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Correlation between Total Extractable Protein and Antigen Contents with Allergen Content of NR Gloves and Chemical Interference on Protein Assays†

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The allergen or allergenic protein (AgP) content of NR latex examination gloves as determined by the IgE-ELISA inhibition method was compared with the total extractable protein (EP) and the antigen or antigenic protein (AP) contents of the same gloves. Results showed poor linear correlation between AgP with EP and AP contents. It was, however, observed that low (<10 AU/mL) to moderate (10 AU/mL – 100 AU/mL) allergen values corresponded to EP(MS 1392:1998) *<100 μg/g, EP*(ASTM D5712-95) *<60 μg/g, EP*(ASTM D5712-99) *<60 μg/dm², EP*(EN 455-3:1999) *<60 μg/g and AP* (ASTM D6499-00) *<10 μg/dm². Above these EP limits the allergen levels were high (>100 AU/mL).*

Comparison between EP(ASTM 99) *and AP showed a high probability of gloves with EP values of <60 μg/dm² contained AP below the recommended limit of 10 μg/dm² although there were still a significant proportion of gloves with EP values of 60 μg/dm² – 100 μg/dm² meeting the AP limit. Above this, the AP content exceeded the limit.*

Interference on AgP or AP determination could be expected when gloves with EP <60 μg/dm² contained AgP >100 AU/mL or AP >10 μg/dm². Likewise interference on EP determination could occur when gloves with EP >60 μg/dm² contained AgP < 100 AU/mL or AP < 10 μg/dm² Although EP determination with correction could check the interference on total EP assay, the interference on allergen and antigen assays could only be ascertained via *carrying out the EP assay as well.*

Key words: latex proteins; standards; chemical interference; allergen; NR gloves; extractable proteins; antigen; correlation

The Type 1 latex allergy has been reported to be associated with the proteins extractable from the NR latex products. A number of methods have thus been introduced to determine these proteins. The most commonly used are the modified Lowry methods, notably the

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RRIM modified Lowry, which has been adopted as *MS 1392:1998*[1], the *ASTM D5712-95*[2] and *ASTM D5712-99*[3] and the *EN 455-3:1999*[4]. These methods, however, measure the amount of total extractable protein (EP) which may not relate well to the actual amount of the allergenic protein (AgP); the proteins which could sensitise individuals to produce IgE antibody and which could bind to the IgE antibody present in the sensitised individuals to elicit latex allergy reactions.

In the process of developing a method to quantify the AgP, the ASTM introduces the *ASTM D6499-00*[5], an IgG-ELISA inhibition method to determine the amount of antigenic protein (AP) in the latex products using rabbit antisera raised against NR ammoniated latex proteins. A recommended AP limit of 10 $\mu g/dm^2$ has been introduced in the standard specification for rubber examination gloves[6].

Currently two IgE-inhibition assays are being used to assess the AgP content, *i.e.* the radioallergosorbent test (RAST) inhibition[7] and the enzyme-linked immunosorbent assay (ELISA) inhibition[8]. Although these two methods adopt the same basic protocols, the ELISA method is more favoured as it offers lower equipment and reagent expenses and free from the hazards of radio-isotopes. Palosuo *et al.*[8] show that these two methods do not only correlate well to each other but also correlate significantly well with the skin prick test (SPT), indicating that the levels of allergens measured by these methods closely paralleled their *in vivo* ability to induce Type I hypersensitivity reactions. Based on the later correlation, the allergen content of <10, 10-100 and >100 AU/mL are classified as low, moderate and high, respectively. The major drawback of these methods is the non-availability of a standard IgE-serum pool and a standard allergen pool that could identify all the AgP with the potential of afflicting allergic reactions to the different groups of sensitised individuals.

FIT Biotech introduces FITkits to quantify four of the thirteen known latex allergens, *i.e.* Hev b1, Hev b3, Hev b5 and Hev b6.02. These are the allergens that could affect the spina bifida kids and the general population.

With variable EP and AP values obtained from the different methods it is thus pertinent to correlate them with a standard allergen concentration. The following study thus compares the EP content as determined by *MS 1392:1998*, *ASTM D5712-95* and *ASTM D5712-99* and *EN 455-3:1999* and the AP content as determined by *ASTM D6499-00* with the allergen content assayed by the IgE-ELISA inhibition method. This will hopefully give some indications as to the level of AgP in the latex products based on their total EP and AP content. It is also the aim of the study to show that the correlation between EP and AgP or AP contents is affected by the presence of substances interfering with the protein assays.

