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Measurements of Total Extractable Proteins in Latex Gloves: A Comparative Study of the RRIM and ASTM *Tests*†

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Extractable protein contents of latex gloves generated by two commonly used methods, the RRIM (MS 1392:96P) *and the* ASTM (D 5712-95) *modified Lowry tests, were examined and their relationship studied.*

Total extractable proteins, EP_{RRIM}, determined by the RRIM test, ranged from 1326 μg/g to < 20 μg/g for 90 gloves. Their corresponding EP_{ASTM} values, obtained by the ASTM test, varied from 1377 μg/g to < 50 μg/g. Statistical analysis showed a very significant correlation between them , with a coefficient of correlation, $r = 0.93$, $P < 0.001$. Generally, EP_{RRIM} values read higher than those of EP_{ASTM}. EP_{ASTM} of 50 μg/g and lower were found to be associated with EP_{RRIM} values ranging from 267 μg/g to < 20 μg/g, suggesting higher sensitivity of the latter measurements. Relevance of the two sets of EP in relation to the allergenicity/allergic potential of latex gloves was discussed.

Accelerated ageing at 70°C for 7 days of latex gloves resulted in the lowering of protein contents. The effect appeared to be more pronounced for EP_{RRIM}, than for EP_{ASTM}.

In view of the latex protein allergy problem related to NR latex products, especially gloves[1], there is a need to evaluate the allergic potential of these products. However, till to-date, a universally standardised test for doing so is still lacking. Many different methods[2–9] are being used in various laboratories in different parts of the world. The methods most commonly adopted in manufacturing countries, particularly Malaysia, are the RRIM test (which has recently become a *Malaysian Standard Test*)[7] and the *ASTM* test[8], both for the determination of total extractable protein content of latex products.

While the RRIM test has been employed for as long as the beginning of the allergy problem, the *ASTM* was established a few years later. It is often asked how different the two sets of values so obtained are and if they are correlated. The present study has therefore been undertaken to compare these values and to see if any relationship exists between them. Furthermore, in view of the ageing requirement

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by the FDA for 'Low Protein Labelling Claim' for latex gloves[10], this effect on total extractable proteins is also investigated.

MATERIALS AND METHODS

Preparation of Gloves Pieces

Square pieces, of dimension 7 cm x 7 cm, were cut from palm area of each glove sample. Care was taken to avoid any contamination.

Accelerated Ageing

Glove pieces were subjected to accelerated ageing at 70°C for 7 days, according to *ASTM 3578-91*.

RRIM Modified Lowry Test[7] (*Malaysian Standard Protein Test, MS 1392-96P*)

Cut pieces of each test sample were extracted in 0.01 *M* phosphate buffered saline (pH 7.4) at 23°C for 3 h. Extract was clarified by centrifugation, to sediment any particulate matter, such as powder. Precipitation of proteins was carried out using trichloroacetic and phosphotungstic acids. This was followed by further centrifugation at 10 000 × g for 30 min. The resulting pellet was redissolved in 0.2 *M* sodium hydroxide, and their concentration determined by the Lowry microassay. Absorbance readings, recorded at 750 nm, were calibrated against standard bovine serum albumin (BSA). Results were expressed as EP_{RRIM} in μg/g of gloves.

ASTM Protein Test[8]

Proteins were extracted in distilled water at 37°C for 2 h. Extract was centrifuged and the proteins were precipitated using deoxycholate, trichloroacetic and phosphotungstic acids. Sedimentation of the protein pellet was carried out by centrifugation at 6000 x g for 15 min. The precipitated proteins were redissolved in 0.01 *M* sodium hydroxide, and their concentration determined by Lowry microassay according to procedure set out in the BioRad *DC* protein kit. Absorbance was recorded at 750 nm and readings calibrated against standard ovalbumin. Results were presented as EP_{ASTM}, in μg/g of gloves.

