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The Factor Contributing to Higher Extractable Protein Content in Natural Rubber Latex Glove as Determined by ASTM D5712-99 *over* ASTM D5712-95 *and its Relation to Allergen Content*

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Values for glove extractable protein content determined by ASTM D5712-99 *were generally higher than corresponding values obtained by* ASTM D5712-95. *This resulted from the different procedures used in the two assays. Among all the variables investigated, it was ascertained that differences in extraction medium were the main contributing factor. Extraction with 25 mM phosphate-buffered saline, as in* ASTM D5712-99*, gave higher protein content than extraction with water, as in* ASTM D5712-95. *The same was true of the allergen values of the extracts.*

Key words: NR latex; gloves; extractable protein; *ASTM D5712-99*; *ASTM D5712-95*; extraction; allergen; content

Extractable proteins (EP) from NR examination gloves which are known to have the potential of eliciting Type 1 latex allergy are generally determined by *ASTM D5712*. This *ASTM D5712-95* method[1] is widely used by glove manufacturers. One of the reasons is that it gave a lower EP value compared to the later revised version of the *ASTM D5712-99* method[2]. The difference could arise from the variable protocols specified in the two standard assays.

Differences in the two assays are mainly in the conditions of extracting proteins from the gloves, the concentration of NaOH used to solubilise the precipitated proteins, the calibration of the standard protein curves and the preparation of the copper and Folin reagents. It is the aim of the study to identify the factor that contributes to the differences in the EP values obtained from the two tests and to determine its effect on the allergen content of the gloves.

MATERIALS AND METHODS

All the gloves used in the study were commercial NR examination gloves. The methods used[1,2] to determine total EP content were *ASTM D5712-95* and *ASTM D5712-99* while the allergen content was assayed based on the method developed by Palosuo *et al*[3].

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The differences in the protocols of *ASTM D5712-95* from *ASTM D5712-99* are tabulated in *Table 1*. Each of the different factors was investigated separately while keeping the other factors constant. Replications were carried out with 5 to 9 different brands of gloves.

RESULTS AND DISCUSSION

EP Values of NR Gloves Determined by *ASTM D5712-95* and *ASTM D5712-99*

The EP content of six brands of NR gloves determined by the *ASTM D5712-99* was higher than the values obtained by *ASTM D5712-95* by a factor of 1 to 3 (*Figure 1*). *Glove 1*, being a non-post-leached powdered glove, gave a higher EP value than the other gloves. The variation in the EP content as determined by the two methods could stem from the different protocols adopted in the two methods (*Table 1*). Each of these was then studied to identify the contributing factor.

Effect of Different Extraction Conditions

Table 2 shows that the EP values obtained by varying the sample dimension, the ratio of sample to extraction medium or the extraction temperature did not differ markedly. However, extracting the gloves with 25 mM PBS as in *ASTM D5712-99* gave twice the higher EP values than that obtained by extracting the gloves with water as in *ASTM D5712-95* method.

Effect of Different NaOH Concentration, Standard Curves and Colour Development Reagents

There seemed to be no difference in EP values obtained by solubilising the protein precipitates in 0.1 M or 0.2 M NaOH, or calibration against precipitated or non-precipitated Ovalbumin (OVBM) standard curves, or complexing the protein with copper and reducing the Folin with the freshly prepared or commercially prepared chemicals.

Thus the major factor that seemed to contribute to the different EP values obtained from *ASTM D5712-99* over *ASTM D5712-95* was the extraction medium. Phosphate Buffered Saline (PBS) is a buffer of pH 7.4 containing 0.138 M NaCl and 0.0027 M KCl. Glove proteins have been shown to be

TABLE 1. DIFFERENCES IN *ASTM D5712-95* AND *ASTM D5712-99* PROTOCOLS

Protocols	*ASTM D5712-95*	*ASTM D5712-99*
Test sample size	7 cm × 7 cm	Whole glove
Extraction medium	Distilled water	25 mM PBS
Ratio of sample to extraction medium	1g to 10 mL	1g to 5 mL
Extraction conditions	37ºC for 2 h	25ºC for 2 h
Standard curve	Non-precipitated OVBM	Precipitated OVBM
NaOH concentration	0.1 M NaOH	0.2 M NaOH
Copper and Folin reagents	Commercial reagents	Freshly prepared

PBS and OVBM: Phosphate Buffered Saline and Ovalbumin, respectively.

