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Allergenicity of Latex Gloves with Reference to Latex Protein Sensitive Individuals in a Canadian Population

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Allergenicity of latex gloves with varying levels of total extractable proteins was studied, by evaluating their allergic responses elicited, if any, in latex sensitive individuals of Canadian origin. Total extractable protein (EP) content of 30 latex medical gloves (powdered and powder-free) was determined by the modified Lowry method, according to both the Rubber Research Institute of Malaysia (RRIM) and the American Society for Testing and Materials (ASTM) *protocols. While* EP_{RRIM} *values ranged from 865 μg/g to <10 μg/g,* EP_{ASTM} *values varied from 870 μg/g to <30 μg/g. A total of 30 latex allergic volunteers were skin-prick tested with the glove extracts.*

Results showed that gloves with high extractable protein contents always tend to elicit positive allergic reactions. On the other hand, much reduced or no allergic responses were associated with gloves having low extractable proteins, regardless of whether the gloves were powdered or powder-free. More than 60% of negative allergic responses were consistently observed at EP_{RRIM} *of about 100 μg/g and lower, and at* EP_{ASTM} *of less than 50 μg/g.*

The total extractable protein content of latex gloves was found to be significantly correlated to their allergenicity, notwithstanding that the protein measurements were not fully allergen specific. Correlation coefficients 'r' of 0.94 for EP_{RRIM} *and 0.88 for* EP_{ASTM}*, were obtained. This finding confirms an earlier report that showed close correlation between allergenicity and* EP_{RRIM} *in a study based on a Finnish population of different genetic make-up.*

The awareness of latex protein allergy of Type I hypersensitivity affecting certain sensitised individuals in Europe and North America, through the use of some latex products has caused great concern to both the medical profession and the latex product manufacturers, especially those of latex gloves. Although incidence of occurrence among the general population has been estimated to be less than 1%, studies have indicated that the prevalence is higher among selected populations, such as the healthcare workers[1–8], spina bifida and multi-operated children[9–11]. However, it may be mentioned that prevalence of the potentially high-risk groups in Malaysia and Thailand is unexpectedly low[12,13], notwithstanding the fact

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that these are the world's leading glove exporters.

As the residual extractable protein fraction of some gloves has been shown to be the cause of the allergic reactions, the presence of this fraction, particularly the allergens, gives rise to an allergenic potential with reference to the sensitive persons. Assessment of this potential has therefore become important, both in the production by manufacturers and the proper selection by users of low risk, low-protein gloves. While there is a need to prevent or minimise further sensitisation among the unaffected population, it is essential that the latex allergic individuals be properly diagnosed and identified. For these patients, latex avoidance is presently the only available treatment.

To date, a number of methods for determining the allergenic potential have been studied. These include the assessment of allergen, antigenic and total extractable protein contents of latex gloves. For practical purposes, the simpler and faster method of measuring total extractable proteins (EP) is often preferred for routine monitoring, especially by the manufacturers. The test methods most commonly adopted for such measurement are those of the Rubber Research Institute of Malaysia (RRIM) (*MS 1392-98*)[14] and the *ASTM (D5712-95)*[15] modified Lowry tests. Both tests are somewhat similar, but the protein values generated for given samples are not identical. Although relatively non-allergen specific, earlier studies based on a latex hypersensitive population in Europe have shown that the total extractable protein values, EP_{RRIM} (as determined by RRIM method) of latex gloves were well correlated with both the allergen content (as determined by the ELISA-inhibition test)[16, 17] and the allergic responses indicated by the clinical skin prick test[17, 18]. Those studies have supported the measurement of EP as an indicator for allergenic potential.

In view of the fact that latex protein allergy can be brought about by more than one allergenic protein, and that specificity of these proteins can vary among different sensitive groups[19–24], it is possible that such variability may also exist between populations of different genetic make-up such as those from European and North American. The present study was therefore undertaken to ascertain if the relationship between EP_{RRIM} and allergic response elicited in latex sensitive individuals is similar in a Canadian latex allergic population. At the same time, the correlation with EP_{ASTM}, if any, was also examined.

METHODS

Latex Gloves

Medical latex gloves to be studied were selected based on their extractable protein content, from the 77 samples made available by the manufacturers for the 1996 glove survey carried out by the RRIM. Glove pieces cut from each glove sample were divided into two lots. While one lot of each sample was extracted and their protein concentrations re-determined at the RRIM in Kuala Lumpur, the samples in other lot were weighed, coded, carefully packed and sent to Wellesley Hospital in Toronto for skin prick testing.

