

Latex allergy: new insights to explain different sensitization profiles in different risk groups

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Summary

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Conflicts of interest

None declared.

Background Differences in latex allergen sensitization profiles have been described between children subjected to repetitive surgical interventions and health care workers (HCW). 'Major' allergens for patients with spina bifida are Hev b 1, 3 and 7, while for HCW, 'major' allergens are Hev b 2, 5, 6.01 and 13. The reason for these differential sensitization profiles is currently unknown.

Objectives To investigate latex allergen profiles on internal and external surfaces of natural rubber latex gloves.

Methods Eighty-two samples of commonly used surgical gloves (41 glove brands) were used for analysis. Specific allergen levels of Hev b 1, 3, 5 and 6.02 on both surfaces of the gloves were quantified using an enzyme immunometric assay, a FITkit® (FIT Biotech, Tampere, Finland).

Results Differences in allergen levels were observed between internal and external surfaces of all glove types. Concentrations of Hev b 1 and Hev b 3 were significantly higher on external surfaces, while internal surfaces had higher allergen levels of Hev b 5 and Hev b 6.02. Analysis of surgical and examination gloves, powdered and nonpowdered gloves also showed that the content of Hev b 5 and Hev b 6.02 was significantly higher on internal surfaces while that of Hev b 1 and Hev b 3 was higher on external surfaces.

Conclusions Our study showed different allergen profiles on internal and external surfaces of natural rubber latex gloves. These results may suggest a relationship between latex allergen localization and sensitization routes in different risk groups.

Latex sensitization is recognized as a serious health problem as latex products are increasingly used, especially among health care workers (HCW) and in patients undergoing multiple surgical interventions, such as patients with spina bifida.^{1,2} Some epidemiological studies have reported a high incidence and prevalence of latex allergy^{1–4} and that it may even have increased during the past 10 years, especially among HCW.¹

The typical allergic reaction to latex is characterized by pruritus, erythema and oedema. As sensitivity increases, patients may develop urticaria initially restricted to the contact area, although it may eventually spread to contiguous areas of the skin. Airborne exposure may lead to nasal, ocular and pulmonary symptoms.⁵ The severe forms of latex allergy are characterized by varying degrees of an anaphylactic reaction ranging from urticaria to respiratory and cardiovascular symptoms.

The most common symptom in HCW is protein-contact dermatitis, a type I hypersensitivity reaction typically involving the hands after wearing gloves.⁵

Natural rubber latex is a complex mixture of several substances such as rubber particles (30–40%), proteins (2–3%) and water (55–65%). In manufactured products, proteins can comprise up to 3% of the contents.^{6,7} Consensus exists that proteins or peptides eluting from natural rubber latex products are responsible for sensitizations among patients and HCW.^{8,9}

There are two risk groups particularly affected by latex allergy: HCW and patients with spina bifida. Interestingly, despite the fact that these two risk groups become sensitized to latex at health institutions, they have different sensitization profiles to latex allergens. Whereas the 'major' allergens for patients with spina bifida are Hev b 1, Hev b 3 and Hev b 7,

for HCW the 'major' allergens are Hev b 2, Hev b 5, Hev b 6.01 and Hev b 13.^{10,11} The reason for this discrepancy in the allergen sensitization profiles is currently unknown. Although genetic factors may influence this differential pattern of allergen recognition in these risk groups, the different reactivity may also be due to differences in the allergen make-up of internal and external glove surfaces as both sides of gloves are submitted to distinct treatment during manufacture procedures.¹² Therefore, the purpose of the present study was to characterize and identify latex allergens on the internal and external surfaces of natural rubber latex surgical and examination gloves.

