

## Extractable latex allergens in airborne glove powder and in cut glove pieces

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### Summary

**Background** Quantification of natural rubber latex (NRL) allergens of NRL glove extracts has been reported in several studies. Similarly, immunoassay studies reporting the level of NRL aeroallergens in air samples have been published. When studying the NRL allergens of gloves, however, little attention has been focused on identifying the relationship between extractable NRL allergens of medical gloves and NRL aeroallergens in indoor air.

**Objective** In an experimental study we analysed NRL aeroallergens of medical gloves in joint relation to total airborne dust concentration and NRL allergen concentration in gloves.

**Methods** NRL aeroallergen level was measured using a chamber setting with 18 lots of powdered medical gloves. In each setting 10 pairs of powdered NRL gloves were swinging in an unventilated chamber (9 m<sup>3</sup>). Air samples were collected using airflow through Millipore filters (pore size 0.8 µm). The filters were weighed before and after the experiment, and total airborne dust concentration in the chamber was calculated. The filter samples were then extracted and the NRL allergen level measured by IgE ELISA-inhibition assay. Furthermore, cut NRL gloves were extracted and analysed by the same method. Finally, levels of two major NRL allergens, Hev b1 and Hev b 6.02, were measured in three selected NRL glove brands.

**Results** The NRL aeroallergen level in the chamber air ranged from <0.9 to 2.9 allergen units (AU)/m<sup>3</sup>. The total airborne dust concentration in the chamber air remained low with all lots of gloves measured (range < 20 to 80 µg/m<sup>3</sup>). The NRL allergen level in cut glove extracts varied over 100-fold (< 10 to 1050 AU/mL). Statistically significant correlation between aeroallergen concentration and airborne dust ( $r = 0.8$ ,  $P = 0.0015$ ) concentration was found. Moreover, significant correlation between aeroallergen levels and allergen content of cut glove pieces was observed ( $r = 0.59$ ,  $P < 0.05$ ). Hev b 1 levels varied from 9 to 25 ng/mL and the levels of Hev b 6.02 from 1720 to 14460 ng/mL in the glove extracts. In the extracts from airborne dust samples, Hev b 6.02 content varied from 61 to 183 ng/m<sup>3</sup>, whereas Hev b 1 levels were very low (0.4 to 3 ng/m<sup>3</sup>).

**Conclusion** An elevated NRL aeroallergen level is rather related to a high level of airborne glove powder than to a high concentration of extractable NRL allergen in medical gloves.

**Keywords** aerosol, allergens, ELISA inhibition, glove powder, medical gloves, natural rubber latex

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### Introduction

Natural rubber latex (NRL) is the most common source material for surgical and examination gloves because of its excellent tensile strength as well as its good barrier properties due to its film forming ability. Over the past decade, however, allergy to latex proteins has become acknowledged as a major occupational problem among glove-using healthcare workers. Allergy to NRL is being recognized with increasing frequency among healthcare workers [1–4] as well as among rubber glove

manufacturing workers [5]. Moreover, children with spina bifida (SB) and other congenital anomalies requiring multiple surgical operations and thereby frequent exposure to various latex devices, are at high risk of developing allergy to NRL [6–9]. Substantial progress has been made in recent years in the purification and molecular characterization of NRL allergens, which has facilitated the assessment of their clinical significance [10, 11]. The International Union of Immunological Societies Allergen Nomenclature Sub-Committee now lists 11 NRL allergens characterized at the molecular level (<http://www.allergen.org>). The most important NRL allergens seem to be Hev b 5 and Hev b 6 for adults, and Hev b 1 and Hev b 3 for children with SB [12].

The total extractable allergen content is highly variable in NRL gloves [11, 13]. Knowledge about the presence of the

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specific allergens in the NRL gloves and other manufactured rubber products is rapidly increasing but still scanty. Two major NRL allergens, Hev b 1 and Hev b 6.02, however, have been identified and purified from the NRL gloves [14, 15]. The NRL allergen content seems, as a rule, to be higher in powdered gloves than in powder-free gloves [16]. Importantly, the powdered NRL gloves are also the major contributor to NRL aeroallergen levels in indoor air, and the contents of NRL aeroallergens are highly variable [17–20]. Use of gloves without powder and gloves with low NRL allergen concentration have been recommended [21]. However, in powdered brands of gloves, the relationship between the NRL aeroallergen content measured from airborne glove powder and NRL allergen level in glove extract is not known.