Extractable Protein Content (EP_{RRIM}) – RRIM Modified Lowry Method (*MS 1392:98*)[14]

Protein extraction. Cut pieces of 7 cm × 7 cm each from the palm area of glove sample were extracted in 0.01 *M* phosphate buffered saline at pH 7.4 at 23°C for 3 h with agitation at 30 min intervals. The clear extract was obtained after removing any insoluble matter that might be present by centrifugation.

Protein precipitation. Proteins in 6 ml of extracts were precipitated using trichloroacetic acid (4.4%, w/v) and phosphotungstic acid (0.2%, w/v). The precipitated proteins were then sedimented by centrifugation at 10 000 g for 30 min. The resulting protein pellet was re-dissolved in minimum volume of 0.2 *M* sodium hydroxide (1 mL – 6 mL) after removal of the supernatant containing interfering substances.

Modified Lowry microassay. 0.8 ml aliquot of each re-dissolved protein solution was treated with 0.3 ml of a reagent containing 6% sodium carbonate and 1.5% copper sulphate in 3% sodium citrate (carbonate: sulphate = 10 : 0.2). After allowing the mixture to stand for 10 min, 0.1 mL of 72% 2 *N* Folin reagent was added. Colour was allowed to develop at room temperature for 30 min, and its absorbency at 750 nm was recorded. If precipitation occurred at this stage, further centrifugation was carried out to give a clear solution for the colorimetric measurements. Results were read against a standard Bovine Serum Albumin (BSA) curve and converted to μg/g or mg/g of gloves, taking into consideration the weight of sample extracted and the volume used in each case.

Extractable Protein Content (EP_{ASTM}) – *ASTM* Modified Lowry Method [*D 5712-95*][15]

Protein extraction. Cut pieces of 7 cm × 7 cm each from the palm area of glove samples were extracted in distilled water at 37°C for 2 h with agitation at 30 min intervals. The clear extract was obtained after removing any insoluble matter that might be present by centrifugation.

Protein precipitation. Proteins in 1 mL of extract were precipitated using deoxycholate (0.014%), trichloroacetic acid (5.5%, w/v) and phosphotungstic acid (5.5%, w/v). The precipitated proteins were then sedimented by centrifugation at 6000 g for 15 min. The resulting protein pellet was re-dissolved in minimum volume of 0.1 *M* sodium hydroxide (0.25 mL – 1 mL) after removal of the supernatant containing interfering substances.

Modified Lowry microassay. 0.2 mL aliquot of each re-dissolved protein solution was treated with 0.1 mL of alkaline copper solution. Protein concentration was determined by the Lowry microassay according to procedure set out in the Bio-Rad *DC* protein kit. Results were read against a standard Ovalbumin curve and expressed as μg/g or mg/g of gloves.

Skin Prick Test (SPT)

Glove pieces from each test sample were extracted in phosphate buffered saline at pH 7.2 (5 mL per gram of gloves) at room temperature for 15 min. A drop of each test extract was applied to the volar surface of the forearm and then pieced with a one-mm one-peaked sterile lancet (Miles, Warwickshire, UK), creating a small break in the epidermis. The drops were wiped clean after 15 min and size of any wheal and flare developed, was carefully outlined and measured. A positive control using histamine dihydrochloride (1 mg/mL), a negative control with physiological saline, and a Bencard Latex Reagent containing 7–10 mg/mL of proteins were also included in the test battery. The skin test protocol has been approved by the Ethical Review Committee of the Ontario Allergy Society.

Test responses were evaluated as measurement of the average of two perpendicular diameters of the wheal in excess of the average diameter of the negative control wheal. A measurement of >4 mm with reference to that of the negative control is considered to be a positive reaction. All positive reactions were transferred with transparent tape as a permanent record.

RESULTS

Extractable Protein Content of Gloves

Thirty commercially available latex medical gloves comprising 33% of powder-free and 67% of powdered types were selected. Their total extractable protein content EP_{RRIM} was found to range from as low as <10 μg/g to as high as 865 μg/g, and their EP_{ASTM} content from <30 μg/g to as high as 870 μg/g. Although the two sets of values are not exactly similar, they are however well correlated, with the coefficient of correlation, r = 0.91, n = 30 at P< 0.001 (*Figure 1*). This confirms results obtained from earlier study[25], where r = 0.93, n = 90.