Materials and methods

Glove extracts

Glove samples were obtained during 2006 from health institutions in Portugal. Extraction of proteins from the gloves was performed using 5 mL of phosphate-buffered saline (PBS) and 2 g of glove (fingers closed with a knot). The extract of internal proteins was obtained after turning the glove inside out. Extraction was carried out by rotation in a rotator for 2 h, at room temperature. After extraction, the rubber products were removed and the extracts were centrifuged at 1900 g for 15 min, filtered through 0.45 µm filters and stored frozen (−20 °C) until analysis was performed. To avoid cross-contamination of natural rubber latex allergens, vinyl gloves were used for all laboratory procedures.

Capture enzyme immunoassay for quantification of allergens

Quantification of Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 was performed using a commercial kit (FITkit[®]; FIT Biotech, Tampere, Finland) according to the manufacturer's instructions. The concentration of extracted allergens in µg L^{−1} was divided by 400 to obtain extractable allergen content in µg g^{−1}, as 2 g of glove was extracted in 5 mL of PBS.

The detection limits of allergens on FITkit[®] were: Hev b 1, 50 ng g^{−1}; Hev b 3, 50 ng g^{−1}; Hev b 5, 25 ng g^{−1}; Hev b 6.02, 25 ng g^{−1}. When allergen levels were below the detection limit they were denoted as zero.

Statistical methods

Data analyses were performed using SigmaStat version 2.03 (Systat Software, San Jose, CA, U.S.A.). The level of statistical significance was considered to be $P < 0.05$. When the data of the allergens showed skewed distributions, a nonparametric distribution-free test was used for comparison between the different categories.

Results

Eighty-two glove samples, corresponding to internal and external surfaces of 41 glove brands commonly used in health

institutions in Portugal in 2006, were analysed. Gloves were categorized as examination or surgical gloves, powdered or powder free. The levels of the four latex allergens studied ranged from not detectable to 0.17 µg g^{−1} for Hev b 1 and Hev b 3, and from 0.04 to 3.82 µg g^{−1} for Hev b 5 and Hev b 6.02 (Table 1). The most difficult latex allergens to detect were Hev b 1 and Hev b 3, which are allergens with hydrophobic characteristics.

In general, for all types of natural rubber latex gloves, our study showed differences in allergen content (Hev b 1, Hev b 3, Hev b 5, Hev b 6.02) between internal and external surfaces. In some glove brands, Hev b 1 and Hev b 3 had allergen levels below the detection limit of the kit and these were denoted as zero (Table 1).

Hev b 1 and Hev b 3 were found at a higher concentration on external surfaces in 63% and 74% of the gloves, respectively. These statistics did not include the values below the detection limit of the kit. On the other hand, internal surfaces had significantly higher allergen levels of Hev b 5 and Hev b 6.02, in 85% and 73% of the gloves, respectively (Table 2; $P < 0.001$, in both cases).

A comparison made between surgical and examination gloves (Table 3) showed that Hev b 5 and Hev b 6.02 were at higher concentration levels on internal surfaces compared with external, whereas the opposite pattern was found for Hev b 1 and Hev b 3. A more detailed examination of the results revealed that in the group of examination gloves, Hev b 5 was found at higher concentration levels on internal surfaces in 17 of 18 (94%) gloves (Table 3). Also, in examination gloves Hev b 1 showed a higher allergen level on internal surfaces in 58% of the gloves, i.e. seven in 12 gloves showed higher values on internal surfaces than external ones (Table 3). This somewhat different behaviour could be related to the extraction conditions of this hydrophobic allergen.

A comparative analysis was also carried out between powdered and nonpowdered gloves. Results were similar to those shown for the comparison between surgical and examination gloves. The hydrophobic allergens Hev b 1 and Hev b 3 were present in significantly ($P < 0.001$) higher amounts in 100% and 78%, respectively, of external surfaces of nonpowdered gloves. In terms of hydrophilic allergens, the study showed that Hev b 5 and Hev b 6.02 levels were significantly ($P < 0.001$) elevated in 86% and 75%, respectively, of internal surfaces of powdered gloves.