The aim of the present study was to quantify NRL allergens in airborne NRL glove powder and in cut latex gloves. Furthermore, we quantified Hev b 1 and Hev b 6.02 levels in airborne glove powder and in extracts from cut glove pieces.

## Materials and methods

### Collection of gloves

In March 1997, altogether 12 brands of medical gloves commonly used in Finland were selected for testing. Seven brands were non-sterile examination gloves, one brand was a sterile examination glove, and three brands were sterile surgical gloves. One non-sterile vinyl glove was selected as a negative control. In December 1997 six additional brands of gloves (nos. 8, 9, 11 and 15, 16, 17) were selected from those on the common market in 1990 to 1996 in Finland (Table 1). Only powdered NRL gloves were chosen for the present study. Airborne glove powder sample collection was conducted at

the Finnish Institute of Occupational Health on separate days for each brand of gloves.

### Glove extracts and air samples

Standard extraction of proteins from NRL gloves (1 g glove cut to small pieces in 5 mL of phosphate-buffered saline, pH 7.2) was carried out at the Laboratory of Immunobiology of the National Public Health Institute.

Air sampling was conducted in an unventilated chamber (9 m<sup>3</sup>). The walls and the floor of the chamber were covered with ceramic tiles. In the chamber, the covering bag of each pair of surgical gloves was opened and 10 pairs of gloves were withdrawn one by one and attached onto a stationary holder. The chamber room was cleaned after each measurement. Similarly, 20 non-sterile examination gloves were taken one by one from their box, withdrawn and attached on the stativa. During the whole air sampling, an electric fan was directed towards the hanging gloves to produce airflow between the gloves. Stationary filter holders and three area air samplers (Reciprotar Model TTL-1, Levy Ky, Espoo, Finland) with constant airflow through filters with a pore size of 0.8 µm (MF-Millipore AAWP 03700, Millipore Intertech, Bedford, MA, USA) were used for each setting of air sampling. The air samplers were operated for 4 h at a flow rate of 20 L/min. The air volume readings of each sampler were recorded. In calculating the total airborne dust concentration the dust weight on each filter specimen was divided by the air volume (L) of the respective sample. In further comparison, the mean of the three determinations of total airborne dust concentration measured by the three samplers was used for each brand of gloves. The detection limit of the dust weight measurement was 0.1 mg, allowing the detection of 20 µg/m<sup>3</sup> in 5 m<sup>3</sup> of air.

**Table 1.** Trade names and manufactures of the 18 medical gloves measured

| Glove no.                              | Trade name                          | Manufacturer/distributor | Town and country          |
|----------------------------------------|-------------------------------------|--------------------------|---------------------------|
| <i>Examination gloves, non-sterile</i> |                                     |                          |                           |
| 1                                      | Glads White non-sterile             | Mölnlycke                | Waremme, Belgium          |
| 2                                      | Sempermed non-sterile               | Semperit                 | Wien, Austria             |
| 3                                      | Baxter 1Y6N015 non-sterile          | Baxter                   | Valencia, California, USA |
| 4                                      | Comfort non-sterile                 | Ansell Medical           | Melbourne, Australia      |
| 5                                      | Top Dent non-sterile                | Oriola hammasväline      | Helsinki, Finland         |
| 6                                      | Golden Hand non-sterile             | Oriola sairaalaväline    | Helsinki, Finland         |
| 7                                      | Nordstock non-sterile               | Nordstock                | Helsinki, Finland         |
| 8                                      | Glads White 612100                  | Mölnlycke                | Waremme, Belgium          |
| 9                                      | Glads Textured Green                | Mölnlycke                | Waremme, Belgium          |
| <i>Examination gloves, sterile</i>     |                                     |                          |                           |
| 10                                     | Comfort sterile examination glove   | Ansell Medical           | Melbourne, Australia      |
| 11                                     | Glads sterile                       | Mölnlycke                | Waremme, Belgium          |
| <i>Surgical gloves</i>                 |                                     |                          |                           |
| 12                                     | Sempermed Derma Plus sterile        | Semperit                 | Wien, Austria             |
| 13                                     | Triflex 2D72541                     | Baxter                   | Valencia, California, USA |
| 14                                     | Surgifit sterile surgical glove     | Nordstock                | Helsinki, Finland         |
| 15                                     | Triflex 2D7153                      | Baxter                   | Valencia, California, USA |
| 16                                     | Triflex                             | NV Travenol              | Lessines, Belgium         |
| 17                                     | Sempermed CR6E5                     | Semperit                 | Wien, Austria             |
| <i>Vinyl gloves</i>                    |                                     |                          |                           |
| 0                                      | Vinyl non-sterile examination glove | Mölnlycke                | Waremme, Belgium          |

