

Content of Asthmagen Natural Rubber Latex Allergens in Commercial Disposable Gloves

C. Bittner, M.V. Garrido, L.H. Krach, and V. Harth

Abstract

The use of natural rubber latex (NRL) gloves in many occupations may lead to latex sensitization, allergic asthma, and skin reactions. Due to their good properties and environmental safety NRL gloves are still being used in the healthcare setting, but also in the food industry, by hairdressers, cleaners, etc. The aim of our study was to assess the protein and NRL allergen content in commercial gloves by different methods, including a new assay. Twenty commercially available NRL gloves were analyzed. Protein extraction was performed according to the international standard ASTM D-5712. Total protein content was measured with a modified Lowry method, NRL content with the CAP Inhibition Assay, the Beezhold ELISA Inhibition Assay, and an innovative ELISA with IgY-antibodies extracted from eggs of NRL-immunized hens (IgY Inhibition Assay). We found a high protein content in a range of 215.0–1304.7 µg/g in 8 out of the 20 NRL gloves. Seven of the 20 gloves were powdered, four of them with a high protein content. In gloves with high protein content, the immunological tests detected congruently high levels of NRL allergen. We conclude that a high percentage of commercially available NRL gloves still represent a risk for NRL allergy, including asthma. The modified Lowry Method allows to infer on the latex allergen content.

Keywords

IgY-antibodies • IgY inhibition assay • Latex allergy • Latex gloves

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1 Introduction

Natural rubber latex (NRL) gloves have been in use since the nineteenth century, initially for protecting patients and healthcare workers

(HCW) from communicable diseases. Over the last 30 years, the use of latex gloves has increased exponentially and its use has spread to other sectors, like the food industry, hairdressing, cleaning services, etc. (Mota and Turrini 2012). Although the allergenicity of NRL was recognized early, international attention to the relevancy of type I allergic reactions to latex was first developed from the beginning of the 1980s as skin problems and respiratory type I allergies rose to a major problem especially for HCW (Cabanes et al. 2012). At the time at which NRL with high allergen content and powdered NRL gloves were still in use, the prevalence of latex allergy among HCW was considerably high (up to 11 %) (Vandenplas 1995; Turjanmaa et al. 1988).

NRL is a cytoplasmic exudate of the latic chyle of the tropical *Hevea brasiliensis* tree and contains most of the known proteins found in any plant cell (Lamberti et al. 2015). The high protein content bears a high allergenic potential of NRL materials. To date, up to 15 allergens and additional isoallergens have been included in the IUIS nomenclature (International Union of Immunological Societies Allergen Nomenclature Subcommittee 2015). One of the main reasons for the high risk of developing respiratory allergies was the powder layer used in the NRL gloves, since the powder acted as a carrier of the NRL allergens facilitating its inhalation (Tarlo et al. 1994). The protein content of NRL gloves and therefore their allergenic potential can be considerably reduced by eliminating the powder and through manufacturing procedures, like leaching (extensive washing processes). Thus, in order to protect HCW from NRL-related allergies, the use of powdered NRL gloves was banned for the healthcare sector in the 1990s. In addition, international regulations (i.e., European DIN standard EN 455-3) have recommended to limit the total protein content in NRL gloves for use in healthcare to the minimum possible. A guide value of a maximum of 30 µg protein per gram of glove material has been recommended in the corresponding German technical standard (BAuA 2008). The powdered gloves ban and the allergen contents limit are restricted to

HCW. Regulations to protect workers in other sectors do not yet exist. Thus, occupations, such as grocers, hairdressers, and cleaners as well as users in non-occupational settings may still remain at relevant risk for developing latex allergy.

In addition, standards for the measurement of latex content in NRL gloves have been introduced (e.g., US-standard ASTM D 6499). The methods recommended in the standards have several disadvantages regarding standardization, reliability, and costs. Moreover, the Beezhold ELISA Inhibition Assay, which is considered the standard for the determination of latex allergen content in NRL products, raises major ethical concerns, since it requires to sacrifice the animals, which makes it difficult to reconcile with the current animal protection awareness.

In the present study we investigated the protein content and NRL allergen content of commercially available gloves. We compared different methods and developed a new test method with the aim to overcome the disadvantages of hitherto existing assays.