TABLE 2. FRACTION OF EP VALUES OBTAINED FROM DIFFERENT PROTOCOLS AS SPECIFIED IN *ASTM D5712-99* AND *ASTM D5712-95*

Protocols	*ASTM D5712-99* specification / *ASTM D5712-95* specification	(EP-99 / EP-95)[a] ± S.E.
Sample dimension	Whole glove / 7 cm × 7 cm	0.99 ± 0.08
Extraction media	25 mM PBS / water	2.19 ± 0.17
Ratio of sample (g) to extraction medium (mL)	1:5 / 1:10	1.07 ± 0.07
Extraction temperature	25ºC / 37ºC	1.04 ± 0.11
NaOH concentration	0.2 M / 0.1 M	1.05 ± 0.02
Standard curves	Precipitated / Non-precipitated OVBM	1.11 ± 0.04
Copper and Folin reagent	Prepared / Commercial	0.94 ± 0.05

[a]Mean of (EP value based on *ASTM D5712-99* specification/ EP value based on *ASTM D5712-95* specification) from five to nine different brands of gloves.

S.E.: Standard error.

mainly acidic proteins[4] and a pH medium of 7.4 could reduce the negative charges on the proteins and together with the NaCl and KCl salts could render them more extractable from the gloves. The mechanism involved could be similar to the low binding affinity of the glove proteins for corn starch in PBS[5]. The results also indicated that more proteins could be extracted from the gloves if leached in water containing some salts than in pure water.

Effect of Extraction Media on Allergen Levels

The same brands of gloves used in the EP determination (except replicate 5, *Figure 1*) were tested for their allergen content. The allergen levels from PBS extract were higher than from the water extract (*Figure 2*) which is as expected, as more proteins were extracted with PBS. However, the high difference of up to 35-fold could indicate that certain chemicals (not the PBS, which had been tested negative) extracted by PBS interfered with the allergen assay. An earlier study[6] showed that different chemicals used in the production of NR gloves gave different allergen values.

CONCLUSION

The different EP values obtained from *ASTM D5712-95* and *ASTM D5712-99* were mainly due to the different extraction media. Extraction of glove with PBS resulted in higher EP values than extraction with water. Similarly the allergen values were higher in the former than in the latter extracts. Based on the study it could be assumed that *ASTM D5712-99* is a better assay for EP content as it uses PBS which simulates the body fluid (sweat) that will be in contact with gloves when in use.

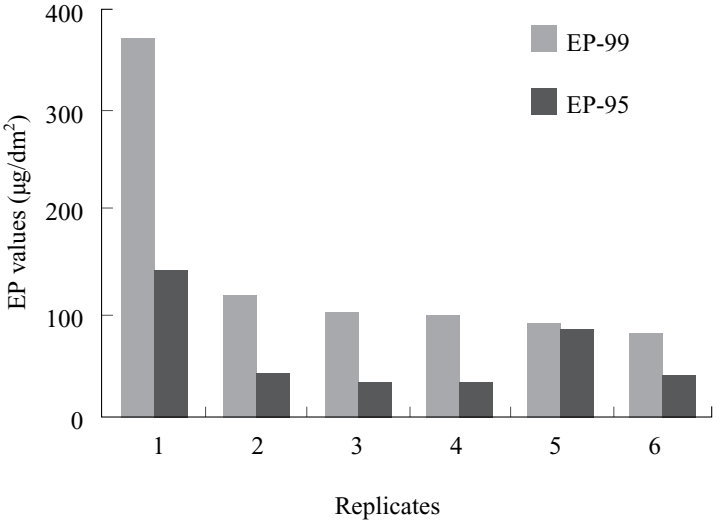

Figure1. The EP values of NR gloves as determined by ASTM D5712-99 *and* ASTM D5712-95.

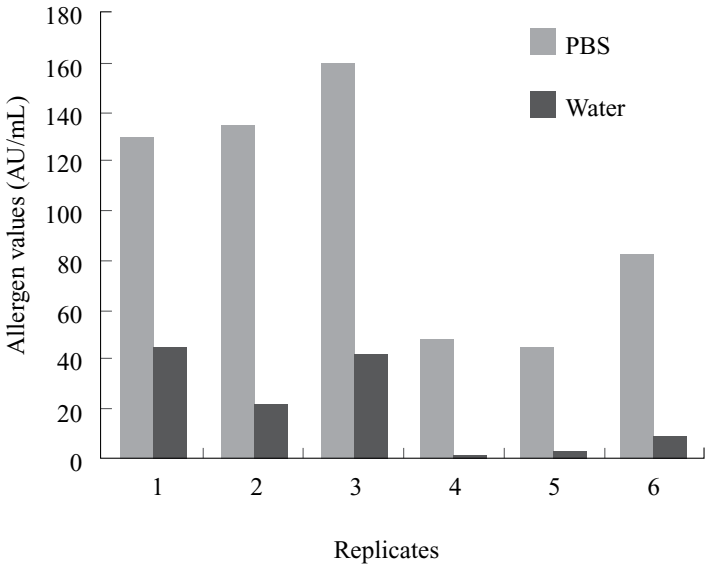

Figure 2. Allergen values from PBS and water extracts of NR gloves.

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