

**LEAP:
Latex ELISA for Antigenic Proteins^{®*}
Preliminary Report**

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Abstract

The use of latex gloves has dramatically increased in the last decade due, in part, to the outbreak of AIDS and the implementation of universal precautions for healthcare workers. Associated with this increased usage has been the increased incidence of reported allergies to latex. This allergy typically manifests itself as dermatitis and urticaria on the hands of healthcare workers, and in some cases severe anaphylaxis and death have occurred. A proportion of these allergies are in response to the natural proteins of the rubber tree. In this paper, we describe a new assay by which to detect these latex proteins. This technique can be used to determine the safest glove (lowest proteins levels) for use by individuals with latex protein allergy and for use in the industry to produce latex products which are free from the allergy inducing proteins.

Introduction

The recent outbreak of allergy to latex products has received considerable attention in the medical¹⁻⁴ and the popular literature.⁵ Sensitivity to latex should be considered a significant risk for health care workers. While many of the reactions are in response to chemical additives in latex, a significant number of reactions occur to natural proteins found within the latex.^{1,6} Reaction to the proteins can be a T cell dependent delayed-type hypersensitivity or IgE mediated immediate-type reactions.⁷ It is the IgE mediated immediate type reactions which are responsible for life-threatening anaphylaxis.

In order to prevent these reactions, it is necessary to manufacture latex products with the lowest possible level of antigenic proteins. The quantification of protein levels in latex products has proved difficult, largely due to interference in the standard protein assays by additives in the latex products. Furthermore, the amount of protein found in latex products is often below the detection limits of these standard laboratory protein assays.

Enzyme linked immunosorbent assay (ELISA) technology takes advantage of the specificity and sensitivity of the antibody-antigen reaction.⁸ Variations of the ELISA method have been developed for the detection and quantification of

a wide range of compounds. In an indirect ELISA, the relevant antigen is absorbed on a test plate, and reacted with a specific antibody. A second enzyme-labeled anti-globulin is then added which attaches to the antigen specific antibody. Next, the enzyme substrate is added and degradation of the substrate by the enzyme results in a color change. The amount of color change is an indicator of the amount of antibody which has bound to antigen. When the antibody concentrations are kept constant, the degree of color reaction product produced is proportional to the amount of antigen present.

Using an indirect ELISA methodology, we have developed an assay by which to measure latex protein levels. This assay is based on polyclonal antiserum which can detect the complete array of latex protein.⁹ This assay can detect and quantitate latex proteins in the nanogram per milliliter range. Thus the test is highly sensitive and suitable for routine use.

Materials and Methods

Extraction of latex proteins

Ammonified latex (Guthrie Latex, Inc. Phoenix, AZ) was poured onto a plastic plate and the emulsion allowed to collapse and air dry for 18 hours. The resulting latex film was cut into small pieces (1 cm²) and placed in a polypropylene bottle. Soluble proteins were recovered from the latex pieces by extracting them overnight (37°C) in 20 ml of distilled water. The latex pieces were removed and the extract centrifuged at 1000 x g to remove particulate matter and then dialyzed against phosphate buffered saline (PBS). Soluble proteins were extracted from latex surgical gloves in a similar manner. Protein concentration of the extracts was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL). The BCA method is a routine laboratory assay for the determination of protein concentration and was performed according to the instructions of the manufacturer.

Anti-latex protein antibody production

A 50/50 mixture (v/v) of latex proteins (10 mg/ml) and TiterMax Adjuvant (CytRx Corp., Norcross, GA) was prepared. A New Zealand White rabbit was injected with a total of 0.8 ml of the mixture in four sites on the back. The rabbit was bled at three weeks, five weeks, and seven weeks. The anti-latex protein titer was determined using the

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indirect enzyme-linked immunosorbent assay (ELISA) and reached a titer of 1/5000 after seven weeks.