### Preparation of air filter samples and measurement of total NRL allergen levels by IgE-ELISA inhibition

The filters were coded and placed in filter holders in random order. Furthermore, the filters were weighed after 24 hours' air conditioning (40% relative humidity and temperature at + 20 °C) before and after the air sampling. For the measurement of aeroallergen level of a single brand of gloves, three air filters and one unused filter as a negative control were prepared and analysed. All the total airborne dust concentrations and aeroallergen levels were means of three determinations. For the immunoanalysis, the filters were cut to small pieces and extracted as above. Enzyme-linked immunosorbent inhibition assay was then performed as described elsewhere [22] by using an automated reader (Titertek Multiskan, Eflab, Espoo, Finland). Briefly, a pool of high-titred sera from three NRL-allergic adults and three NRL-allergic children with spina bifida was used as a source for NRL-specific IgE antibodies to the most clinically significant NRL allergens (including hevein, prohevein, hevein C-domain and rubber elongation factor). The source material, i.e. liquid latex from the rubber tree (total protein concentration 10 mg/mL), was assigned to contain 100 000 AU/mL [22]. The readings were transformed to allergen units (AU) per mL using a standard inhibition curve. Finally, the NRL aeroallergen level was calculated by dividing the measured AU/mL concentration in an air filter specimen with the sampled air volume.

### Analysis of Hev b 6.02 (hevein) and Hev b 1 (rubber elongation factor)

Further ELISA inhibitions, using polyclonal monospecific rabbit antibodies to Hev b1 and to Hev b 6.01, respectively [22], were performed with the glove extracts and the air filter samples to detect Hev b 1 and Hev b 6.02 concentration. Samples of three brands of gloves with detectable aeroallergen concentration (gloves nos. 8, 9 and 11 in Table 1) were analysed.

### Statistical analysis

Bivariate correlation test (Pearson) was used to compare the airborne allergen level with total airborne dust concentration and total allergen level in cut glove pieces. Fourteen NRL gloves with detectable extractable allergen levels (> 10 AU/mL; see Table 2) were selected for correlation analysis. Strength of the linear relationship between the variables was calculated, and a *P*-value smaller than 0.05 was considered statistically significant.

## Results

### Airborne dust measurements

Altogether 54 air samples were collected during the measurements. In a single sample, the air volume varied from 4007 to 7432 L, with a mean of 5186 L. In the present chamber experiment, the total airborne dust concentration remained low with all 17 brands of medical NRL gloves and one control brand of vinyl examination gloves measured (Table 2). The maximum weight of the total airborne dust on a single filter was 0.6 mg with an air volume of 5074 L representing a dust concentration of 120 µg/m<sup>3</sup>. With four brands of examination gloves the total airborne dust concentration in the chamber air was more than

