The development and use of a polyclonal sandwich enzyme immuno assay in occupational exposure assessment of Natural Rubber Latex proteins

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Nederlandse samenvatting

Latex allergie is inmiddels een beruchte beroepsgebonden aandoening, met name onder ziekenhuispersoneel waar de prevalentie tussen de 5% en 15% ligt. Voor de bepaling van de dermale en inhalatoire blootstellingdeterminanten en de vaststelling van een mogelijke dosisrespons relatie is een gevalideerd assay noodzakelijk. Wegens succesvolle eerdere ervaringen is voor de detectie van latexallergenen in monsters gekozen voor de ontwikkeling van een polyklonaal sandwich EIA. Hiertoe is anti latex IgG, dat een breed spectrum aan latex allergenen detecteert, uit het serum van een konijn gebruikt

Het assay is gevalideerd door middel van verscheidene laboratoriumexperimenten, waaronder sensitiviteit- en specificiteittesten en luchtmonsternamen en huidpads, en een vergelijkende analyse met een bestaande IgE inhibitie assay.

De resultaten tonen aan dat het assay zeer specifiek is en een sensiviteit heeft tussen de 2,5 en 3,0 ng/mL, of 6 ng/m³ voor 8-uurs metingen. De vergelijkende analyse met het bestaande IgE inhibitie assay liet een hoge mate van correlatie zien voor de luchtmonsters (r^2=0,7-0,8) en de handschoenenextracten (r^2=0,9)

Omdat het polyklonale immunoassay een wijde range aan verschillende latex allergenen oppikt, is het een mogelijk waardevol instrument in blootstellingmetingen. Verdere validatie en toetsing van het assay in de praktijk zijn echter noodzakelijk.

Summary

Latex allergy among health care workers remains a serious occupational health problem with a recently reported prevalence of latex allergy between 5% and 15%. The main determinants of air and dermal latex allergen exposure have been identified, but clear exposure-response relations have not yet been developed, partly because of a lack of a valid assay.

Because of positive previous efforts, a polyclonal sandwich EIA for the measurement of latex allergens in sample extracts was developed. Anti-latex IgG, detecting a wide range of latex proteins, was obtained using serum from a rabbit.

Our assay was validated through several laboratory experiments, including sensitivity and specificity testing, measuring reactivity of extracts from gloves and air samples, and a comparison analysis with an existing IgE inhibition assay.

The results show the assay to be specific and to have a sensitivity of 2.5-3.0 ng/mL, or 6 ng/m³ for full shift inhalation exposure measurements. The assay is successful in measuring latex proteins in extracts from powdered gloves, filters from airborne sampling and skin pads. Furthermore, the comparative analysis with the existing IgE inhibition assay shows a high correlation for the airborne dust samples (r^2=0.7-0.8) and glove extracts (r^2=0.9).

It is concluded that a polyclonal immuno assay might be a valuable tool in latex exposure assessment as it describes the overall allergenicity of latex products, normally containing a range of different latex proteins. Further validation of the assay is necessary and practical value should be tested.

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Abbreviations used:

EIA: Enzyme immuno assay
LOD: Level of detection
NRL: Natural rubber latex
OD492: Optical density at 492 nm

Introduction

Despite measures to reduce latex allergen exposure, latex allergy remains a serious occupational health problem, especially for health care workers. A recently published review (Poley and Slater, 2000) indicated a reported prevalence of latex allergy among health care workers between 5% and 15%.
Exposure to natural rubber latex (NRL) proteins appears to be the most significant risk factor for developing latex allergy (Poley and Slater, 2000). Exposure to airborne NRL allergens and direct skin contact with NRL is therefore an occupational risk affecting all workers using latex-containing products. Airborne exposure to latex is probably enhanced by corn starch particles found inside powdered latex gloves to which the latex proteins can adhere (Brehler *et al.*, 1997). Substitution of powdered latex gloves by low allergen or non-powdered latex gloves was shown to reduce aerogen NRL allergen loads 10 fold (Heilman *et al.*, 1996) or below the detection limit (Allmers *et al.*, 1998). Other known determinants of airborne exposure to NRL allergens are the frequency of changing of latex gloves (Sri-akajunt *et al.*, 2000) and resuspension from reservoirs of powder in the room and clothing (Swanson *et al.*, 1994). Dermal exposure to products such as latex gloves has the potential to contribute to latex sensitisation (Woolhiser *et al.*, 1999). It is argued that during prolonged wearing of surgical gloves proteins are liberated from latex by body sweat. These proteins are subsequently absorbed through the skin. Hand cream increases the amount of latex protein that is transferred from gloves to the hands of the wearer (Beezhold *et al.*, 1994) and might lead to more cases of hypersensitivity. (Baur *et al.*, 1998).
The relative importance of dermal exposure compared to airborne exposure in developing latex allergy is not known and has never been measured. Other determinants of exposure to NRL allergens remain unclear. Further identification of the most relevant occupational exposure routes therefore remains an important step in developing and evaluating control strategies to reduce these risks. Assays are important as they give insight to these exposure determinants.