Population Tested

Thirty otherwise healthy patient volunteers who were previously diagnosed as latex protein allergic with a positive history and latex skin test positive to a standard latex preparation (Bencard Laboratory, Mississauga, Ontario, Canada) were studied. These included healthcare (60%) and non-healthcare workers (40%) as shown in *Table 1*. Their average age was 35.6 years ranging from 23 years to 66 years. Except for one participant, all the others were females. All demonstrated cutaneous reaction to latex, while 56.7% of them showed symptoms that were inhalant, and 13.3% of them were anaphylactic.

A control population of 10 patients, not known to be latex allergic was also tested at the allergy clinic. These patients were, however, diagnosed to have allergy symptoms such as allergic rhinitis, urticaria, chronic sinusitus and anaphylaxis due to other allergens. All control subjects tested negative to latex and the diluent (negative control), but positive to histamine (positive control).

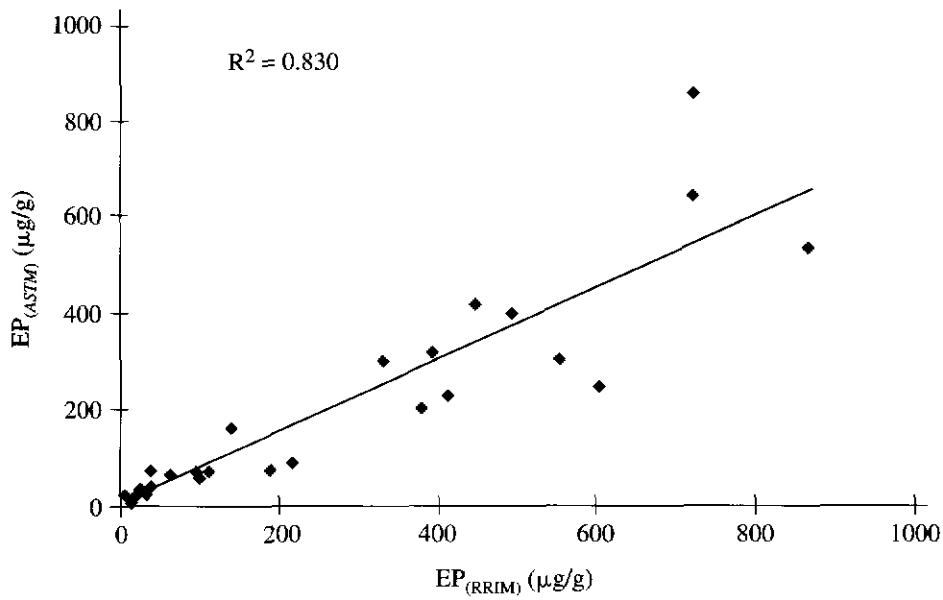

Figure 1. Correlation between total extractable protein contents as determined by the RRIM modified Lowry test and those determined by the ASTM *modified Lowry test, for 30 latex medical gloves.* Correlation coefficient r = 0.91.

TABLE 1. CHARACTERISTICS OF POPULATION TESTED

Number of patient volunteers tested		30
Age range		23 yrs. – 66 yrs.
Mean age		35.6 yrs.
Healthcare workers		18 (60%)
Laboratory workers		4 (13.3%)
Non-healthcare workers		8 (26.7%)
Female		29
Male		1
Allergy symptoms:	*Cutaneous*	30 (100%)
	Inhalant	17 (56.7%)
	Anaphylactic	4 (13.3%)

Skin Prick Responses

Each of the 30 latex sensitive volunteers was clinically skin-tested with extracts of the 30 latex glove samples. It may be mentioned that although protein extraction time for the skin tests was 15 min as compared to 2–3 h as in the case of the Lowry tests, extracts of 15 min have been shown to contain more reactive antigenic components and elicit stronger reactions in latex sensitive subjects than the 24-h extracts[26]. Results, expressed as percent positive SPT responses in relation to total extractable protein contents of latex gloves, are shown in *Table 2*.