**Table 2.** The means of the total airborne dust concentrations during the experiment in the chamber, the NRL allergen concentrations in glove extracts, and the means of airborne NRL allergen levels in the chamber measured with 17 brands of NRL gloves and one vinyl glove

| Glove no.                              | Dust concentration (µg/m <sup>3</sup> ) | Allergen concentration in glove extract (AU/mL) | Airborne level of allergen (AU/m <sup>3</sup> ) |
|----------------------------------------|-----------------------------------------|-------------------------------------------------|-------------------------------------------------|
| <i>Examination gloves, non-sterile</i> |                                         |                                                 |                                                 |
| 1                                      | < 20                                    | 127                                             | 0.4                                             |
| 2                                      | < 20                                    | 31                                              | < 0.1                                           |
| 3                                      | < 20                                    | < 10                                            | < 0.1                                           |
| 4                                      | 30                                      | 18                                              | < 0.1                                           |
| 5                                      | < 20                                    | 113                                             | 0.1                                             |
| 6                                      | 80                                      | 153                                             | 2.6                                             |
| 7                                      | 70                                      | < 10                                            | < 0.1                                           |
| 8                                      | 80                                      | 1050                                            | 2.9                                             |
| 9                                      | 40                                      | 507                                             | 2.6                                             |
| <i>Examination gloves, sterile</i>     |                                         |                                                 |                                                 |
| 10                                     | 110                                     | < 10                                            | < 0.1                                           |
| 11                                     | 80                                      | 332                                             | 1.2                                             |
| <i>Surgical gloves</i>                 |                                         |                                                 |                                                 |
| 12                                     | 30                                      | 35                                              | < 0.1                                           |
| 13                                     | < 20                                    | 15                                              | < 0.1                                           |
| 14                                     | 30                                      | 446                                             | 0.2                                             |
| 15                                     | < 20                                    | 31                                              | < 0.1                                           |
| 16                                     | < 20                                    | 705                                             | 0.2                                             |
| 17                                     | < 20                                    | 95                                              | < 0.1                                           |
| <i>Vinyl gloves</i>                    |                                         |                                                 |                                                 |
| 0                                      | < 20                                    | < 10                                            | < 0.1                                           |

60 µg/m<sup>3</sup> (Table 2). The levels of total airborne powder dust concentration of all surgical gloves were low ( $\leq 30$  µg/m<sup>3</sup>) in the present chamber experiment.

### Total NRL allergen content

In glove extracts, more than 100-fold variation in the levels of NRL allergens was found (Table 2). Altogether, three (18%) glove extract samples of these 17 NRL gloves had a low level of extractable NRL allergens (< 10 AU/mL), six samples (35%) had a moderate level (10 to 100 AU/mL) and eight samples (47%) had a high level (> 100 AU/mL) of extractable NRL allergens.

In filter extracts, NRL allergen content varied from < 0.1 to 21 AU/mL. Divided by sampled air volume, this resulted in 29-fold variation (from < 0.1 to 2.9 AU/m<sup>3</sup>) of NRL aeroallergen concentration in the chamber air (Table 2). In seven filter extracts (41%) detectable levels of aeroallergens were found but in 10 extracts aeroallergen levels remained below the sensitivity of the assay.

### Hev b 1 and Hev b 6.02 measurements

Three brands of NRL gloves (nos. 8, 9 and 11) with detectable levels of NRL allergens in airborne glove dust were selected for determination of Hev b 1 and Hev b 6.02 content. The concentrations of these hevein compounds in the selected glove extracts and respective filter specimen are provided in Table 3.

**Table 3.** Hev b 1 and Hev b 6.02 allergen concentrations in glove extracts and airborne levels in selected NRL gloves

| Glove No. | Hev b 1                  |                                     | Hev b 6.02               |                                     |
|-----------|--------------------------|-------------------------------------|--------------------------|-------------------------------------|
|           | In glove extract (ng/mL) | Airborne level (ng/m <sup>3</sup> ) | In glove extract (ng/mL) | Airborne level (ng/m <sup>3</sup> ) |
| 8         | 25                       | 1.1                                 | 1720                     | 183                                 |
| 9         | 9                        | 0.4                                 | 8480                     | 61                                  |
| 11        | 14                       | 3.4                                 | 14460                    | 113                                 |