Part of the problem in this field might be due to a lack of validated assays for determining the amount of extractable allergens in exposure samples (European Commission: Scientific Committee on Medical Products and Medical Devices, 2000). Analysis of the allergen level is not well validated (Brehler *et al*, 2002). As yet, the established method for the analysis of NRL gloves is the Lowry assay, which does not differentiate between proteins and latex allergens.
NRL exposure assessment studies have employed two different assays for analysis of latex allergens in samples: IgE inhibition assays using human sera from latex allergic patients (Swanson *et al.*, 1994, Swanson *et al.*, 1999, Liss *et al.*, 1997, Sri akajunt et al., 2000, Tarlo et al., 1994, Baur et al., 1998, Heilman *et al.*, 1996) and a monoclonal IgG sandwich enzyme immuno assay (EIA) (Slater *et al.*, 1994 and Raulf-Heimsoth *et al.*, 2000). IgE inhibition assays use the serum of latex allergenic patients and are therefore hard to standardize. Monoclonal EIA's have the disadvantage of measuring only one latex allergen, while several latex allergens have been shown to produce allergic reactions in humans.
The main objective of this study was to develop and evaluate an assay, that is more sensitive than the current ones, measures a wider variety of latex allergens at the same time, and has more potential of being standardized.
Because of positive previous experiences in α-amylase (Houba *et al.*, 1997) and rat- and mouse urine allergen (Hollander *et al.*, 1997) occupational exposure assessment studies, we attempted to develop a polyclonal sandwich EIA for measuring latex proteins.

Material en methods.

Isolation and labeling of rabbit anti-latex IgG.
Anti-NRL antiserum was obtained from a rabbit raised and used previously at the Food and Drug Agency (FDA), Centre for Devices and Radiological Health, Maryland, USA, for the analysis of latex proteins in gloves. Earlier immunoblotting studies showed that the anti-serum to be able to detect a wide range of latex proteins (Tomazic-Jezic et al., 1999).
The IgG fraction was isolated from 11 mL rabbit anti-serum through precipitation with 33% ammonium sulphate. The precipitate was centrifuged and resuspended in and dialysed against 0.1 M phosphate buffer (pH 6.3, 0.02% azide), and applied to a column (25 x 2.5 cm) of DEAE cellulose (DE-52; Whatman, Maidstone, Kent, UK) equilibrated with 0.1 M phosphate buffer solution, pH 6.3, 0.02% azide. Elusion was carried out in a batch-wise gradient of 0, 50, 100, and 500 mM NaCl with volumes of 3-5 ml in the same phosphate buffer. Protein peaks, collected at the four different salt concentrations, were pooled and concentrated by reversed osmosis using poly-ethyleneglycol (PEG-2000) and tested for their IgG anti-latex titre. High specific activity (IgG anti-latex titres > 20,000) was detected only in fractions eluated with 0 and 50 mM NaCl. Part of the different IgG fractions was labeled with biotin at 1 mg/mL through incubation with biotin-N-hydroxysuccinimide ester to be used as detector antibody (Hollander *et al.*, 1997; Houba *et al.*, 1997).