Latex protein standard

To produce a quality preparation which could be used as a standard in the LEAP assay, latex proteins were extracted overnight from raw latex using 50 mM ammonium bicarbonate as a volatile buffer. The extract solution was centrifuged to remove particulate matter and was extensively dialyzed (110 volumes with three changes of water) to remove salts and low molecular weight peptides. The latex proteins were then lyophilized and the powdered protein was weighed. Based on the gram weight of the powder, the protein was reconstituted to a concentration of 1 mg/ml in distilled water. The reconstituted protein solution was then assayed for protein by the BCA method. The gram weight of protein powder and the BCA determination differed by 12%. The latex protein standard solution was then adjusted to 1 mg/ml based on the BCA determination. Finally, the protein was diluted to 10 µg/ml in 0.1 M carbonate buffer pH 9.6, divided into aliquots, and stored at -20°C until use as the standard in the ELISA assay.

LEAP: Latex Protein ELISA assay

Latex protein was absorbed onto the wells of 96 well easy wash polystyrene ELISA plates (Corning) by adding 100 µl aliquots of latex protein in 0.1 M carbonate buffer pH 9.6 to the wells and incubating the plates for five hours at 37°C. The standard curve was made by two-fold serial dilutions, in triplicate, of the latex protein standard (1 µg/ml) through eight wells. Similarly, samples containing unknown concentrations of latex proteins were also serially diluted. The plates were washed three times with PBS containing 0.1% Tween 20 (TPBS) and the unreacted sites blocked with 10% bovine serum albumen (BSA) in TPBS for one hour. The plates were then incubated with 100 µl of anti-latex protein antisera (1/5000 dilution) overnight at 37°C, washed three times with TPBS and reacted with 100 µl of HRP-labelled anti-rabbit IgG (1/1000 dilution) for one hour. After washing five times, a colored reaction product was produced by development in orthophenyldiamine (OPD, 1 mg/ml) in water containing 0.001% hydrogen peroxide. Finally, the reaction was stopped by the addition of 50 µl of 4N sulfuric acid and the orange colored reaction product was quantitated by reading its absorbance at 492 nm using a TiterTek Multiskan ELISA plate reader (Flow Laboratories). The concentration of latex protein in unknown samples was determined by comparison to the latex protein standard curve.

Results

To determine the anti-latex protein titer, microtiter wells were coated with 1 µg of latex proteins and reacted with serial dilutions of the antisera. The results summarized

in Figure 1 demonstrate the specificity of the antisera for latex proteins. The solid line demonstrates the reactivity of anti-latex antisera with latex protein coated wells. No reactivity was observed with the normal rabbit serum control. From these experiments, a working dilution of the primary antisera (1/5000) that gave a half maximal reading was chosen for use in further studies.

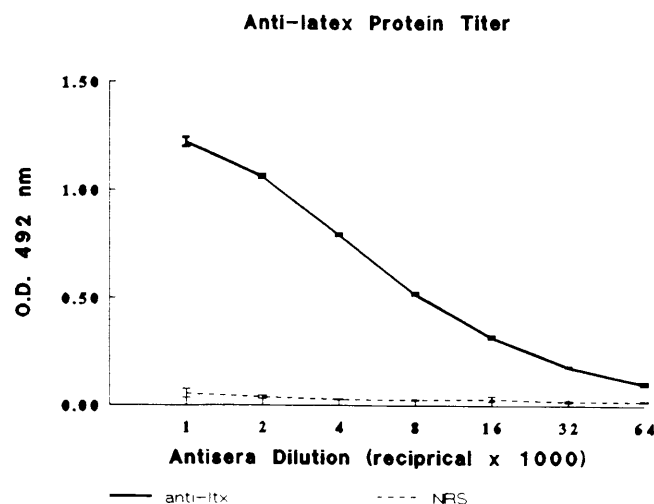


Figure 1.

Comparison of anti-latex protein antiserum to normal rabbit serum for reactivity with latex proteins. Data points represent the mean $\pm$ standard deviation of triplicate determinations.

Next, we established a protein standard with which to compare unknown samples of latex extracts. Proteins isolated from raw latex were extensively dialyzed against water to remove salts and small peptides, and then lyophilized to a powder. The resulting powder was weighed, reconstituted, and the protein concentration determined by the BCA method. This standard was diluted to 1 µg/ml in carbonate buffer before use in the LEAP assay. Figure 2 demonstrates the reproducibility of the standard curve generated by two-fold serial dilutions of this latex protein standard when reacted with 1/5000 dilution of the anti-latex protein antisera. These conditions were found to be optimal and used for subsequent assays of latex protein levels.