RESULTS

Total Extractable Protein Contents

A total of 90 commercial gloves were extracted and tested using both the RRIM and *ASTM* methods. Results are summarised in *Table 1*. It can be seen that total extractable protein content, EP_{RRIM}, ranged from as high as 1326 μg/g to as low as to 19 μg/g. Their median and overall mean values were 505 μg/g and 519 μg/g, respectively. Similarly, values of EP_{ASTM}, varied from 1377 μg/g to 36 μg/g, with a median of 372 μg/g and a mean of 423 μg/g. Comparison of the overall means indicated EP_{ASTM} was 18.5% lower than that of the EP_{RRIM}, while in the case of the medians, the decrease was 26.3%.

Closer examination of the data at different EP_{RRIM} ranges, revealed that means of EP_{ASTM} read 13.8% – 25% lower than those of EP_{RRIM} when the latter values were greater than 100 μg/g. However, at EP_{RRIM} of 100 μg/g and lower, the mean of EP_{ASTM} indicated 17.6% higher values than that of EP_{RRIM} (*Table 2*).

Comparison of data based on different EP_{ASTM} ranges, on the other hand, was found to vary somewhat as shown in *Table 3*. Although

TABLE 1. COMPARISON OF EXTRACTABLE PROTEIN CONTENTS BY RRIM AND *ASTM* METHODS FOR 90 GLOVE SAMPLES

Item	EP_{RRIM} (μg/g)	EP_{ASTM} (μg/g)	% diff.
Range	19 – 1326	36 – 1377	–
Median	505	372	26.3
Mean	519	423	18.5
S.d.	407	348	–

S.d.: Standard deviation

TABLE 2. MEANS OF EP_{RRIM} AND EP_{ASTM} AT DIFFERENT EP_{RRIM} RANGES FOR 90 GLOVE SAMPLES

EP (μg/g)	EP_{RRIM} range (μg/g)				
	100 & less	>100 – 400	>400 – 700	>700 – 1000	>1000
No. of samples	20	21	16	18	15
Corresponding EP_{ASTM} Range	36–107	49–408	219–673	360–1377	557–1238
EP_{RRIM} Mean (S.d.)	51 (28)	212 (63)	551 (86)	846 (83)	1148 (102)
EP_{ASTM} Mean (S.d.)	60 (23)	159 (106)	451 (120)	729 (228)	877 (184)
% diff.[a]	–17.6	25.0	18.1	13.8	23.6

[a] % with reference to EP_{RRIM} values

lower EP_{ASTM} values than those of EP_{RRIM} were still apparent, the difference was more marked at the lower ranges. Nevertheless, statistical analysis of the two sets of data showed that they are very closely related, with the coefficient of correlation, $r = 0.93$, $P < 0.001$ (*Figure 1*).

Effect of Accelerated Ageing

EP_{RRIM} and EP_{ASTM} contents of latex gloves were determined with and without accelerated ageing. Of the 77 lots of commercial gloves tested, 61 were powdered gloves, and 16 were powder-free of which 4 were siliconised,

TABLE 3 MEANS OF EP_{RRIM} AND EP_{ASTM} AT DIFFERENT EP_{ASTM} RANGES FOR 90 GLOVE SAMPLES

EP (μg/g)	EP_{ASTM} range (μg/g)				
	50 & less	>50 – 100	>100 – 400	>400 – 700	>700
No of samples	14	16	19	21	20
Corresponding EP_{RRIM} Range	19–206	51–267	113–835	434–1017	817–1326
EP_{RRIM} Mean (S d)	66 (71)	136 (79)	353 (142)	743 (167)	1065 (162)
EP_{ASTM} Mean (S d)	45 (6)	76 (18)	289 (83)	576 (82)	924 (178)
% diff [a]	31 8	44 1	18 1	22 5	13 2

[a] % with reference to EP_{RRIM} values

3 polymer coated and the rest chlorinated Results are summarised in *Table 4* below.