Sandwich EIA

Optimisation of the sandwich assay was carried out in experiments with non-labeled IgG coated at 0.2 – 16 µg/ml and incubated with standard NRL, provided by the American Society for Testing of Materials (ASTM), at 8 to 50,000 ng/ml. NRL protein binding was detected after incubation with biotinylated IgG diluted 1/500 through 1/13,000. Optimal binding and detection of NRL was achieved using labeled and unlabelled IgG from the fraction obtained at 50 mM NaCl at a coating concentration of 1 µg/ml and the biotinylated antibody diluted 1/500. Based on these experiments, the procedure for the analysis of latex allergens used in further experiments was as follows. Microtiterplates (Greiner art. nr 655093, Nuertingen, Germany) were coated overnight at 4°C with 100 µl/well of a 1 µg/ml (protein) rabbit polyclonal anti-NRL antibody in PBS and blocked with PBS-Tween (PBT) containing 0.2% gelatine (PBTG). Test samples were incubated for 1 hour on a shaking plat-

form at 37°C.
Biotin was detected after incubation with avidine-peroxidase and 0-phenylenediamine (OPD) for 30 minutes at 20°C in the dark. As a final step 50 µl HCl was added to terminate the reaction. A standard curve of the optical density at 492 nm (OD_{492}) against the log concentration of standard allergen was calculated using 4-parameter curve fitting with the aid of SOFTmax software package (Molecular Devices Corporation; Menlo Park, CA, USA). Specificity of the assay was tested with extracts of wheat, soy, potato and corn; rodent and mite allergens; milk protein, human and animal albumin.

Glove extraction

Glove extraction was tested in an experimental set-up, in which three brands of gloves were bedded in 50 mL PBS-Tween/azide per glove. The three glove brands were coded A, B, and C. Glove A and B were powdered latex gloves and glove C was a non-powdered latex glove. Different types of gloves were chosen, because of the broad range of gloves used amongst health care workers.
Two procedures were followed. Three gloves of each brand were filled with 50 mL PBS-Tween/azide per glove, closed and then shaken for 4 hours at 37°C. Alternatively the gloves were cut with clean scissors in small (roughly 1 cm^2) pieces. The extraction fluid was centrifuged for 15 minutes at 2,000 g. Supernatant was collected and frozen at –20°C for later analysis in the EIA.

Measuring airborne exposure

Air sampling was first performed in a ventilated hood, in which glove brands A, B and C were mechanically manipulated for 1.5 hours to create airborne latex allergens. The latex gloves were strung to the whisk of two blenders both attached to variable resistor, through which the rotational speed could be adjusted. The blender was positioned so that the glove would beat against a tripod. The gloves were changed every five minutes, thus 18 pair of gloves were used per measurement.

Dust was captured on pre-weighed polytetrafluoroethylene (Teflon) filters (Millipore, pore size 10 mm, diameter 25 mm), in a PAS6 sampling head, connected to a GilAir3 personal air sampling pump (Gilian Product Sensidyne, Inc., USA) operated at a flow rate of 2 L/min (ter Kuile, 1984). To compare efficiency dust was also captured parallel using a Gilair5-sampling pump (3,5 L/min), attached to a GSP sampler (Deha-Haan&Wittman GmbH, Friolzheim, Germany) containing a Teflon filter (Millipore; pore size 10 µm, diameter 37 mm). Filters were conditioned for 24 hours, weighed, stored at –20°C, and extracted in 15 mL Greiner tubes with 1 and 2 mL of PBS-Tween-azide for the 25 µm and 37 mm filters respectively. The extracts were tested in the sandwich EIA assay at dilutions of 1/3, 1/9 and 1/27. Gravimetric determination of the dust on the filters was performed in a conditioned room (constant temperature (22-25 °C) and moisture percentage (35-45 %)) on a Mettler® MT-5 microbalance.

Validity tests

A comparison was made with an existing NRL-specific IgE inhibition assay used for the detection of latex allergen exposure (Baur *et al.*, 1996), using 0.5 - 1.5 mL of extracts from the above mentioned glove extraction and air sampling experiments.

Results

Sensitivity and specificity
Figure 1 shows the EIA reactions of serially diluted NRL standard and a high allergen latex glove extract. An S-shaped dose-response curve was obtained using the standard NRL. The glove extract showed a parallel curve making the assay useful for the measurement of latex allergens in glove extract samples.