2 Methods

The study was approved, according to the German Law on Animal Protection, by the Hamburg Health Authority. An approval from the Ethics Committee was waived since no humans were involved in the study.

2.1 Latex Protein Extraction from NRL Gloves

A sample of 20 commercially available NRL gloves was drawn randomly from several German drugstores, supermarkets, pharmacies, and hospital material providers. NRL was extracted according to the method recommended in the US-Standard ASTM D 5712. Briefly, gloves weight was determined using an electronic balance. The gloves were cut into 1 × 1 cm pieces. The pieces were soaked in a glass container with

1 ml phosphate-buffered saline (PBS) per g glove material. The closed container was stirred during 2 h in a 37 °C water bath. The eluate was transferred to centrifugation tubes and centrifuged over 20 min at 4000 rpm. The aliquot was stored at –20 °C until protein or allergen analysis. In order to avoid any contamination, latex-free gloves were worn during the whole process.

2.2 Protein Quantification With a Modified Lowry Method

We quantified the total protein content with the Lowry Micro DC Protein Assay Kit (BioRad Laboratories; Munich, Germany) according to the manufacturer's instruction and the ASTM standard D 5712. Briefly, samples and standards were incubated in 1.5 ml Eppendorf-tubes stepwise with trichloroacetic acid (72 % TCA-solution), phosphor wolfram acid (72 % PTA-solution), and sodium deoxycholate (0.15 % DOC-solution) during 10–20 min at room temperature. The samples were centrifuged during 15 min at 6000 g. The supernatant was discarded and the pellet was solved in 250 µl 0.1 N sodium hydroxide. After 15 min incubation with 100 µl Reagent A at room temperature, Reagent B was added. The photometrical measurement was done in a disposable cuvette at 750 nm.

2.3 CAP Inhibition Assay

The latex allergen content was determined indirectly by IgE determination in the ImmunoCAP-system (FisherScientific; Freiburg, Germany) following IgE-inhibition of glove extracts as described earlier (Baur et al. 1997). Briefly, a standard dilution of Malaysian latex milk in concentrations from 0.005 to 4.0 µg/ml, on the one hand, and a pool of serum from 5 latex allergic volunteers, on the other hand, were incubated at 4 °C over night with the extracts of the 20 different latex gloves each. Subsequently, unbound NRL antibodies in the supernatant were measured by ImmunoCAP with NRL (k82;

FisherScientific). The content of allergenic protein was determined from the standard curve of defined NRL content.

2.4 Beezhold ELISA Inhibition Assay

Two rabbits were vaccinated with 500 µl NRL-standard-antigen (1 mg/ml) and 500 µl complete Freund's adjuvant and 4 weeks later with incomplete Freund's adjuvant. The rabbits were exsanguinated 6 weeks after the first vaccination by transthoracic heart puncture and the serum was obtained. The inhibition assay was done according to the ASTM standard D 6499. Briefly, an inhibition step was performed as described above using the serum pool of the 2 immunized rabbits instead of the human serum pool. Unbound antibodies were measured by ELISA with anti-rabbit-IgG antibodies. As described in the standard, repeated determination was performed.

2.5 IgY Inhibition Assay

Two chicken were vaccinated with 500 µl NRL-standard-antigen solution (1 mg/ml) and 500 µl complete and incomplete Freund's adjuvant in an interval of 10 days. The eggs laid 2 weeks or later after vaccination were collected and stored at 4 °C. IgY antibodies were obtained from the egg yolk with the EGGSTract® IgY Purification System (Promega; Mannheim, Germany). The ELISA IgY-inhibition assay was built according to the above mentioned ASTM Standard D 6499, whereas IgY-antibodies were used instead of IgG-antibodies. The second antibody was an anti-Chicken-IgY conjugated-antibody (Promega; Mannheim, Germany).

2.6 Statistical Elaboration

The results of the tests were compared with Spearman's rank correlation coefficient (r_s). The accuracy of the allergen determination

methods was assessed using the receiver operating characteristic (ROC) curve. The results of the modified Lowry Method were taken as reference to calculate sensitivity and specificity. A degree of agreement between tests was evaluated calculating kappa. All calculations were made with the statistical software package PASW Statistics 18.0.0 (IBM Germany).

