional RAST-inhibition assay developed by Ceska et al. [1975] was used.

One hundred µl (50 µl of latex IgE positive pool serum +50 µl PBS) and 100 µl (50 µl of non allergic patients pool

serum +50 µl PBS, as negative control) were added to tubes, each containing a piece of glove (1 cm²) for a 2 hr pre-contact. In this way a part or all latex specific IgE antibodies bind themselves to latex proteins possibly present into the piece of glove, so they are no more available. For positive control, tubes containing 50 µl of latex IgE positive pool serum and 50 µl PBS without any piece of glove, were used.

Subsequently, 50 µl were harvested from each tube (in this solution latex specific IgE could have been lost during the 2 hr pre-contact, depending on the presence of latex proteins in the glove's fraction) and transferred in another tube containing a latex-coated bead and 50 µl PBS (a volume of 100 µl is necessary to completely cover the bead). After an overnight incubation, beads were washed and 100 µl of I125 labelled goat anti-human IgE serum were added to each tube. After a further overnight incubation and other subsequent washings, beads' residual radioactivity was determined by gamma-counter. The calculation of the inhibition percentage was done as follows: %inhibition = (positive control cpm – sample cpm)/(positive control cpm – negative control cpm) × 100.

To exclude that possible decreases of the inhibition percentages were due to an aspecific link between IgE antibodies and glove latex protein experiments, using a pool of human sera from patients with positive RAST for mites and grass extracts and negative RAST for latex extract, were also performed. In this case mites or grass extracts-coated beads were used instead of latex extract beads. The calculation of the inhibition percentages was done as described above.

RAST-inhibition experiments were performed also with glove extracts. In this case a 2 hr pre-contact was performed mixing in a tube 30 µl of glove extract and 20 µl of latex IgE positive pool serum. Then, the above described procedure was used but 30 µl PBS instead of 30 µl of glove extract were added in the positive control and 20 µl of normal human serum instead of 20 µl of latex IgE positive pool serum were added in the negative control.

Statistical Analysis

A linear regression analysis was performed in accordance with the STATVIEW-SAS program to evaluate:

- the correlation between data obtained directly from glove samples and the data obtained from the respective glove extracts
- the correlation between the total protein contents as measured by modified Lowry-assay and the allergenic latex protein contents measured as a percentage of RAST-inhibition. The inhibition percentage was expressed with the following equation:

$$2 * \arccos [\text{sqr} (\% \text{ inhibition})].$$

In order to validate the RAST-inhibition assay, the 10 brands of gloves with the highest inhibition percentages were considered, and a linear regression, using the data obtained from 10 glove extract graduated dilutions for each glove, was calculated.

RESULTS

Measurement of the total protein levels through the modified Lowry-assay revealed concentrations (expressed as the average of the two samples analyzed) ranging from 5.17 µg/g to 917.38 µg/g. Generally, the highest concentrations were found in latex powdered examination gloves (concentrations ranging from 17.45 µg/g for glove R to 917.38 µg/g for glove U) and in latex powdered surgical gloves (concentrations ranging from 17.45 µg/g for glove A to 610.98 µg/g for glove G).

In latex powder-free surgical gloves the concentrations ranged from 5.7 µg/g for glove H to 33.86 µg/g for glove N.

In nitrile gloves the total proteins ranged from 5.17 µg/g for glove Z to 22.62 µg/g for glove K. The results are shown in Figure 1.

Measurement of extractable allergenic protein levels through RAST-inhibition assay directly on glove samples

revealed an average inhibition percentage ranging from 0% in nitrile gloves Z and vinyl gloves 2, 3, and 5 up to 82.5% in gloves U (latex powdered examination gloves). The results are shown in Figure 2. The highest concentrations were found in latex powdered examination gloves (with a range from 57.44% to 82.25%) and in latex powdered surgical gloves (with a range from 20.97% to 59.73%). Vinyl gloves, as expected, showed no inhibition or else negligible inhibition percentages.

Comparison between the inhibition percentages obtained by the RAST-inhibition assay performed directly on the glove samples and the percentages obtained on the glove extracts showed a good correlation. The extract inhibition percentages were generally higher since their protein content was more concentrated.

Figure 3 shows the regression line ($R^2 = .77, P - v < 0.0001$).

A good correlation ($R^2 = .677, P - v < 0.0001$) resulted also between the total protein content of the 24 different types of latex gloves (revealed by Lowry-assay) and the inhibition percentages revealed by RAST-inhibition assay on the glove samples (Fig. 4).