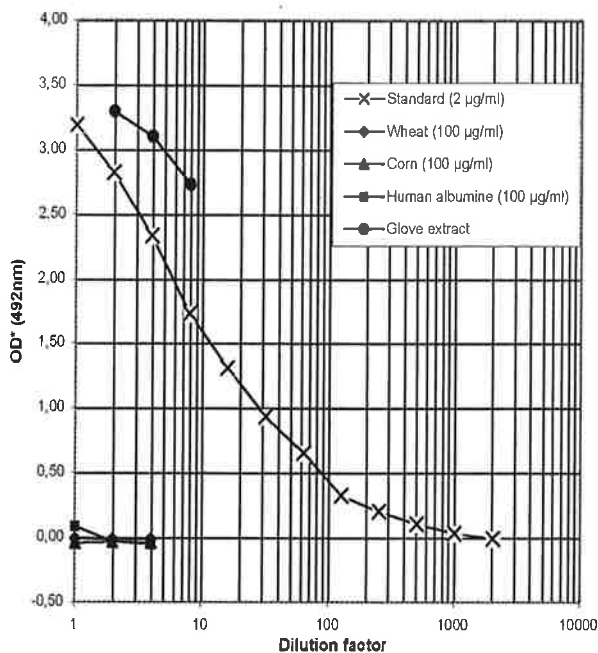

Figure 1. Dose response curve showing dilutions of standard NRL and dilutions of a high allergen latex glove extract against OD492 in a polyclonal sandwich EIA. The reactivity of three other extracts is shown to visualize the specificity of the assay.

Sensitivity of the assay was defined as the concentration NRL resulting in an OD_{492} of 0.050 above the average blanc value. This corresponded with a NRL concentration of 2.5 ng – 3.0 ng/mL.
Specificity was tested for by measuring reactions to wheat, soy, potato and corn extracts; rodent and mite allergens; milk protein and human and animal albumin. Figure 1 shows the results of a selection of the specificity tests done with the polyclonal sandwich EIA. None of the extracts reacted with the assay (OD492 < 0.050 above blanc value), indicating a high specificity of the assay.

Glove extraction

With the sandwich EIA assay NRL could be detected in all extracts of both brands of powdered gloves, as shown in table 1. Concentrations of the extracts ranged from 69 to 9348

Table 1 NRL proteins in gloves A, B and C (coding is identical to figure 2), measured with a polyclonal sandwich EIA, extracted in two different methods.

Glove	Concentration in extract (ng/mL): mean ± S.D. Cut (n)	Intact (n)	NRL (mg) per glove
A	7,909 + 1,439 (n = 3)	8,329 + 709 (n = 3)	~ 400
B	131 + 16 (n = 3)	91 + 22 (n = 3)	~ 5
C	8 (n=1); ND* (n=2)	ND* (n=3)	< 0.5

*) ND : non-detectable

ng/mL. No systematic difference was found between the results from extraction of intact and cut up gloves.
For the non-powdered glove brand C the assay detected NRL in only one out of six latex gloves extracts (8 ng/mL). All three different brands of gloves showed parallel dose-response curves in a series of dilutions, from which a mean NRL content of 400 (glove A) and 4 (glove B) and < 0.5 (glove C) micrograms latex protein per glove was calculated.

Table 2. Airborne dust and latex allergen concentrations after mechanical glove manipulation in a ventilated hood, using two different sampling methods. Glove coding is identical to figure 2.

Glove	Sampling method: GSP Dust (mg/m³) mean ± S.D.	NRL (ng/m³) mean ± S.D.	Sampling method: PAS6 Dust (mg/m³) mean ± S.D.	NRL (ng/m³) mean ± S.D.
A	3.24 ± 0.36	1,975 ± 223 (n=4)	1.44 ± 0.30	1,569 ± 226 (n=5)
B	2.25 ± 0.43	234 ± 31 (n=5)	1.24 ± 0.38	138 ± 44 (n=5)
C	0.08 ± 0.05	54 (n=1) ND* (n=3)	0.08 ± 0.03	25 ± 17 (n=2) ND* (n=2)