It is apparent that accelerated ageing resulted in a lowering of 25 2% in the overall means of EP_{RRIM} and 16.5% reduction in the corresponding EP_{ASTM} measurements The magnitude of changes was relatively greater in the case of the powdered gloves (reduction of 25 4% in EP_{RRIM}, and 16.9% in EP_{ASTM}), and less so in the powder-free gloves (10.6% and 4 2%, respectively)

The aged EP values were very well related to those of the unaged in both cases, especially the EP_{RRIM}, as illustrated in *Figures 2* and *3*. The higher coefficient of correlation of *0 98* for EP_{RRIM} as compared to 0.92 for EP_{ASTM} (both at $P < 0.001$) supports the higher sensitivity of the RRIM test.

DISCUSSION

Total Extractable Proteins

Although both the RRIM and *ASTM* tests involved the Lowry colorimetric assay technique, their protocols are not the same Briefly, the general procedure comprises three parts· (1) Protein extraction, (2) Protein precipitation and (3) Protein quantitation A number of variations have been adopted by both tests (*Appendix 1*) These have consequently resulted in differences not only in the sensitivity of the tests, but also in the extractable protein (EP) values so generated This is particularly so in the case of the calibration curves, where absorbance readings are much lower for the *ASTM* test than the RRIM test for the same protein concentration Sensitivity limits of the methods are, in fact, 20 μg/g and 50 μg/g for the RRIM and the *ASTM* tests, respectively. In agreement with

TABLE 4. EFFECT OF ACCELERATED AGEING ON EP_{RRIM} AND EP_{ASTM} OF LATEX GLOVES

Glove samples	EP_{RRIM} (μg/g) Unaged	Aged	% change	EP_{ASTM} (μg/g) Unaged	Aged	% change
All gloves (n = 77)						
Range of EP	5 – 1540	3 – 1186		14 – 1966	8 – 1537	
Mean EP	591	442	– 25.2	484	404	– 16.5
Powdered gloves (n = 61)						
Range of EP	33 – 1540	21 – 1186		48 – 1966	25 – 1537	
Mean EP	733	547	– 25.4	598	497	–16.9
Powdered-free gloves (n = 16)						
Range of EP	5 – 140	3 – 121		14 – 159	8 – 130	
Mean of EP	47	42	–10.6	48	46	– 4.2

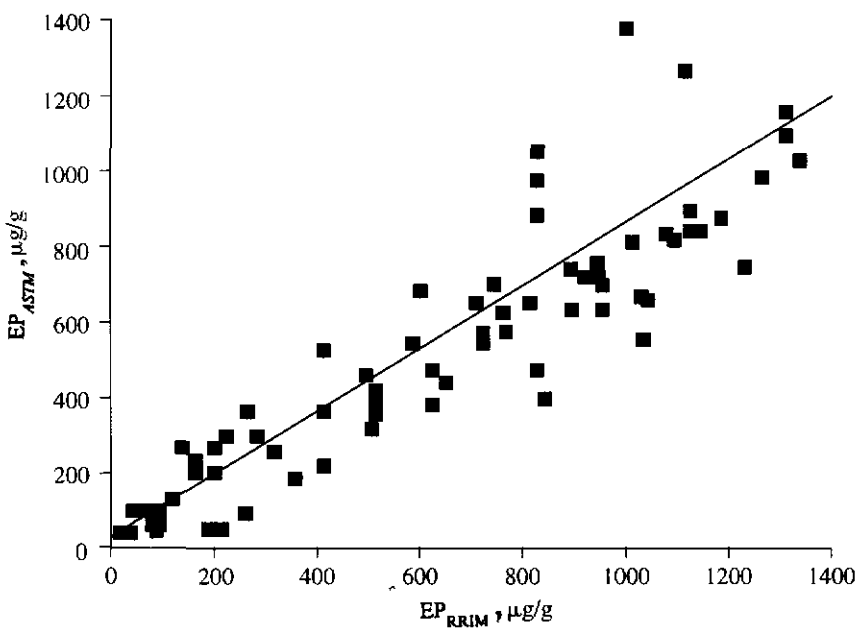

Figure 1. Correlation between EP_{RRIM} and corresponding EP_{ASTM} values of latex gloves. Coefficient of correlation, r = 0.93, P<0.001, n = 90.