No relevant differences were found between the inhibition percentages measured in different areas (wrist,

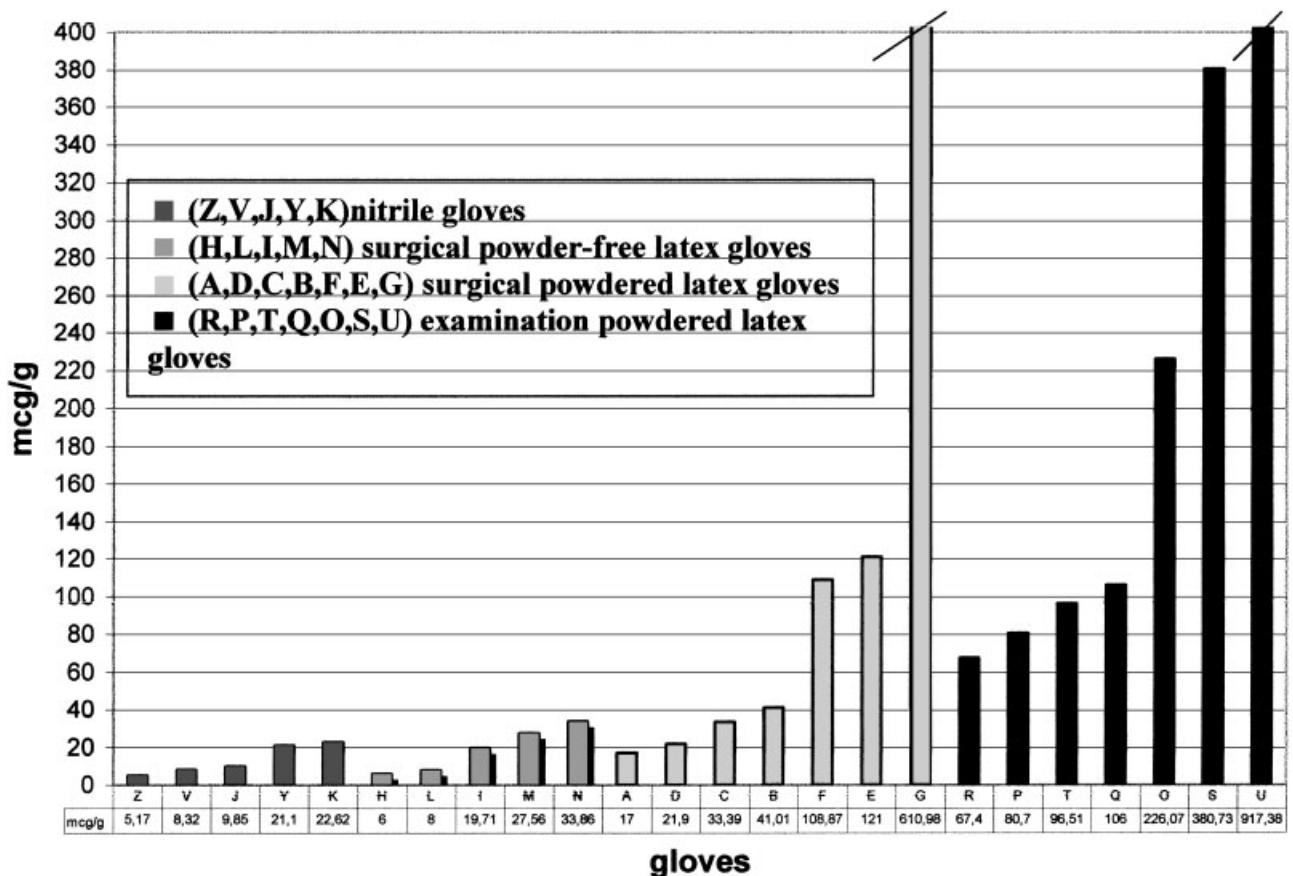


FIGURE 1. Average total protein concentrations in the gloves examined subdivided by type.