Our results showed that in all types of gloves, Hev b 1 and Hev b 3 were in higher content on external surfaces whereas Hev b 5 and Hev b 6.02 were mainly located on internal surfaces.

Discussion

The present study showed the interesting and novel observation that the internal and external surfaces of latex gloves have different allergen profiles. These results could provide useful information regarding the sensitization patterns developed in latex-allergic patients.

Table 1 Allergen levels of internal and external surfaces in different glove brands

Glove brand®	Hev b 1 ($\mu\text{g g}^{-1}$), internal/external	Hev b 3 ($\mu\text{g g}^{-1}$), internal/external	Hev b 5 ($\mu\text{g g}^{-1}$), internal/external	Hev b 6.02 ($\mu\text{g g}^{-1}$), internal/external
Nonsterile examination gloves (powdered)				
Basto Viegas	0.00/0.00	0.06/0.06	0.81/0.33	0.95/0.88
Bioshield	0.05/0.05	0.00/0.06	2.17/0.97	1.10/0.96
Eurotubo Deltalab	0.00/0.00	0.07/0.09	1.17/0.64	1.01/0.60
Gant	0.05/0.05	0.00/0.00	2.79/1.40	0.36/0.33
Mediquet	0.05/0.05	0.00/0.00	2.60/1.60	0.31/0.25
Romed	0.00/0.00	0.08/0.11	1.06/0.88	0.81/0.46
Ruthe	0.05/0.05	0.00/0.00	2.50/0.67	0.29/0.22
Sanger PrimaGrift	0.05/0.05	0.00/0.00	3.74/0.38	0.38/0.31
Star	0.05/0.06	0.00/0.00	3.82/2.39	0.41/0.38
Topstardispendent	0.05/0.06	0.00/0.00	2.76/2.16	0.36/0.36
UlmaClassic	0.05/0.05	0.00/0.00	3.26/1.04	0.29/0.30
Vasco	0.00/0.05	0.08/0.09	0.54/0.33	0.59/0.87
VWR	0.05/0.05	0.00/0.00	0.73/0.39	0.12/0.11
Nonsterile examination gloves (nonpowdered)				
Eurotubo Deltalab	0.09/0.00	0.07/0.12	0.36/0.25	0.35/0.34
Manufix	0.00/0.00	0.07/0.10	0.30/0.28	0.42/0.38
Ruthe	0.00/0.00	0.04/0.07	0.27/0.26	0.35/0.35
Topstar	0.05/0.05	0.00/0.00	0.49/1.00	0.04/0.04
Vaz Pereira	0.00/0.00	0.11/0.11	0.59/0.45	0.36/0.33
Sterile surgical gloves (powdered)				
Comfit	0.00/0.05	0.12/0.11	0.61/0.28	0.67/0.37
Hassemed	0.05/0.11	0.00/0.00	0.72/0.42	0.78/0.38
Maxitex	0.05/0.06	0.00/0.08	0.67/1.08	0.12/0.12
Maxitex duplex PF	Brown: 0.00/0.05 Yellow: 0.00/0.05	Brown: 0.06/0.08 Yellow: 0.08/0.11	Brown: 0.44/0.37 Yellow: 0.29/0.27	Brown: 0.34/0.33 Yellow: 0.37/0.34
Medi-Grip Plus	0.07/0.05	0.00/0.05	0.31/0.42	0.52/0.52
Motex	0.00/0.05	0.00/0.00	2.27/2.66	0.35/0.35
Neoglove	0.05/0.05	0.00/0.00	1.35/0.55	0.41/0.25
Nugard	0.05/0.05	0.00/0.00	2.74/1.69	0.09/0.06
Romed	0.00/0.00	0.00/0.00	0.26/0.25	0.37/0.33
Sempermed Classic	0.00/0.05	0.00/0.00	2.16/1.52	0.37/0.35
Sempermed	0.05/0.05	0.00/0.00	0.60/0.37	0.10/0.06
Surg G	0.05/0.05	0.00/0.00	0.50/0.70	0.09/0.06
Triflex	0.05/0.06	0.00/0.00	1.01/0.92	0.21/0.26
Vasco Surgical	0.05/0.00	0.00/0.00	0.79/0.67	1.10/0.98
Sterile surgical gloves (nonpowdered)				
Cardinal Health	0.00/0.05	0.12/0.17	0.40/0.39	1.10/0.92
Garal	0.00/0.00	0.10/0.11	0.27/0.27	0.34/0.33
Motex	0.00/0.00	0.07/0.08	0.27/0.26	0.34/0.33
Nugard	0.00/0.00	0.09/0.08	0.71/0.57	0.93/0.74
PMH	0.00/0.00	0.10/0.11	0.43/0.32	0.35/0.34
Profeel	0.05/0.06	0.00/0.00	1.30/0.48	0.24/0.04
Protegrity	0.05/0.06	0.00/0.00	0.93/0.70	0.24/0.29
Sensiflex	0.05/0.06	0.00/0.00	0.70/0.39	0.04/0.04