*) ND : non-detectable

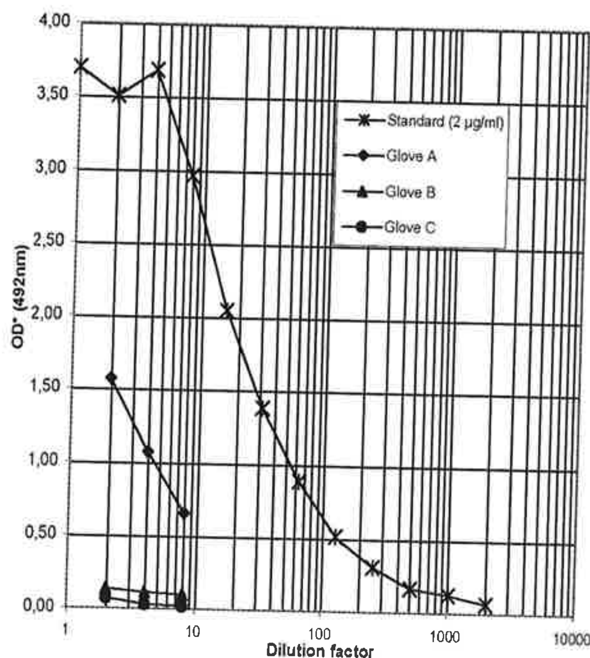

Figure 2. Latex aero allergen concentrations after the use of three different latex gloves in an experimental set-up. Gloves A and B are powdered latex gloves. Glove C is a non-powdered latex glove.

Measuring airborne exposure

The laboratory experiments manipulating gloves to produce an airborne latex allergen concentration showed the assay was successful in measuring latex proteins on filters from airborne sampling. As shown in table 2, dust concentrations in the ventilated hood were highest using glove brand A with average dust concentrations of 3.24 mg/m³ and 1.44 mg/m³, using GSP and PAS6 sampling heads respectively.
Figure 2 shows the reactivity of the extracts of the PAS6 filters for the three brands of gloves. All three brands of gloves show curves parallel to the standard curve making the assay useful for the measurement of airborne latex allergens. NRL aero-allergen concentration levels varied from non-detectable to 2292 ng/m³ using GSP sampling heads and from non-detectable to 1794 ng/m³ using PAS6 sampling heads. The exposure measurements using the GSP sampling head resulted in capturing more dust on the filter compared to measurements using the PAS6 sampling head. This is likely to have been caused by the higher flow of the Gilair5 pump. Sensitivity of the exposure assessment did not differ, as GSP sampling head filters had to be extracted in twice or three times the amount of PBS-Tween-azide, because of the filter's bigger diameter. PAS6 filters normally had to be extracted in 2-3 ml PBS-Tween-azide, meaning a sample sensitivity of 5–10 ng/mL, or a LOD of 6 ng/m³, given a personal air sampling period of 8 hours at 2 L/min.

Validity tests

Of the 19 airsample filter extracts with a detectable NRL concentration in the sandwich EIA, 18 reacted positive with the IgE inhibition assay at the BGFA. All 14 extracts which had tested non-detectable in the sandwich EIA, also did not react in the IgE inhibition assay. None of the sixteen glove extracts send, tested differently in qualitative terms. When clustered by glove brand, the Pearson correlation (r^2) for the positive airborne dust samples was 0.7 to 0.8 and for the positive glove extracts was 0.9.
Because of the limited sample size no comparative analysis was performed. From the graphs a systematic difference in the absolute values for the glove extracts is notable. The IgE inhibition assay roughly picked up 10 times higher concentrations of latex proteins compared to the IgE inhibition assay used at BGFA (figure 3).