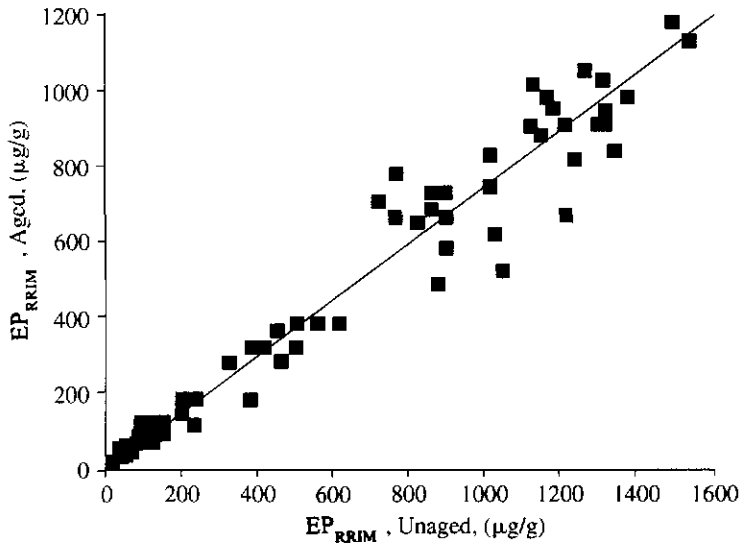

Figure 2. Relationship between aged and unaged EP_{RRIM} values for 77 lots of latex gloves. Correlation coefficient r = 0.98, P<0 001.

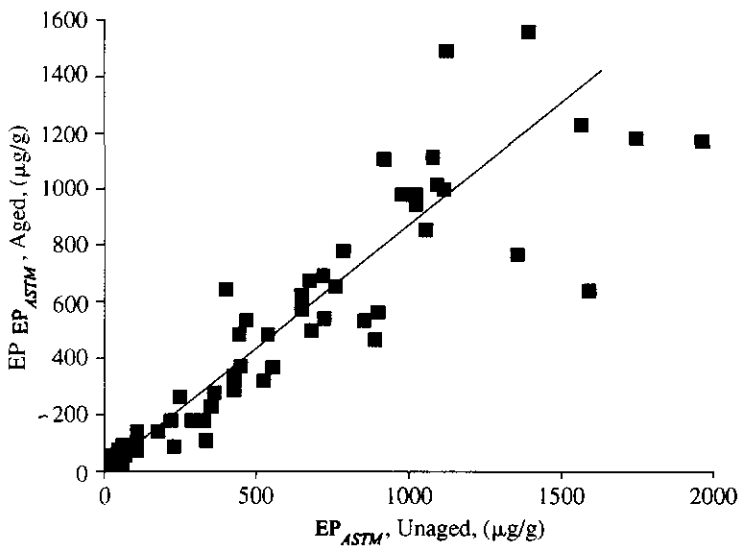

Figure 3. Relationship between aged and unaged values of EP_{ASTM} for 77 lots of latex gloves. Coefficient of correlation r = 0.92, P<0.001.

this is also the fact that the EP_{RRIM} values read higher than those of the corresponding EP_{ASTM}, for the same latex glove samples tested Furthermore, it is noteworthy that, as shown by the present findings, low EP_{RRIM} of up to 100 μg/g corresponded to EP_{ASTM} of similar range, *i e* up to 107 μg/g (Table 2), while low EP_{ASTM} values of 100 μg/g and less were found to be associated with a much wider range of EP_{RRIM}, *i e* 267 μg/g and lower *(Table 3)*. Of special interest is the fact that gloves with EP_{ASTM} of 50 μg/g (sensitivity limit of the *ASTM* test) and less, were shown to have EP_{RRIM} varying from 206 μg/g to < 20 μg/g. This could mean that the RRIM test is capable of yielding more sensitive measurements than the *ASTM* test On the other hand, it could also be argued that such behaviour might be due to differences in response to interference by the two tests However, there is presently no data to substantiate this The two sets of EP data are, in fact, very well correlated, coefficient of correlation, r = 0 93, P < 0.001 and n = 90