Latex sensitization is linked to proteins from natural rubber latex gloves. Several hundred proteins have been described in latex and 16 of them (Hev b 1 to Hev b 13) have been recognized as latex allergens by the Allergen Nomenclature Sub-Committee of the World Health Organization/International Union of Immunological Societies.¹³

Researchers commonly assess allergens levels of the whole glove to determine their potential risk for latex allergy. Koh *et al.*¹⁴ performed a study in gloves used by HCW in Singapore to determine allergen levels in examination, surgical, pow-

dered and nonpowdered gloves. They concluded that natural rubber latex allergens were present in the majority of examination gloves at levels high enough to cause latex allergy in sensitized patients.

Among HCW, the most important latex allergens are Hev b 2, Hev b 5, Hev b 6.01 and Hev b 13. Hev b 6.01 (prohevein) is the precursor protein of Hev b 6.02 (mature hevein) and hevein carries the 'major' IgE epitope of prohevein. Hev b 1, Hev b 3 and Hev b 7 are 'major' allergens for patients with spina bifida. In our study, Hev b 6.01, Hev b 7 and Hev b 13

Table 2 Comparison of allergen levels between internal and external surfaces of all types of gloves

Allergen levels ($\mu\text{g g}^{-1}$)	Type of surface		P-value ^a
	Internal (n = 41), n (%)	External (n = 41), n (%)	
Hev b 1	11 (37) ^b	19 (63) ^b	<0.001
Hev b 3	5 (26) ^b	14 (74) ^b	<0.001
Hev b 5	35 (85)	6 (15)	<0.001
Hev b 6.02	30 (73)	11 (27)	<0.001

^aWilcoxon signed rank test. ^bWe did not consider values of zero on allergen levels.

allergens were not analysed in the glove samples because there is no commercial test available for measurement of these allergens. Nevertheless, studies performed by several researchers support the view that Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02 represent useful and sufficient marker proteins reflecting allergenicity of natural rubber latex products.^{3,15}

Our results showed that there are qualitative as well as quantitative, statistically significant differences on allergen profiles between internal and external surfaces of the analysed latex gloves. The 'major' allergens for HCW, Hev b 5 and Hev b 6.02, were found in higher amounts on internal surfaces of the gloves. HCW are a very important risk group for latex allergy as they regularly wear surgical rubber gloves and thereby contact with the internal surface of the gloves and the lubricant powder which is rich in internal latex proteins.^{16–18} According to Ahmed *et al.*,⁵ the most frequent latex exposure-related symptom in HCW after wearing gloves is IgE-mediated contact dermatitis, a reaction usually involving the hands. This reaction to latex proteins includes eczematous lesions that could become chronic as the exposure persists. In line with this, some studies have reported that avoidance of latex products dramatically reduces the number of HCW who had been unable to work because of latex exposure.^{18,19} Thus, consensus exists that the environment plays a crucial role in determining the development of allergic diseases. The sensitization profile of HCW suggests that they may contact preferentially with their 'major' allergens, such as Hev b 5, Hev b 6.01 and Hev b 13. Our study demonstrated that, in 41 latex glove

brands, Hev b 5 and Hev b 6.02 were in higher amounts on internal surfaces. These novel results suggest that the sensitization route may play an important role in latex allergy in this risk group.