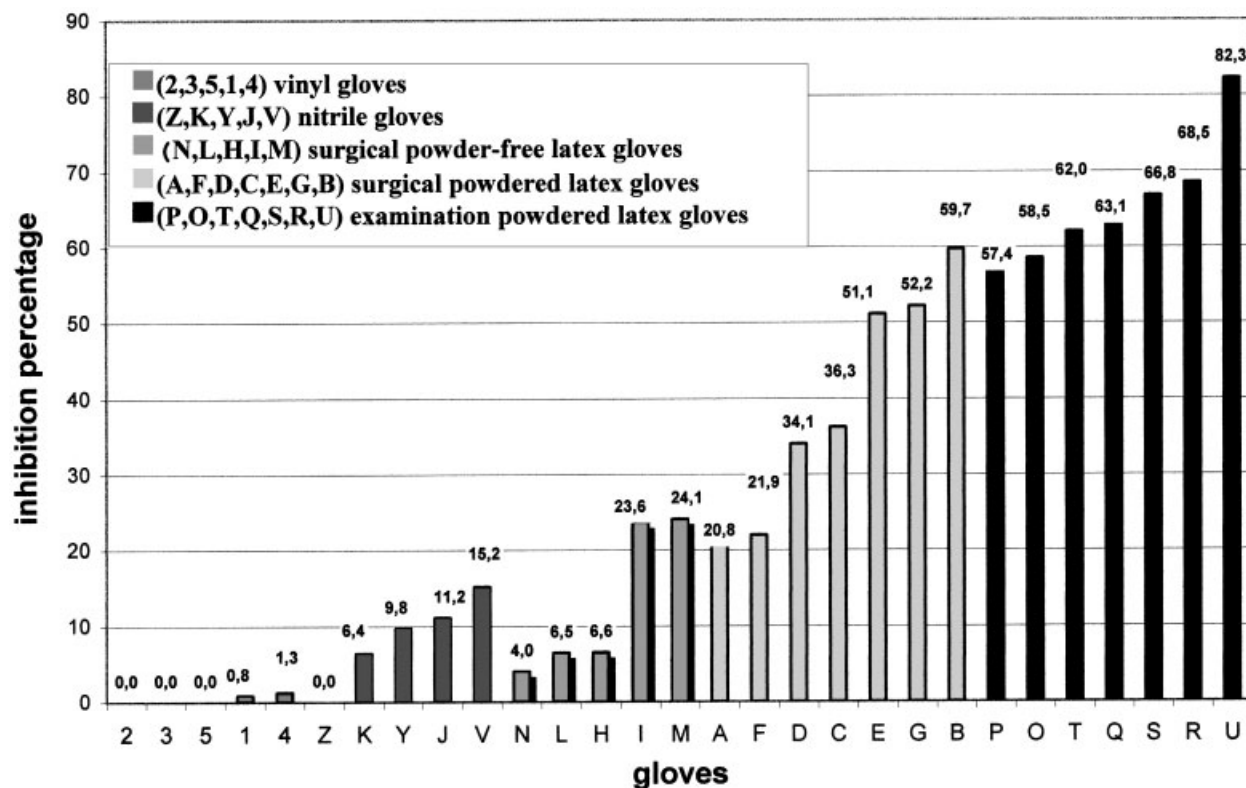


FIGURE 2. Inhibition percentages on glove samples, subdivided by type.

palm, and fingers) of the same glove. The measurements were performed in both latex powder-free surgical gloves and nitrile gloves. There were also no relevant differences between the inhibition percentages in gloves of the same brand belonging to different lots (Fig. 5).

Comparison between the inhibition percentages of three types of latex gloves (L, D, and U) and one nitrile glove (V) after contact with a pool of sera containing anti-mites and anti-Grainaceae-pollen IgE, revealed no relevant aspecific reactions to the glove surfaces. The average inhibition