MATERIALS AND METHODS

Glove Samples

About 100, 87, 40, 32 and 55 NR powdered and powder-free examination gloves were used in the comparative studies between EP as determined by *MS 1392:1998*, *ASTM D5712-95*, *ASTM D5712-99* and *EN 455-3:1999* and AP by *ASTM D6499-00* with the AgP contents, respectively. Another 107 gloves were used in the study on EP (*ASTM 99*) and AP contents.

Total Extractable Protein Determinations

Proteins were extracted from the gloves and assayed following the protocols drafted in the *MS 1392:1998*[1], *ASTM D5712-95*[2], *ASTM D5712-99*[3] and *EN 455-3:1999*[4]. Although there were some variations (*Table 1*), the basic procedures were the same. These involved sample preparation, protein extraction from the test samples, precipitation of proteins in the extracts, solubilising the precipitated proteins in sodium hydroxide, complexing the proteins with copper reagent and measuring the absorbance of reduced Folin at 750 nm. The amount of the total EP was extrapolated from the standard curve. The optional correction method provided in the *ASTM D5712-99*[3] was applied in the investigation of chemicals interfering with the EP assay.

Antigenic and Allergenic Protein Determinations

For the AP determination, the whole glove extraction was as drafted in *ASTM D5712-99*. The AP content in the extracts was assayed by the IgG-ELISA inhibition method outlined in *ASTM D6499-00*[5]. The standard antigen and the primary antisera were the Industry Reference Material obtained from Guthrie Research Institute, USA.

In the allergenic protein determination, cut pieces of glove were extracted following the extraction procedure of *MS 1392:1998*. The allergen content in the extracts was determined by the RRIM IgE-ELISA inhibition method adapted from that of Palosuo and co-researchers[8]. The IgE antibodies were from pooled human

TABLE 1. DIFFERENCES IN THE STANDARD MODIFIED LOWRY METHODS TO DETERMINE EP CONTENT IN NR GLOVES

Protocols	*MS 1392:1998*	*ASTM D 5712-95*	*ASTM D5712-99*	*EN 455-3:1999*
Test piece	7 cm × 7 cm	7 cm × 7 cm	Whole glove	Two glove surfaces
Extraction buffer	0.01 M PBS	Distilled/ deionised water	0.025 M PBS	0.1 M TES
Volume buffer/g sample	5mL/g	10 mL/g	5 mL/g –10 mL/g	Fixed volume of 25 mL
Extraction conditions	23°C/3 h	37°C/2 h	25°C/2 h	25°C/2h
Precipitating reagents	TCA, PTA	DOC, TCA, PTA	DOC, TCA, PTA	DOC, TCA, PTA
Standard proteins	BSA	Ovalbumin	Ovalbumin	Ovalbumin
Standard curve	Non-ppt curve	Non-ppt curve	Ppt curve	Ppt curve
Limit of detection			23.5 μg/g[a]	
Limit of Quantitation	20 μg/g	50 μg/g	70.5 μg/g[a]	

PBS: Phosphate buffered saline; TES: N-tris-[Hydroxymethyl]-methyl-2-aminoethanesulphonic acid; DOC: sodium deoxycholate; TCA: Trichloroacetic acid; PTA: Phosphotungstic acid; BSA: Bovine serum albumin

Ppt: precipitated

[a] Based on extraction ratio of 5 mL buffer to 1 g of rubber.

sera which had been tested to contain specific antibodies to latex proteins while the allergen source was derived from fresh NR latex serum proteins The differences between the two ELISA inhibition methods were shown in *Table 2*

Evaluating Chemical Interference on the Protein Assays

The interference of chemicals on the protein assays was evaluated by testing the protein content in solutions spiked with the chemicals or in the extracts of cured dipped films incorporated with the chemicals

Protein test of the chemical solution or dispersion Most of the chemicals were prepared following normal procedures for the manufacture of latex products[9] Casein, potassium oleate and potassium laurate were prepared in KOH solution while sodium dodecyl sulphate (SDS) and $Ca(NO_3)_2$ were dissolved in water Diphenylguanidine (DPG®), antioxidant Vulkanox BKF (antioxidant 2246®), accelerator zinc diethyl dithiocarbamate (ZDEC®) and Titanium dioxide (TiO_2) were prepared in dispersion Savinase was diluted from its concentrated solution with water The control sample was mixed with the clear fraction of the chemicals and the protein content in the mixture determined The concentration of the EP and AP in the mixture was expressed in μg/g by multiplying the concentration in μg/mL with 5 This would reflect on the probable amount of the proteins contributed by the interfering materials, if present in the glove extracts, assuming that 1g glove was extracted with 5 mL buffer