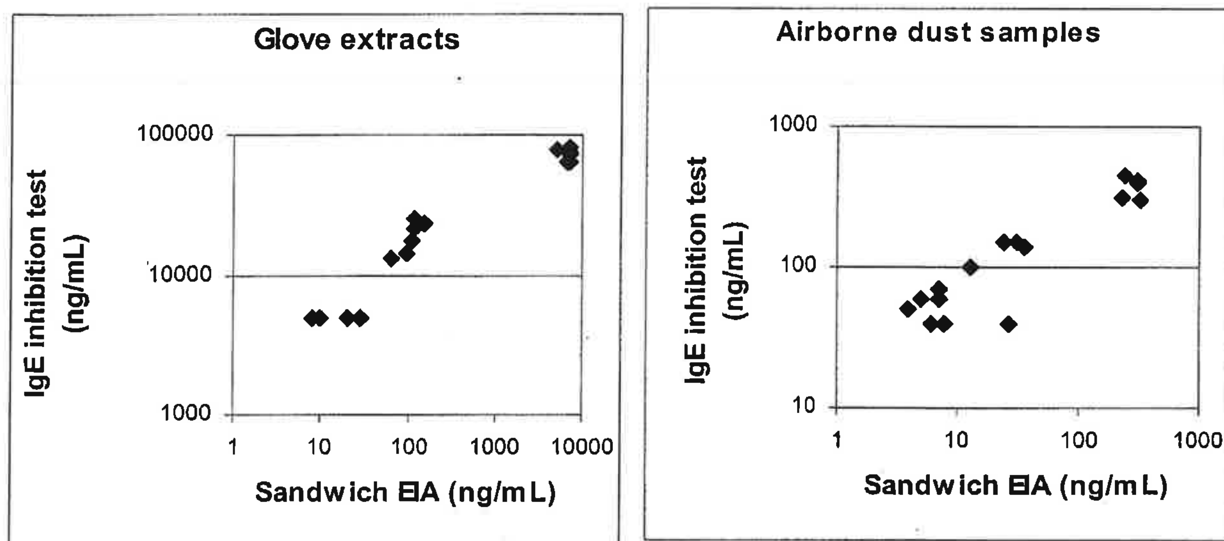

Figure 3. Comparison of two methods for measuring latex proteins in extracts of latex gloves and airborne dust: the IgE inhibition assay and the polyclonal sandwich EIA assay. Although both graphs show a moderate to high correlation, a systematic difference is notable.

Discussion

Since total protein measurement by modified Lowry method remains unsatisfactory and the human IgE-inhibition method is limited by the use of sera from type I allergic patients (Chabane *et al,* 1999), a polyclonal sandwich EIA using the anti-serum of a rabbit was developed for the detection of latex allergens in workplace exposure assessment. The assay has the distinct advantage over the total protein assay that it can discriminate NRL proteins from nonlatex proteins.

A polyclonal sandwich EIA also has the advantage of detecting a wide range of latex allergens, thus giving a more realistic view of the total exposure of a worker. Monoclonal EIA assays are highly specific for one single allergen molecule, which raises concerns towards validity: does the content of one particular major allergen reflect the 'overall' allergenicity? Natural rubber latex appear to contain a range of proteins able to arouse allergic reactions on humans. Different research methods show variable results in defining major latex allergens. Three allergens (Hev b5, Hev b6 and possibly Hev b1), have been defined as major allergens, meaning that IgE anti-bodies, specific for Hev b5 and Hev b6, can be seen in over 50% of humans allergic to latex (Vandenplas *et al.,* 1999). By skin prick testing, recombinant latex allergens Hev b5, 6 and 7 were found to be the most common allergens for health care workers (Yip *et al.,* 2000). Using direct binding to rHev b5/maltose binding protein by IgE of latex-allergic patients, the majority (92%) of sensitized health care workers, were shown to have IgE specific for Hev b5 (Slater *et al.,* 1996). Comparative studies between monoclonal and polyclonal sandwich EIA assays for the analysis of allergens in other occupational exposure studies, show variation in results between the two approaches.

The polyclonal EIA was found to be sensitive and highly specific in α–amylase- (Houba *et al.,* 1997) and in a rat- and mouse urine allergen exposure assessment study (Hollander *et al.,*1997). In the latter study a monoclonal sandwich EIA used at the National Institute of Working Life in Sweden gave 4.1 times lower levels of rat urine allergen levels compared to the polyclonal sandwich EIA developed by the Institute for Risk Assessment Sciences (IRAS) in the Netherlands. The polyclonal rabbit antibodies used in the polyclonal sandwich EIA reacted with all allergens present in rat urine, where as the monoclonal immunoassay used antibodies against *Rat n* I only.