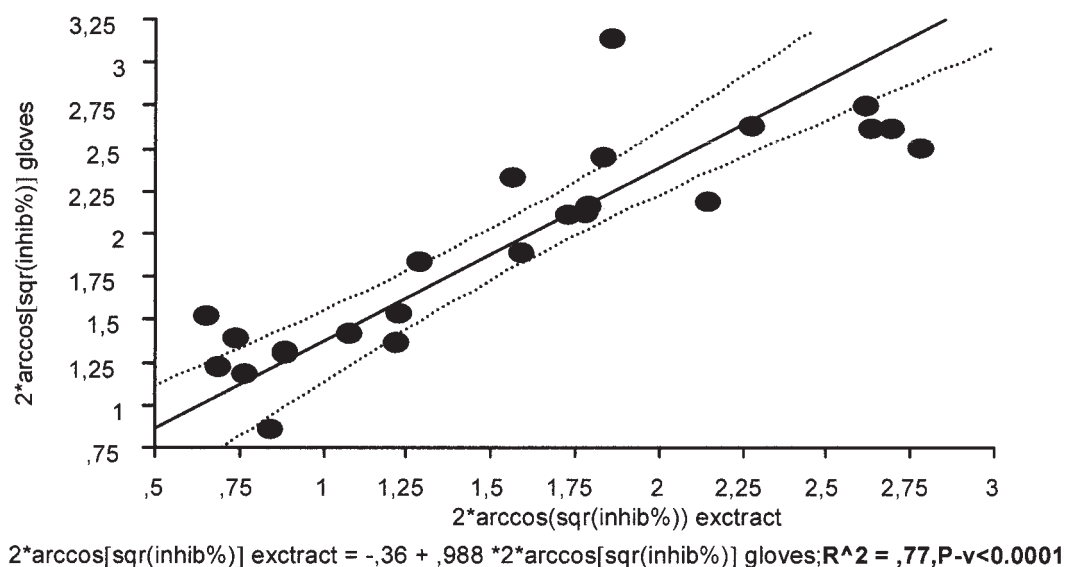
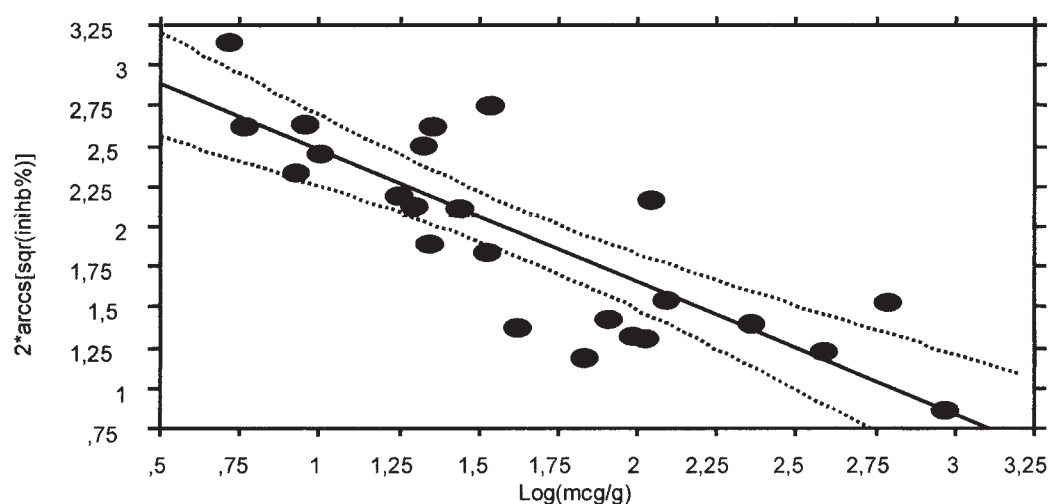


FIGURE 3. Correlation between inhibition percentages in glove samples and glove extracts, expressed as $2 \cdot \arccos[\sqrt{\text{inhib\%}}]$.



$$2 \cdot \arccos[\sqrt{\text{inhib}\%}] = 3,287 - ,815 \cdot \text{Log}(\mu\text{g/g}); R^2 = ,677, P\text{-}v < 0.0001$$

FIGURE 4. Correlation between the total protein contents and the inhibition percentages in all latex gloves.

percentages for the anti-mites IgE ranged from 0% (SD 7.1) in glove V to 4.1% (SD 0.5) in glove U, and for the anti-Grainaceae-pollen IgE the percentages ranged from 1.22% (SD 7.31) in glove D to 7.04% (SD 6.26) in glove V. The highest percentages pointed out (gloves U and V) must be considered negligible.

DISCUSSION

To reduce the risk of latex allergy, especially among health-care workers, most latex glove manufacturers are

trying to reduce the total protein content in the gloves to a level as low as possible.

Currently, there is no agreement among scientists on the parameter of “total protein content” that can be predictive of the allergenic potential of latex gloves. Some studies have shown a clear correlation between the amount of total proteins in the gloves and the allergenic latex protein contents as measured by the RAST-inhibition assay [Yunginger et al., 1993; Beezhold et al., 1996; Palosuo et al., 1998]. However, other authors found little correlation between these two parameters, concluding that the total