Although both tests measure only the total extractable proteins, and not the allergens alone, the EP data so obtained could be of relevance if they relate well to the allergen contents or allergenicity of the test samples This has, in fact, been shown to be the case for EP_{RRIM} [11,12] Gloves with EP_{RRIM} contents varying from > 1000 μg/g to < 20 μg/g were both evaluated for their allergen levels using the IgE ELISA-inhibition test[11] , as well as tested by the skin prick test on latex hypersensitive subjects from a European population[12] . Highly significant relationships were obtained in both cases More importantly, EP_{RRIM} contents of about 100 μg/g and lower were shown to be associated with not only very low allergen levels, but also with very little allergic response by the sensitive persons Such findings have rendered the EP_{RRIM} measurements useful as indicators of allergic potential of latex gloves

There are no similar studies reported on EP_{ASTM}, but according to Sussman *et al* [13] who recently skin prick tested some latex sensitive persons in Canada with gloves of EP_{ASTM} contents up to 50 μg/g , more than 50% of the subjects responded positively In the light of the present observations, it may not be impossible that the gloves tested included some with EP_{RRIM} levels of as high as 200 μg/g, hence contributing to the relatively high positive responses observed. To confirm this, more studies are needed

Effect of Accelerated Ageing

The Food and Drug Administration (FDA) of the USA, in addressing the latex allergy issue, allowed a 'Low Protein Labelling Claim' for latex medical gloves[10] in May 1995 As one of the requirements relating to the claim, accelerated ageing is necessary prior to analysis of the test sample for total extractable protein content. This is in view of the fact that the EP content might increase during storage

The present study has in fact shown that decreases in the extractable protein contents of latex gloves were detected after the ageing process. The drop in EP could likely be due to denaturation of some extractable proteins when the dry gloves were subjected to prolonged heating, leading to certain amount of insolubilisation of these proteins Such decreases were more apparent in the case of the powdered gloves than the powder-free gloves, suggesting that some loss of powder during the ageing process might have partly contributed to this difference

Very occasional increases in EP have also been observed The presence of water in the not so dry test sample could be the cause In such cases, heating during the ageing process could have facilitated further migration of the soluble proteins towards the surface of the film along side with the water, resulting in higher EP contents

CONCLUSION

It may be concluded that both the RRIM and the *ASTM* tests are suitable for measuring total extractable proteins of latex products. Protein values by the former test are generally higher than those of the latter test, and the two sets of data are significantly correlated However, it should be pointed out that the EP values in both cases are not absolute, being referred to arbitrary protein standards. As such, their comparison could only be deemed more precise if done in reference to a standardised method, which is currently not yet available . But in view of the good correlations between EP_{RRIM} and allergenicity/allergen content, the EP_{RRIM} may be considered to be a useful indicator for allergic potential of latex gloves, particularly for the manufacture of low protein gloves. On the other hand, more data is required in the case of EP_{ASTM}

Accelerated ageing of latex gloves generally has no adverse effect to their extractable protein contents

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REFERENCES

1 *Proceedings of International Latex Conference Sensitivity to Latex Medical Devices (1992), November 5 – 7, Baltimore, Maryland, USA*

2 TURJANMAA K , LAUREN K , MAKINEN-KILJUNEN S AND REUNALA T (1988) Rubber Contact Urticaria Allergenic Properties of 19 Brands of Latex Gloves *Contact Derm* , **19**, 362