We found our assay to be specific, as the polyclonal sandwich EIA did not react (OD_{492} < 0.050 above blanc value) with several extracts. It should be noted though, that tests with cross-reacting allergens (avocado, bananas and pollen) have not yet been performed.

The assay was also found to be sensitive, able to measure personal air concentrations of latex allergens up to 6 ng/m^3, using a flow of 2 L/min for 8 hours. The LOD in other airborne latex allergen exposure assessment studies range from 0.014 ng/m^3 to 0.28 ug/m^3 (Swanson *et al.,* 1994, Swanson *et al.,* 1999, Liss *et al.,* 1997, Sri-akajunt *et al.,* 2000, Tarlo *et al.,* 1994, Baur *et al.,* 1998, Heilman *et al.,* 1996). The broad range in the LOD mentioned can mostly be explained by the wide variety in air sampling methods used. Lower LOD are mostly due to much larger air volumes obtained during stationary measurements. A higher LOD naturally has a negative impact on the success of any exposure assessment study investigating airborne latex allergen exposure, since concentrations have been reported to be below 1 ng/m^3 when using low allergen or non-powdered latex gloves in the work environment (Swanson *et al.,* 1994, Liss *et al.,* 1997, Tarlo *et al.,* 1994, Baur *et al.,* 1998, Heilman *et al.,* 1996).

In conclusion our study found a polyclonal sandwich EIA to be a possible useful tool in determining exposure to latex proteins. The assay was successful in determining the amount of all potential allergenic NRL proteins in latex gloves and airsample filters. However, it is clear that the assay needs further validation as reproducibility of the assay and statistical correlation with other existing assays were not tested for. Although the tests were successful a higher level of sensitivity might be necessary for occupational exposure assessments in settings where low or non-powdered latex gloves are used.

References

Allmers, H., Brehler, R.,Chen, Z., Raulf-Heimsoth, M., Fels, H., Baur, X. Reduction of latex aeroallergens and latex-specific IgE antibodies in sensitized workers after removal of powdered natural rubber latex gloves in a hospital. J. Allergy Clin. Immunol. 1998; 102: 841-846.

Baur, X., Chen, Z., Allmers, H., Beckmann, U. Can a threshold limit value for natural rubber latex allergens be defined? J.Allergy Clin. Immunol. 1998; 101, 24-27.

Baur, X., Chen, Z., Allmers, H., Beckmann, U., and Walther, J.H. Relevance of latex aeroallergen for healthcare workers. Allergology international 1996; 20: 105-111.

Baur, X., Chen, Z., Allmers, H., Raulf-Heimsoth, M. Results of wearing test with two different latex gloves with and without the use of skin-protection cream. Allergy 1998; 53: 441-444.

Beezhold, D.H., Kostyal, D.A., Wiseman, J. The transfer of protein allergens from latex gloves. AORN Journal 1994; 59-3: 605-613.

Brehler, R., Kolling, R., Webb, M., Wastell, C. Glove powder – a risk factor for the development of latex allergy? Eur. J. Surg. 1997; suppl 579: 23-25.

Brehler, R., Rütter, A., Kütting, B. Allergenicity of natural rubber latex gloves. Contact dermatitis. 2002; 46: 65-71.

Chabane H, Barbara J, Hrabina M, Leynadier F. Competitive immunoassay for antigenic latex protein measurement: rabbit antiserum-based assay compared to modified Lowry and human IgE-inhibition methods. J Investig Allergol Clin Immunol. 1999 Nov-Dec;9(6):372-9.

European Commission: Scientific Committee on Medical Products and Medical Devices. Opinion on Natural rubber latex allergy. June 2000.

Heilman, D.K., Jones, R.T., Swanson, M.C., and Yunginger, J.W. A prospective, controlled study showing that rubber gloves are the major contributor to latex aeroallergen levels in the operating room. Journal of Allergy and Clinical Immunology. 1996; 98, 325-330.

Henning, A., Brehler, R., Chen, Z., Raulf-Heimsoth, M., Fels, H., Baur, X. Reduction of latex aeroallergens and latex-specific IgE antibodies in sensitized workers after removal of powdered natural rubber latex gloves in a hospital. J. AllergyClin. Immunol 1998:Vol. 102, 8, 841-846.