Using the LEAP assay, we studied the extractability of protein from latex. First, we determined the time required for extraction of proteins from surgical gloves. As shown in Figure 3, proteins are rapidly solubilized from latex gloves. Surprisingly, within one minute, 50% of the latex protein is solubilized. Protein extraction reaches a maximum at 30 minutes after which time little additional protein is extracted. In additional experiments, we found that the temperature of extraction (22°C vs 37°C) did not significantly

influence the amount of protein which was extracted; however, slightly higher protein levels were found when extraction was performed at 37°C.

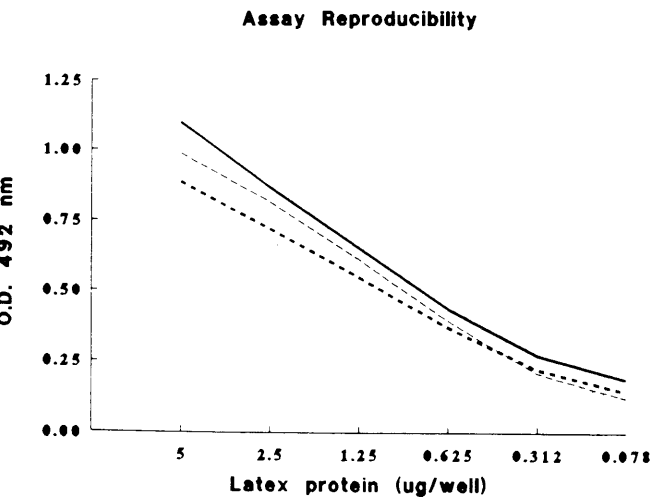


Figure 2. Demonstration of assay reproducibility. The standard protein preparation was assayed on four separate days. Data points represent the mean of triplicate determinations.

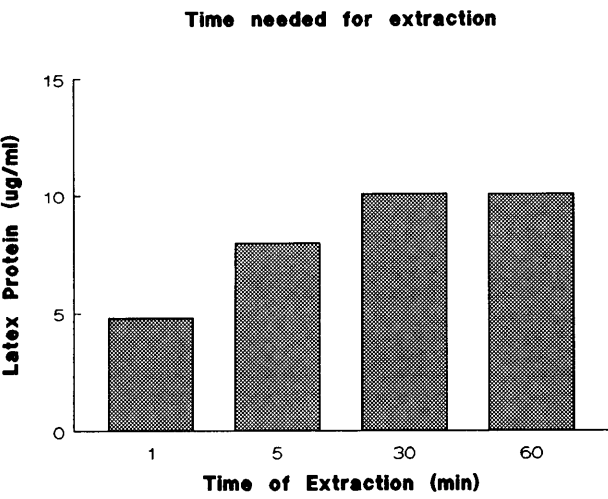


Figure 3. Examination of the time required for extraction of latex proteins. Four surgical gloves of the same brand were extracted separately in polypropylene bottles for the indicated times. For consistency each glove was extracted with PBS pre-warmed to 37°C.

Next, we examined soluble protein levels in 16 brands of surgical gloves by both the LEAP and the BCA method. For these experiments, one glove, from each of the various

manufacturers was extracted in 10 ml of PBS. Extraction was done overnight at 37°C to ensure recovery of as much of the protein as possible. To test for interfering substances, each extract solution was divided into two fractions. One fraction from each glove was dialyzed against water (molecular weight cut off membrane, 1000 Da) to remove the small molecules and peptides which are known to interfere in protein determination assays.^{10,11} The other fraction (neat) did not undergo further treatment. As shown in Table 1, the surgical gloves varied considerably in the amount of protein found on the gloves. The BCA and LEAP methods varied dramatically in the levels of protein observed. The BCA method produced values in the milligram range of protein recovered from the gloves. However, following dialysis, the protein levels observed by the BCA method were reduced by two-fold or greater indicating the presence of substances which interfere in the BCA method. In contrast, the LEAP method produced values in the microgram range of protein per glove. Importantly, LEAP was not influenced by dialysis, suggesting that the substances which interfere in the BCA assay do not influence the LEAP method. As a negative control, we included a surgical glove (Smartpractice, Tactylon) that was latex free. While the BCA method measured small amounts of protein in the latex free glove, no latex proteins could be detected with the LEAP assay.