3 BEEZHOLD, D H (1993) Measurement of Latex Protein by Chemical and Immunological Methods *Proceedings of Latex Protein Allergy The Present Position, Amsterdam*, 25

4 YUNGINGER, J W , JONES, R T , FARAWAY, A F , KELSO, J M , WARNER, M A AND HUNT, L W (1994) Extractable Latex Allergens and Proteins in Disposable Medical Gloves and Other Rubber Products *J Allergy Clin Immunol* , **93**, 836

5 HAMILTON, R G AND ADKINSON, N F (1994) Serologic Assays for Antigens and Antibodies *Immunol Allergy Clin N Amer* **14**, 351

6 FARIDAH YUSOF AND YEANG H Y (1992) Quantitation of Proteins from Natural Rubber Latex Gloves *J nat Rubb Res* , **7(3)**, 206

7 Test Method for the Analysis of Extractable Proteins in Natural Rubber Products (1996) *Malaysian Standard Test, MS 1392 96P*

8. Standard Test Method for Analysis of Protein in Natural Rubber and its Products (1995) *ASTM Designation 5712 – 95*

9 PALOSUO, T , MAKINEN-KILJUNEN, S , ALENIUS, H , REUNALA, T , ESAH YIP AND TURJANMAA, K (1997) Measurement of Natural Rubber Latex Allergen Levels in Medical Gloves by Allergen-specific IgE ELISA-inhibition, RAST-inhibition and Skin Prick Testing Submitted for publication

10. Interim Guidance on Protein Content Labelling Claim for Latex Medical Gloves (1995) Food and Drug Administration (FDA), Centre for Devices and Radiological Health (CDRH) and Office of Device Evaluation (ODE) Document.

11. ESAH YIP, PALOSUO, T., ALENIUS, H. AND TURJANMAA K. (1997) Correlation between Total Extractable Proteins and Allergen Levels of Natural Rubber Latex Gloves. *J. nat. Rubb. Res.*, **12(2)**, 120.

12. ESAH YIP, TURJANMAA, K., NG, K.P. AND MOK, K.L. (1995) Residual Extractable Proteins and Allergenicity of Natural Rubber Products. *Proceedings of International Conference on Latex Protein Allergy: The Latest Position, Paris*, 33.

13. SUSSMAN, G.L. AND BEEZHOLD, D.H. (1997) Safe Use of Natural Rubber Latex. *Allergy and Asthma Proc.* **17(2)**, 101.

APPENDIX 1 RRIM AND ASTM *PROTEIN TESTS· MAJOR DIFFERENCES*

No.	Procedure	RRIM Protocol	*ASTM* Protocol
1	Extraction		
	Extraction medium	PBS (0.01 M, pH 7.4)	Distilled/deionised water
	Volume/g of sample	5 ml/g	10 ml/g
	Temperature	23° ± 2°C	37° ± 2°C
	Time	3 h	2 h
2	Protein precipitation/concentration		
	Precipitants	TCA/PTA	DOC/TCA/PTA
	Final concentration of TCA	4.4%	5.5%
	PTA	0.2%	5.5%
	DOC	–	0.014%
	Resolubilisation – medium	0.2 *M* NaOH	0.1 *M* NaOH
	– volume	1 ml to 6 ml	0.25 ml/1.0 ml
	Concentration	6× – 1×	4× – 1×
	Sedimentation rate	10 000 × g/30 min	3000 × g/30 min or 6000 × g/15 min
3	Lowry microassay		
	Volume of protein solution	800 µl	200 µl
	Alkaline copper solution	300 µl (citrate)	100 µl (tartrate)
	Reaction time	10 min	0 min
	Folin reagent – strength	72% of 2N	Dilute (not specified)
	– volume	100 µl	800 µl
	Colour development	30 min	15 min – 30 min
	Calibration standard protein	BSA	Ovalbumin

TCA: Trichloroacetic acid; PTA: Phosphotungstic acid; DOC: sodium deoxycholate; PBS: Phosphate buffered saline; BSA: Bovine serum albumin