Statistical analyses of the data revealed that total extractable protein contents of latex gloves are well correlated with both EP_{RRIM} and EP_{ASTM}. Semi-log plot of the two parameters in each case can be seen in *Figures 2a* and *2b*. The coefficient of correlation 'r' for EP_{RRIM} is 0.94, and for EP_{ASTM} the coefficient is 0.88, at $P<0.001$ for both instances.

DISCUSSION

Latex protein allergy is a Type I immediate hypersensitivity response, which is IgE mediated. The allergic reactions are elicited with the degranulation of mast cells resulting in the release of preferred and newly generated chemical mediators. Mast cell degranulation is triggered off by the crosslinking of specific IgE or IgG_4 antibodies with latex allergenic peptides on the surface receptors found in latex sensitised individuals.

Although skin prick testing confirms that certain proteins or polypeptides can elicit the allergic reactions *in-vivo*, and can be used for assessing allergenic potential of latex gloves[16,18, 27], it is an impractical test due to the requirement of latex hypersensitive subjects that are not easily available. The use of *in-vitro* serological immunoassays such as the allergen-specific radioallergiosorbent test with inhibition (RAST-inhibition) and the allergen specific enzyme-linked immunosorbent assay (ELISA-inhibition) is relatively more convenient[16,17, 27–29] In these assays,

TABLE 2. ALLERGIC RESPONSES ELICITED IN LATEX SENSITIVE POPULATION BY GLOVES WITH VARYING TOTAL EXTRACTABLE PROTEIN CONTENTS

No.	EP_{RRIM} (μg/g)	EP_{ASTM} (μg/g)	SPT Response (% of 30 subjects tested)	
			Positive response	Negative response
1*	5	29	6.7	93.3
2*	8	30	0	100
3*	9	29	6.7	93.3
4*	11	27	0	100
5*	22	30	10.0	90.0
6	33	48	20.0	80.0
7*	39	29	20.0	80.0
8*	46	40	23.3	76.7
9	47	87	20.0	80.0
10*	49	52	23.3	76.7
11*	68	79	23.3	76.7
12	71	72	13.3	86.7
13	84	68	40.0	60.0
14	95	63	36.3	63.3
15	102	53	53.3	46.7
16	113	62	23.3	76.7
17	140	159	50.0	50.0
18	188	78	46.7	53.3
19	216	93	56.7	43.3
20	330	306	70.0	30.0
21	379	208	66.7	33.3
22	392	325	56.7	43.3
23	412	235	66.7	33.3
24	446	423	53.3	46.3
25	492	405	76.7	23.3
26	553	312	66.7	33.3
27	603	253	70.0	30.0
28	719	650	70.0	30.0
29	726	870	63.3	36.7
30	865	545	70.0	30.0
a	Diluent	–	0	100
b	Histamine**	–	86.7	13.3
c	Latex*** (Bencard)	–	93.3	6.7

* Denotes powder-free gloves. All other gloves were powdered.

** 4/30 subjects showed very mild responses of <4mm to histamine, but demonstrated clearly positives responses to some glove extracts.