In our previous studies²⁰ we have demonstrated the existence of a differential distribution of allergens in two natural rubber latex glove brands, using sodium dodecyl sulphate–polyacrylamide gel electrophoresis, immunoblotting, hydrophobic interaction chromatography and enzyme-linked immunosorbent assay (ELISA). We showed that internal surfaces were enriched in Hev b 5 and Hev b 6.02 and external surfaces had higher contents of Hev b 1 and Hev b 3. In the present study, we have extended these observations to a wide array of glove brands and observed by ELISA that internal and external surfaces of 41 latex glove brands have different allergen profiles (Table 1). This nonuniform allergen distribution may be associated with different degrees of sensitization risk for HCW and patients with spina bifida, which can result in the different reactivity patterns to latex allergens observed in these two groups.^{10,11} Therefore, we propose that these two risk groups become sensitized to latex by different routes of exposure. HCW contact directly with the internal surfaces of latex gloves where we found higher contents of Hev b 5 and Hev b 6.02, 'major' allergens for this risk group. Additionally, they may inhale aerosols of glove powder coated with natural rubber latex allergens of inner surfaces that contact with the mucous membrane of the nose, throat and airways of the lung.^{17–19,21} On the other hand, the 'major' allergens for patients with spina bifida were detected at higher levels on the external surface of latex gloves, and patients may become sensitized by contact during surgical procedures.²²

The differences in the allergen make-up of internal and external surgical glove surfaces, found in the present work, may result from the manufacture procedure. The method of production including the means used to coat gloves to make donning them easy can influence the eventual exposure to latex allergens on the glove. In several manufacturing processes of latex gloves, during the vulcanization process the water trapped in the latex film is turned into steam and escapes through it. As the steam passes the film, it carries residual nitrates and soluble proteins. Consequently, the surface of the latex glove that is on the outside of the mould (which will be the internal surface when the glove is put on) is rich in these materials.¹²

Table 3 Comparison of allergen levels between internal and external surfaces of surgical gloves and examination gloves

Allergen levels ($\mu\text{g g}^{-1}$)	Surgical gloves (n = 23), n (%)			Examination gloves (n = 18), n (%)		
	Internal	External	P-value ^a	Internal	External	P-value ^a
Hev b 1	4 (22) ^b	14 (78) ^b	<0.001	7 (58) ^b	5 (42) ^b	0.063
Hev b 3	3 (30) ^b	7 (70) ^b	<0.001	2 (22) ^b	7 (78) ^b	<0.001
Hev b 5	18 (78)	5 (22)	<0.001	17 (94)	1 (6)	<0.001
Hev b 6.02	17 (74)	6 (26)	<0.001	13 (72)	5 (28)	<0.001

^aWilcoxon signed rank test. ^bWe did not consider values of zero on allergen levels.

Interestingly, our study also showed higher concentrations of allergens on internal surfaces of the gloves (Table 1). As these surfaces had most of the extractable latex allergens, the results may explain the high rate of cutaneous manifestations observed in latex-allergic HCW.²⁰

The latex allergy epidemic has had its highest peak in the last two decades but to ensure continuing good management of the problem, natural rubber medical devices should contain low allergen levels. The data presented here indicate that there are quantitative as well as qualitative differences in allergen profile on internal and external surfaces of latex gloves. The major allergen profiles for HCW and patients with spina bifida found, respectively, on internal and external surfaces of the gloves suggest a relationship between latex allergen localization and sensitization routes in different risk groups.

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