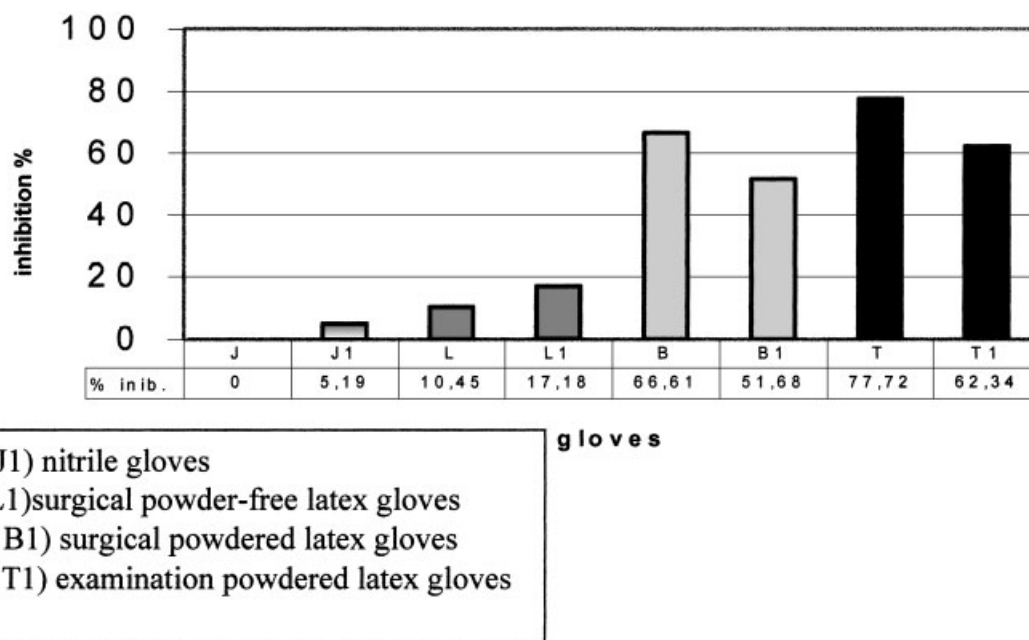


FIGURE 5. Comparison between inhibition percentage in gloves of the same brand belonging to different lots.

protein content alone cannot predict the sensitizing potential of a gloves [Alenius et al., 1994]. It is possible that these different conclusions are based on the different methods used for the preparation of glove latex extract for the RAST-inhibition assay.

In this study the total protein content was measured in a large variety of gloves, and the results show that powdered latex gloves, both surgical and for examination, have significantly higher protein content than powder-free gloves. This observation agrees with the data published in the literature [Yunginger et al., 1993; Beezhold et al., 1996; Palosuo et al., 1998], and it confirms the hypothesis that the lubricant powder is an excellent carrier of latex proteins [Fischer, 1986; Assalve et al., 1988; Seggev et al., 1990; Heese et al., 1991; Crippa and Pasolini, 1997].

Latex proteins were found also in some nitrile gloves. A possible explanation of this is that latex is added to nitrile gloves during production to increase their elastic properties [Villanta et al., 2000]. The presence of latex proteins in these gloves was confirmed by RAST-inhibition assay. This data was further confirmed by clinical observation: subjects allergic to latex could not "tolerate" nitrile gloves, either.

However, among the different nitrile gloves examined in this study, some showed an absolutely negligible protein content, which leads to the conclusion that the way nitrile gloves are produced differs from one manufacturer to another.

It should be underlined that the total protein contents of each brand of glove examined were nearly the same as those declared in the product information sheets.

We used the RAST-inhibition assays directly on glove samples instead of glove extract as an alternative, reliable, and faster way to evaluate the allergenic potential of latex gloves.

We compared the results obtained on glove samples with those obtained on glove extract and found a high correlation, although the inhibition percentages on glove extract were generally higher, probably as a consequence of its concentration (from an initial volume of 4 ml it was reduced to a final volume of 200 µl).

There is a clear correlation between the total protein levels and the allergenic latex protein levels. This fact suggests that the gloves with highest total protein content can have the greatest allergenic potential. On the basis of our experimental model we can exclude that the inhibition percentages that we observed are due to IgE aspecific bindings on the surfaces of the latex or nitrile glove samples.

In conclusion, we believe that the total protein content is relevant from a preventive point of view for a correct glove choice. Therefore, it is advisable that manufacturing companies should provide package inserts containing at least the total protein contents and, possibly, allergenic latex protein levels and the measurement methods used. Before buying a

large amount of gloves, it would be useful to at least verify their total protein content, to assure that the information provided by the manufacturing companies is correct. Health care institutions should select gloves according to the lowest content of extractable protein lowest content and, possibly, powder-free. Our data confirm that powdered latex gloves show generally the highest protein content and glove powder is also a documented irritant factor.

Moreover, the manufacturers must declare whether they have added latex to their nitrile gloves. Meanwhile it is necessary to pay attention in choosing nitrile gloves, especially as an alternative to natural rubber gloves for subjects with latex allergy, since some brands may contain significant amounts of allergenic latex proteins.

Vinyl gloves, on the other hand, seem to be completely latex-free. Therefore, they are a good alternative to latex gloves in situations without high biological risk (since they do not provide the same protection against biological agents) or the need for sterility.

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