#### *Comparison of airborne dust content, total NRL allergen content, and Hev b 1 and Hev b 6.02 contents*

Fourteen NRL gloves, with detectable extractable NRL allergen levels ( $>10$  AU/mL), were selected for analysis. A statistically significant correlation ( $r=0.8$ , Pearson's correlation coefficient,  $P=0.0015$ ) between aeroallergen concentration and airborne dust concentration was found. No significant correlation between airborne dust concentration and allergen content of cut glove pieces was found. Significant, though modest, correlation between aeroallergen concentration and allergen content of cut glove pieces was observed ( $r=0.59$ ,  $P<0.05$ ).

## Discussion

For evaluation of allergic respiratory responses to occupational NRL exposure, the information regarding allergen content in medical gloves in use is useful, but the quantification of NRL aeroallergen concentration is also needed. The results of the present study show that although the NRL allergen content varies considerably among the glove extracts, the association between the amount of extractable NRL allergens in powdered gloves and the level of NRL aeroallergens in the space where gloves have been used is quite low. However, in all measurements a high level of airborne NRL allergen was associated with a high level of NRL allergen concentration in glove extract. A high level of airborne NRL allergens may exist when using powdered gloves having an allergen concentration more than 150 AU/mL in glove extract.

Market surveillance studies on different brands of medical gloves with information on the content of NRL allergens of latex gloves are now available, at least in Finland and in Sweden. The powdered medical glove types that were measured in the first stage of the present study were selected on the basis of their previous high allergen content [22]. However, only four of these 12 brands of gloves still had a high allergen content ( $>100$  AU/mL) in 1997, while in eight brands of the gloves the allergen content had decreased remarkably. During 1994 to 1997 the proportion of high-allergen gloves out of all medical gloves commercially available in Finland decreased from 30% to 14%, while the proportion of moderate-allergen gloves increased. Low-allergen gloves retained more than half of the market [23]. The aim of the present study was to measure NRL aeroallergen content among gloves with high allergen content. Therefore six additional brands of high-allergenic NRL gloves available on the common market during 1990 to

1996 were selected for the experimental chamber measurement in the second stage of the study.

In the present study the concentration of NRL allergens in glove extracts was, as expected, highly variable. However, both the total airborne dust and the NRL aeroallergen concentrations were rather low in all of the gloves measured. It is generally agreed that there may be considerable differences between the allergen levels of NRL gloves made by different manufacturers, between different glove brands, and even between gloves of different batches from a single manufacturer [16, 22, 24]. Moreover, it has been recently suggested that a NRL aeroallergen level of  $0.6$  ng/m<sup>3</sup> is a critical threshold for appearance of symptoms for healthcare workers who are sensitized to NRL [25].

In a previous report, a positive correlation was found between the number of NRL gloves used and the level of NRL aeroallergens [19]. In the present study, the number of NRL gloves was the same in each measurement of the aeroallergen level and the airborne samples were collected and measured by a standardized method (Finnish standard SFS 3860). In the previous intervention study, the change of high-allergen-containing NRL gloves to low-allergen-containing NRL gloves reduced levels of latex aeroallergens to less than 1/10 in a medical operating room [19]. NRL aeroallergen concentration depends both on the total airborne dust concentration and on the level of NRL allergens in medical gloves. In evaluating the risk of airborne allergies these both have to be taken into account. Interestingly, of the two specific NRL allergens analysed, only hevein (Hev b 6.02) could be detected in the filter samples collected from the airborne glove dust. Our results therefore suggest that Hev b 6.02 may play a major role in NRL-induced allergic rhinitis and asthma.

In accordance with previous findings, airborne NRL aeroallergens can be detected by air sampling and specific immunoassay of the samples [17, 25]. Although the concentrations of NRL aeroallergens showed a nearly 30-fold variation, the present results suggest that the NRL aeroallergen level depends rather on the total airborne dust level than on the concentration of extractable NRL allergens in gloves. Further studies are needed to determine levels of specific NRL allergens both in glove extracts and in airborne glove dust.

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