*** 2/30 subjects indicated very mild reactions to latex, but exhibited clear positive responses to some glove extracts.

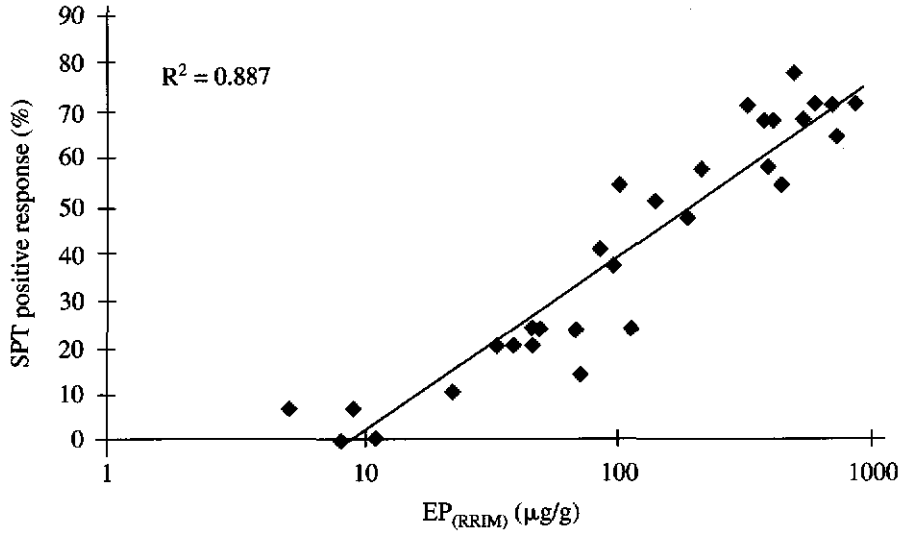

Figure 2a. Correlation between results of EP_{RRIM} of 30 latex gloves and positive SPT responses elicited in 30 latex sensitive individuals in Canadian population. Coefficient of correlation, r = 0.94, P<0.001.

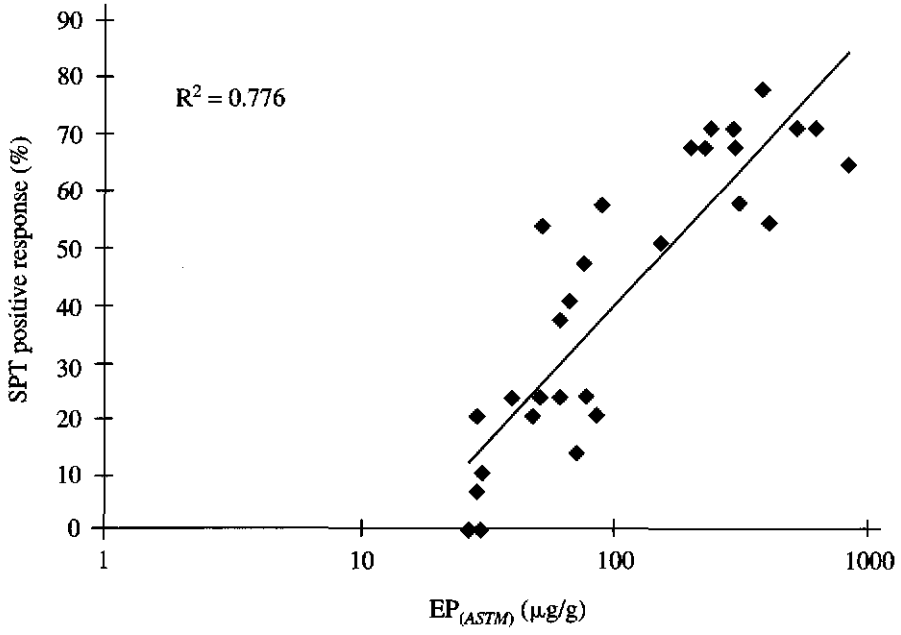

Figure 2b. Correlation between results of EP_{ASTM} of 30 latex gloves and positive SPT responses elicited in 30 latex sensitive individuals in Canadian population. Coefficient of correlation, r = 0.88, P<0.001.

serum IgE antibodies from latex sensitive persons are used. These tests involve the binding of allergen-specific IgE antibodies with the specific allergen. However, two effective reference standard mixtures are required, namely, a pool of the relevant allergenic proteins and a complement of the corresponding specific IgE antibodies. Unfortunately, due to the lack of complete information regarding the spectrum of allergenic proteins present particularly in latex products, such standard reference mixtures are currently not yet available. As a result, considerable variation exists in such measurements by different laboratories employing different reference mixtures. These tests are also relatively less specific and sensitive than the clinical skin prick test. Furthermore, the test procedures are rather sophisticated. Therefore, the technically easy measurements of total extractable proteins that can be standardised are adopted today for routine monitoring of allergenic potential of latex products[14,15]. Since not all proteins present in the residual extractable protein fraction analysed are allergenic, the main shortfall of these tests is the non-specificity for these allergens.

In our present study, the relevance of the total extractable protein measurements in relation to the skin prick test response shown by latex hypersensitive subjects was examined. The allergic responses, elicited by glove extracts containing varying amount of proteins, in latex hypersensitive individuals of Canadian origin demonstrated a very significant relationship between the two parameters (*Figure 2a*). High total extractable protein contents of latex gloves (EP_{RRIM} , as measured by the RRIM test method) were associated with higher percentage of the tested subjects showing positive allergic responses. On the other hand, lower total extractable protein contents tended to show much reduced or no positive allergic responses. This clearly confirms the correlation reported in earlier studies[18,30], particularly the one conducted with latex hypersensitive individuals from a Finnish population. As EP levels at which majority of hypersensitive subjects does not react to are of great interest in relation to low risk products, EP values were plotted against allergic SPT reactions expressed as percent negative responses (*Figure 3*).