Hollander A., van Run, P., Spitshoven J., Heederik, D., Doekes, G. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. Clin. Exp. Allergy 1997; 27: 617-626.

Houba, R., van Run, P., Doekes, G., Heederik D. Airborne levels of a-amylase allergens in bakeries. J. Allergy Clin. Immunol. 1997; 99: 286-292.

Kenneth, T., Kim, Wellmeyer, E.K., Miller, K.V. Minimum Prevalence of latex Hypersensitivity in Health Care Workers. Allergy and Asthma Proc. 1999; 20, 387-391.

Kuile W.M. ter. Vergleichsmessungen mit verscheidenen Geraeten zur Bestimmung der Gesamtstaubkonzentration am arbeidsplatz: Teil II. Staub Reinh. Luft 1984;44:211-216.

Liss G.M., Sussman, G.L., Deal, K., Brown, S., Cividino, M., Siu, S., Beezhold, D.H., Smith, G., Swansson, G.M., Yunginger, J., Douglas, A., Holness, D.L., Lebert, P., Keith, P., Wasserman, S., and Turjanmaa, K. Latex Allergy: epidemiological study of 1351 hospital workers. Occup. Environ. Med. 1997; 54, 335-342.

Poley, G.E., Slater, J.E. Latex allergy. J. Allergy Clin. Immunol 2000; 105:1054-62.

Raulf-Heimsoth, M., Sander, I., Chen Z., Borowitzki, G., Diewald, K., van Kampen, V., Baur, X. Development of a monoclonal anti-body based sandwich ELISA for the detection of the latex allergen Hev b1. Arch. Allergy Immunol 2000; 123(3): 236-241.

Slater, J.E., Vedvick, T., Arthur-Smith, A., Trybul, D.E., Kekwick, R.G.O. Identification, Cloning, and sequence of a Major Allergen (Hev b5) from Natural Rubber Latex (Hevea brasiliensis). The J. of Biological Chemistry 1996; 41, 25394-25399.

Sri-akajunt, N., Sadhra, S., Jones, M., and Burge, P.S. Natural rubber latex aeroallergen exposure in rubber plantation workers and glove manufacturers in Thailand and health care workers in a UK hospital. Ann.Occup.Hyg. 2000; 44, 79-88.

Swanson, M.C., Bubak, M.E., Hunt, L.W., Yunginger, J.W., Warner, M.A., and Reed, C.E. (1994). Quantification of latex aeroallergens in a medical center. J. Allergy Clin. Immunology. 94, 445-451.

Swanson, M.C., Yunginger, J.W., Lam V., and Truscott, W., (1999). Airborne Natural Rubber Allergen Concentrations Within a Malaysian latex glove production facility. Unknown (abstract).

Tarlo, S.M., Sussman, G., Contala, A., and Swanson, M.C. Control of airborne latex by use of powder-free latex gloves. J.Allergy Clin.Immunol. 1994; 93, 985-989.

Tomazic-Jezic, V.J.,Woolhiser, M.R., Beezhold, D. Quantification of latex proteins by inhibition ELISA. J. Allergy and Clin. Immunol 1999; 103: S162. (Abstr. 55th annual meeting Am. Acad. Allergy Astma Immunol., 1999; Orlando)

Vandenplas, O., Charous, B.L., and Tarlo, S.M. (1999). Asthma in the workplace (Bernstein, I.L., Chan-Yeung, M., Malo, J.-L., and Bernstein, D.I., Eds.) second edition. Marcel Dekker, Inc., New York, Basel. 425-444.

Woolhiser, M.C., Munson, A.E., Meade, B.J. Role of sensitization routes in the development of type I hypersensitivity to Natural Rubber Latex in mice. Am. J. of Ind. Med. Supp. 1999; 1:139-141.

Yip, L., Hickey, V., Wagner, B., Liss, G., Slater, J., Breiteneder, H., Sussman, G., Beezhold, D. Skin prick test reactivity to recombinant latex allergens. Int.Arch. Allergy Immonol. 2000; 121, 292-299.