3 Results

The results of the protein and allergen content analysis with the modified Lowry Method, the Beezhold ELISA Inhibition Assay, the CAP Inhibition Assay, and the IgY Inhibition Assay are presented in Table 1. The total protein content exceeded the recommended threshold for medical use gloves of 30 µg per gram glove material in 8 out of the 20 gloves, ranging from 215.0 to 1304.7 µg per gram glove material. Powdered gloves exceeded the recommended threshold more frequently than powder-free gloves (57 % vs. 31 %).

The results from the NRL allergen test methods correlated well with the results of the modified Lowry Method which measures total protein content (Table 2). The highest correlation with the total protein content was seen for the results of the Beezhold ELISA Inhibition Assay ($r_s = 0.883$). Our IgY Inhibition Assay showed a lower, but still satisfactory, correlation ($r_s = 0.735$). The results of the IgY Inhibition Assay and of the CAP Inhibition Assay showed also a high correlation with the results from the Beezhold ELISA Inhibition Assay, which is considered to be the gold standard for determining latex allergen content (Fig. 1, Table 2). Again, the IgY Inhibition Assay showed a lower correlation than the CAP Inhibition Assay, although statistically significant.

Taking the modified Lowry Method as reference, the test characteristics of the three methods to measure the NRL allergen content are summarized in Table 3. According to the ROC curve analysis, the accuracy, determined by the area under the curve (AUC) of the IgY Inhibition Assay, was lower than that of the CAP Inhibition

Assay and the Beezhold ELISA Inhibition Assay. At the optimal cut-off the CAP Inhibition Assay and the Beezhold ELISA Inhibition Assay were similar. The strength of agreement was very good between the CAP Inhibition Assay and the Beezhold ELISA Inhibition Assay (kappa = 0.80; 95 % CI 0.54–1.00) and good between the IgY Inhibition Assay and the other analytical methods (kappa 0.60; 95 % CI 0.26–0.94).

4 Discussion

Allergic reactions to NRL proteins are known since the 1920s, when the allergy became a well-recognized health problem particularly among HCW due to the frequent and prolonged use of protective gloves (Turjanmaa et al. 1996). In addition, powdered NRL gloves have also been a major carrier of NRL aeroallergens leading to respiratory allergies (Heilman et al. 1996). In order to prevent sensitization and clinical relevant allergies to NRL, national and international recommendations regulate the use of NRL gloves in the health care setting. In Germany, the technical standard for handling occupational hazards TRGS 401 bans the use of powdered NRL gloves and recommends a maximum protein content of 30 µg/g glove material for the health care setting (BAuA 2008). Different methods to reduce the protein content of NRL materials, like leaching and enzyme treatment have been developed (Perrella and Gaspari 2002). Indeed, the incidence of NRL allergy (latex allergic skin diseases and especially respiratory diseases) among HCW has decreased exponentially since the implementation of the recommendations (Latza et al. 2005).

Nevertheless, nowadays the general incidence of NRL allergy seems to have reached a steady state without a further decrease (Boonchai et al. 2014; Merget et al. 2010). Meanwhile, the use of NRL gloves is very common also in other industrial sectors, like the food industry, hair dressing, and cleaning, as well as in glove manufacturing (Sanguanchaiyakrit et al. 2014). The current regulations to limit allergenicity of NRL gloves protect only HCW, witnessing a lack

Table 1 Results of protein and natural rubber latex (NRL) allergen content

Glove ID	Type	Extracted weight (g)	Total protein content (Lowry) ($\mu\text{g/g}$) ^a	Latex allergen content ($\mu\text{g/g}$ glove material)		
				CAP Inhibition Assay	Beezhold ELISA Inhibition Assay ^b	IgY Inhibition Assay ^c
1	PF	6.30	<LOD	15.90	<LOD	85.37
2	PF	8.60	1304.65	478.15	273.75	268.96
3	PF	12.30	<LOD	2.52	<LOD	<LOD
4	PF	6.90	<LOD	4.10	<LOD	<LOD
5	PF	7.01	<LOD	1.39	<LOD	<LOD
6	PF	7.85	<LOD	12.10	<LOD	<LOD
7	PF	14.10	1207.34	868.90	87.75	581.98
8	PF	5.40	1080.00	129.77	33.14	34.39
9	PF	6.47	<LOD	3.47	<LOD	<LOD
10	PF	5.82	<LOD	9.39	<LOD	<LOD
11	PF	6.48	<LOD	31.96	<LOD	<LOD
12	PF	6.39	<LOD	3.37	<LOD	<LOD
13	PF	5.74	974.00	67.34	32.76	11.71
14	P	6.68	<LOD	30.17	11.15	<LOD
15	P	5.27	<LOD	16.35	<LOD	<LOD
16	P	5.89	<LOD	16.60	6.35	<LOD
17	P	6.10	1057.38	88.00	40.60	<LOD
18	P	5.63	195.38	55.25	44.55	179.59
19	P	5.70	215.00	30.51	17.89	71.18
20	P	5.85	260.00	48.43	9.85	35.60