Although the two sets of results indicated similar statistical significance between EP_{RRIM} and SPT response, the rate at which test subjects reacted negatively to skin prick testing with decreasing EP content appeared to be higher among the Finnish population than in the Canadian population. This could be partly attributed to variation in allergic response by the two test populations, and partly due to some differences in allergen content of the two batches of latex gloves studied. It is of interest to note that the two regression lines intersected at EP_{RRIM} of 130 μg/g where approximately 55% of both groups of test subjects were predicted to show no allergic response. It is however apparent that, at lower EP levels less than 100 μg/g, both populations showed higher percentages of *negative* SPT responses, often reaching 100% as EP_{RRIM} continued to decrease. This observation is consistent with that reported for the allergen content[17], which was shown to be very low at EP_{RRIM} of about 100 μg/g and less. Such levels are certainly markedly lower than those of gloves encountered during the early nineties, which often had EP as high as 1000 μg/g or more.

It is generally accepted that powder-free gloves, especially those chlorinated ones, have very low extractable protein contents, and hence their low allergenicity. However, our results showed that powdered gloves with similarly low extractable protein levels could also exhibit low allergenicity, as reflected in

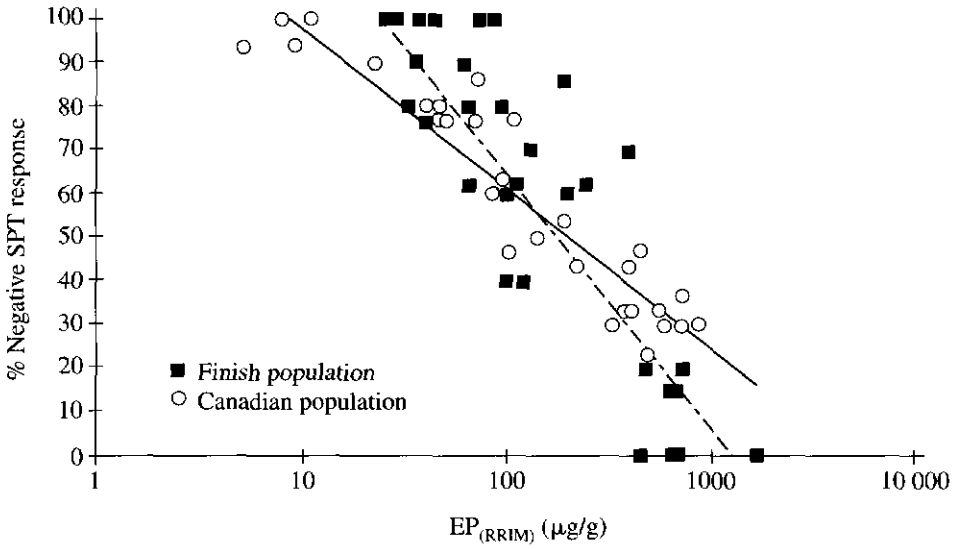

Figure 3. Correlation between total extractable proteins (EP_{RRIM}) of latex gloves and percentage negative SPT responses shown by latex hypersensitive individuals from Canadian population as compared to that from Finnish population[18]. Canadian population: $r = -0.94$, $n = 30$; Finnish population: $r = -0.87$, $n = 39$.

the similarly high percentages of *negative* allergic response observed (*Table 2*). Although there is virtually no information regarding threshold EP levels for causing sensitisation or allergic reactions in sensitive individuals, the above findings could help to provide useful guideline for the manufacture of low-risk products for the non-affected users, which constitute majority of the general population.

The *ASTM* modified Lowry test [*ASTM (D5712-95)*] is quite often employed for protein analysis of latex gloves, especially for the purpose of 'low protein labeling claim' in the marketing notification application in USA. The protein values generated (EP_{ASTM}) are not identical as the EP_{RRIM}, nor are they significantly different from each other. However, they are well correlated (*Figure 1*). The relationship of EP_{ASTM} with allergic response, studied simultaneously with EP_{RRIM}, revealed certain degree of similarity. Owing to the lower sensitivity of *ASTM* test method particularly at the lower end, EP_{ASTM} values for the same glove samples are seen to spread over a shorter range as compared to EP_{RRIM} (*Figures 2a* and *2b*). It is also noteworthy that in view of the wider scatter of the EP_{RRIM} values, levels whereby higher percentage of negative responses (>60%) were consistently observed, seem to be at EP_{ASTM} lower than 50 μg/g instead of 100 μg/g as shown by EP_{RRIM} (*Figure 4*).