Table 1

Company	Brand	Protein (ug/glove)			
		BCA		LEAP	
		neat	dialy	neat	dialy
Ansell	Medi-grip	21800	900	1	b.d.
	Sensi-touch	2700	700	87	47
Baxter	Ultraderm	4000	840	140	136
B.D.	Integron	5250	2100	253	171
	Eudermic	600	360	45	32
Fuji	Pristine	1200	30	28	34
J & J	Neutralon	2700	1400	159	194
Regent	Biogel	6000	450	43	25
	Biogel M	9000	700	0.6	0.6
Smartpractice	Tactylon	400	100	b.d.	b.d.
Travenol	Trifelx	11000	5750	2078	2046
Perry	White latex 42	10000	4250	111	112
	White latex 47	350	120	6	7
	Microptic	11200	4400	772	735
	Dermaguard	7000	225	163	149
	Orthopedic	13600	8200	168	137

Abbreviations:

b.d. = below detection
dialy = dialyzed
B.D. = Becton Dickinson
J & J = Johnson and Johnson

In the latex gloves, protein levels measured by the LEAP assay were approximately 100 fold lower than those determined by the BCA method. The microgram level of protein determined with the LEAP method is consistent with the protein levels which we have estimated in latex glove extracts using SDS-polyacrylamide gel electrophoresis. Given the strong interference observed in the BCA method, and the fact that LEAP is a specific antibody-based assay, it appears that the LEAP assay measures latex proteins in glove extracts more accurately than does the BCA method.

Discussion

Latex allergy has been reported in up to 7.4% of surgeons and 5.6% of operating room nurses.¹ In addition, a high percentage of dental practitioners¹² and 39-65% of spina bifida patients^{13,14} have latex sensitivity. Latex allergy can be life threatening for latex sensitive patients undergoing medical procedures and thus is considered a significant risk factor for healthcare workers. In this report, we describe the development of a new assay which may well aid in the study of the latex proteins responsible for some of these latex allergies.

The immunoassay LEAP can reliably detect low levels (nanograms) of latex proteins in extracts from surgical gloves and other latex containing products. The assay does not appear to be affected by the presence of interfering substances in the glove extracts. The LEAP method detects latex protein in the ng/ml and is thus 100 times more sensitive than the BCA method. Furthermore, because of the sensitivity of the LEAP, most extract solutions required further dilution for the protein levels to be in the range of the assay. This further reduces the possibility for substances to interfere in this assay. In contrast, the BCA method was found to estimate extremely high levels of protein, levels which we could not substantiate by other methods such as SDS-polyacrylamide gel electrophoresis. We believe that the LEAP method is the more accurate method by which to quantitate the amount of latex protein in extracts from latex materials.

A surprising finding was the speed at which the latex proteins are solubilized. Within one minute, we observed 50% of the maximal protein levels in the glove extracts. It is widely believed that the latex glove wearer is exposed to the latex proteins by sweating in the gloves and absorption of the proteins through compromised skin. Our data suggest that the latex proteins may be liberated in a very short period and thus an allergic reaction may appear rapidly. Conversely, it also suggests that latex glove contact with moist mucous membranes or body tissues for even a relatively short time may be enough to liberate substantial amounts of latex proteins from the gloves of the surgical personnel.

The soluble proteins found by this method do not account for all of the latex protein at risk in latex gloves.

Beside the soluble protein measured in the LEAP assay, latex proteins can be demonstrated bound to the starch particles which serve as donning agents on the gloves.⁹ The starch particles serve as a vehicle for the attached proteins to become aerosolized when the gloves are donned. Aerosolized starch particles have been reported to induce asthma, conjunctivitis, and rhinitis in latex sensitive people.^{15,16,17} Additionally, latex proteins may be chemically bound within the latex.⁷ These bound proteins may not leach out of the gloves in washing steps of the manufacturer. To produce truly protein-free latex gloves, latex proteins must be removed from the raw latex prior to manufacture.

Latex sensitive individuals must be made aware of the health problems associated with latex sensitivity as must those who provide healthcare.

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