P powdered, *PF* powder free, *LOD* limit of detection

^aLOD for modified Lowry Method = 10 $\mu\text{g/g}$

^bLOD for Beezhold ELISA Inhibition Assay = 5 $\mu\text{g/g}$

^cLOD for IgY Inhibition Assay = 10 $\mu\text{g/g}$

Table 2 Correlation between the different analytical methods

Correlation	r_s	p
Modified Lowry Method – CAP Inhibition Assay	0.866	<0.01
Modified Lowry Method – Beezhold ELISA Inhibition Assay	0.883	<0.01
Modified Lowry Method – IgY Inhibition Assay	0.735	<0.01
Beezhold ELISA Inhibition Assay – CAP Inhibition Assay	0.892	<0.01
Beezhold ELISA Inhibition Assay – IgY Inhibition Assay	0.674	<0.01
CAP Inhibition Assay – IgY Inhibition Assay	0.672	<0.01

of awareness to the risk of NRL allergy in other professions in which NRL gloves are also frequently used. For these industrial and service sectors there are no regulations or recommendations in place regarding the protein or allergen and powder content of gloves, the same is true for the general public. We found a relatively high amount of gloves with high protein and NRL allergen content within the 20 randomly bought latex gloves. These gloves, available to the general public in drugstores,

pharmacies, etc., bear the risk of NRL sensitization and consequently of skin allergic reactions. Above all, nearly half of the gloves with high protein content were powdered, posing a risk to the development of allergic asthma.

A further problem is the measurement of the NRL allergen content in NRL materials. A number of different test procedures have been described. Due to its simplicity, the modified Lowry Method is commonly in use and it is the standard procedure in Germany (Deutsches

Fig. 1 Correlation between tests results

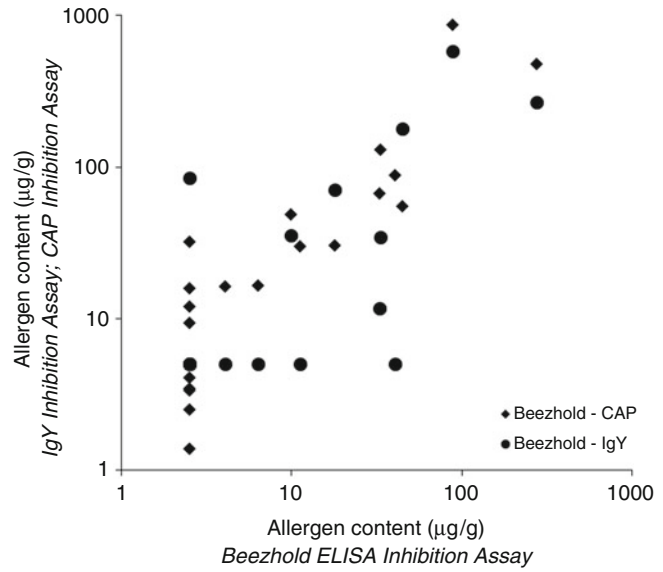


Table 3 Characteristics of tests to measure the natural rubber latex (NRL) allergen content using the modified Lowry Method as reference

	Cut-off values (µg/g)	Sensitivity (%)	Specificity (%)	AUC
CAP Inhibition Assay	10	100	50	0.99
	30	100	83	
Beezhold ELISA Inhibition Assay	5	100	83	0.99
	10	88	92	
	30	75	100	
IgY Inhibition Assay	10	88	92	0.89
	30	75	92	