CONCLUSION

The measurement of total extractable protein content of latex gloves may not be fully allergen specific, protein values obtained in the

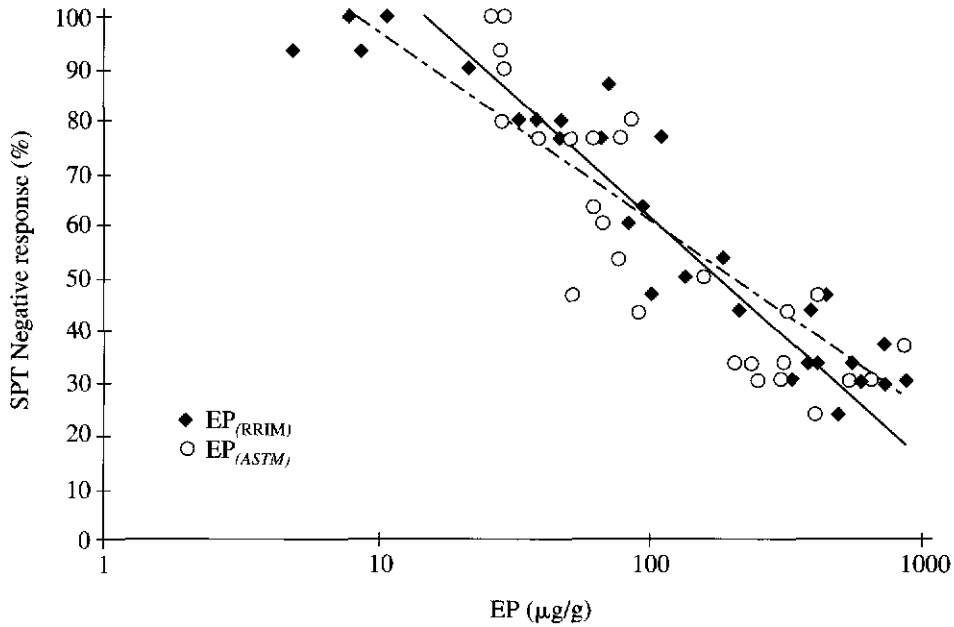

Figure 4. Total extractable protein content of 30 latex gloves and corresponding percentage negative allergic response shown by 30 latex hypersensitive individuals of Canadian origin, when clinically skin prick tested. EP_{RRIM} denotes protein values by RRIM modified Lowry test; and EP_{ASTM} denotes protein values by ASTM modified Lowry test. 'r^2' is 0.887 for EP_{RRIM} and 0.776 for EP_{ASTM}.

present study have once again been shown to be significantly correlated to the allergenic potential as evaluated by skin prick responses elicited in latex hypersensitive individuals. This relationship persisted regardless of origin of the latex hypersensitive population tested. As observed earlier, the test subjects also consistently showed high percentages of no allergic reaction when EP_{RRIM} fell below 100 μg/g of gloves, regardless of the powder status of gloves. However, rate of increasing negative allergic response with decreasing EP level seemed to be relatively higher among the Finnish/European population than that of Canadian/North American population. This implies that the Finnish/European hypersensitive population tested was more sensitive to changes in extractable protein content of latex gloves than that of the Canadian/North American.

Similarly, the total extractable protein content of latex gloves, EP_{ASTM}, is also well correlated with the skin prick testing response elicited in the same test subjects of Canadian origin.

It is well recognised that allergenicity of latex gloves has become an important parameter, in relation to minimising further sensitisation among users. An adequate assessment of this parameter is therefore necessary. In the absence of an ideal method, the determination of total extractable protein content using the modified Lowry assays is a

good interim compromise while accurate specific methods of quantification of latex allergens are being developed. The Lowry methods, being technically easy, sensitive, reproducible and possible to standardise, are indeed very useful in monitoring the various phases of development and manufacturing of latex products with low allergenic potentials.

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