AUC area under the curve

Institut für Normung e.V. 2007). A fundamental limitation of this method is that it measures total proteins instead of NRL allergens under the assumption that both positively correlate. Indeed, several authors question this assumption and refuse the use of the modified Lowry Method as a marker for allergenicity of NRL materials (Palosuo et al. 2002). Available methods to measure the NRL allergen content are the CAP Inhibition Assay and the Beezhold ELISA Inhibition Assay, among others. The advantages of the CAP Inhibition Assay are the timesaving and little interference-prone semi-automated ImmunoCAP®-method. Unfortunately, the CAP Inhibition Assay requires the use of sera from NRL allergic subjects, which are naturally finite, not standardized, with

variable antibody patterns and levels. Thus, the reproducibility of the method has been questioned, since the spectrum of allergens covered varies depending on the pool of sera (Koch 2002). It has been proposed to enhance the standardization of the CAP Inhibition Assay by measuring Hev b 1 as a marker allergen, since Hev b 1 is one of the known major allergens (Raulf-Heimsoth et al. 2000). However, the use of marker allergens for allergen content description is again questionable, since Hev b 1 is not always present in NRL gloves (Mabe et al. 2009).

In order to cover the whole spectrum of NRL allergens, Beezhold developed an inhibition assay with NRL IgG-antibodies from rabbits immunized with the complete spectrum of latex

proteins (ASTM D 6499) (Tomazic-Jezic et al. 2002). The method has, however, important disadvantages. First of all, it requires to sacrifice the animals (rabbits) in order to obtain the anti-NRL-antibodies for the assay. In addition, the amount and the spectrum of antibodies produced by the rabbits is also frequently limited (Schade et al. 2001). Also, IgG antibodies are known to tend to react non-specifically, leading to false positive results (Hansson et al. 2008). Finally, this method is time-consuming and cost-intensive.

Our aim was to develop an assay overcoming the disadvantages of the above mentioned methods. We produced an assay with hen IgY-antibodies. The production of antibodies in the eggs of immunized hens was already described in 1893 (Klemperer 1893). With the growing sensibility for animal protection and increasing legal restrictions to the use of animals in research, the extraction of IgY-antibodies from hen eggs gained academic interest in the 1990s. The method has several advantages. First, immunization of the hen elicits IgY-immunoreactivity reliably. Second, unspecific reactions of IgY antibodies are known to be unlikely (Schade et al. 2001). Third, after immunization, the eggs laid by the hen will contain the appropriate antibodies lifelong. With this method, no animals need to be sacrificed, thus the method raises no major ethical concerns related to animal protection issues in contrast to the Beezhold ELISA Inhibition Assay. In addition, the antibody production is much more effective than in rabbits; the antibody content in one egg is approximately the same that the one in the whole blood of one rabbit (Wooley and Landon 1995). Finally, the antibodies can be stored simply for years by storing the refrigerated undamaged egg.

Our results demonstrate that the new IgY based test assay correlates well with the hitherto widespread methods such as the modified Lowry Method, the CAP Inhibition Assay, and the Beezhold ELISA Inhibition Assay. Indeed, in comparison to the other test methods, sensitivity and specificity of the IgY assay was high, although lower than that of the other tests. The

IgY Inhibition Assay also showed a good degree of agreement with the other tests. Overall, test accuracy of our assay was good.

Considering the advantages and disadvantages of the heretofore tested procedures and in view of test accuracy, our results confirm that the cost-effective and easy to perform the modified Lowry Assay is the test of choice for preventative purposes. As described earlier (EN 455-3), we saw a good correlation of protein and allergen content in NRL gloves. Particularly, if the modified Lowry Assay delivers results under the limit of detection, it can be reliably inferred that the NRL allergen content would be at very low levels, and thus risk of sensitization would also be low. Nevertheless, in situations where there is a need to specifically measure the whole range of NRL allergens instead of total proteins, we recommend the use of the IgY Inhibition Assay. In our view, that test allows a respectful and considerate use of animals while achieving a satisfactory accuracy.

In conclusion, our results indicate that there is a need to extend the restrictions of allergen contents, ideally to all available NRL gloves. Otherwise, further decreases in the incidence of latex allergy will hardly be achieved.

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Conflicts of Interest The authors declare no conflicts of interest regarding this publication.

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