Protein test of coagulant dipped latex films containing the chemicals HA latex concentrate compounded with various chemical solutions or dispersions at the concentrations (*Table 7*) was matured for 2 days at ambient temperature Coagulant dipped films were prepared from the latex following common procedures adopted in glove production These involved dipping the former (glass test-tubes) in a coagulant mixture (10% $Ca(NO_3)_2$ and 10% $CaCO_3$) for 30 sec, oven dried at 70°C for 5 min before dipping the former in the latex for 30 sec This was followed by partial drying at 70°C for 2 min, wet gel leaching in 70°C water for 1 min, curing at 110°C for

TABLE 2 MAJOR DIFFERENCES BETWEEN *ASTM D6499-00* AND RRIM ELISA INHIBITION METHODS

Parameters	*ASTM D6499-00*	RRIM ELISA inhibition
Antibody	IgG from rabbit	IgE from human adult
Antigen/allergen source	Lyophilised NR ammoniated latex proteins	Aqueous NR fresh latex serum proteins
Proteins determined	Antigenic proteins	Allergenic proteins
Test specimen	Whole glove	Cut glove pieces
Extraction procedure	*ASTM D5712-99*	*MS 1392-1998*
Limit of quantitation	0 25 μg/g rubber	0 5 AU/mL
Recommended Limit	10 μg/dm^2	
Allergen levels		Low <10 AU/mL Moderate 10 AU/mL –100 AU/mL High >100 AU/mL

30 min, post-leaching in 70°C water for 1 min and finally oven dried at 70°C for 10 min. Cornstarch powder was applied dry on the film. A piece of dipped film of dimension 6 cm × 6 cm was extracted with 25 mM PBS at a ratio of 1 g film to 5 mL buffer. The extracts were clarified before being assayed for the protein content.

RESULTS AND DISCUSSION

Although the RRIM IgE-ELISA inhibition method differed from the Finnish method[8], mainly in the IgE-sera pool, the two methods gave comparable allergen values when the same glove samples were assayed for their allergen content (*Table 3*). The variation in Glove 3 could be due to the different response of the Finnish IgE-sera pool to these particular glove proteins compared to the response of adult human IgE-sera pool used in the present study. The IgE-sera pool used in the RRIM IgE-ELISA inhibition assay contained specific IgE antibodies to 14, 20 (prohevein), 30, 36, 45 and 75 kD NR latex proteins but not to 14 kD rubber elongation factor nor to 27 kD protein affecting the spina bifida kids. Similar marked differences in the allergen values of certain gloves were observed by Palosuo *et al.*[8] when comparing the adult from the spina bifida sera pools.

The AgP content of the other four gloves were, however, within the same allergen levels: 2 low and 2 high. Based on the comparable results with Palosuo *et al.*[8], the allergen levels in the present study were also classified under the same categories: low (<10 AU/mL), moderate (10 AU/mL – 100 AU/mL) and high (>100 AU/mL). Gloves 1, 2 and 5 were always used as internal references in all the RRIM IgE-ELISA inhibition assays.

Comparison Between Total Extractable and Allergenic Protein Content

EP determined by MS 1392:1998. *Figure 1a* shows a poor linear correlation with $r^2 = 0.11$ between EP (MS) and the AgP of 100 glove samples. However, it was observed that all the 20 gloves with EP <100 µg/g contained low to moderate allergen. On the other hand only 15% of the remaining 80 gloves with EP >100 µg/g contained moderate allergen. Majority contained high allergen.

The present data contradicts a report by Yip *et al.*[10] of a good correlation ($r^2 = 0.79$) between EP (RRIM) and AgP contents of 46 glove samples and of a high proportion of gloves (14/14) with EP <100 µg/g containing low allergen. These variations could be due to the different IgE-sera pool or to the different types

TABLE 3. THE ALLERGEN CONTENT OF GLOVES DETERMINED BY IGE-ELISA INHIBITION FROM TWO DIFFERENT LABORATORIES

Gloves	Allergen content (AU/mL)	
	Finnish Laboratory	RRIM Laboratory
1	<0.5	0.8
2	2	5
3	17	124
4	300	350
5	1500	1052

and sample sizes of gloves used in the two studies.

EP determined by ASTM D5712-95. From 87 gloves studied, EP *(ASTM 95)* also showed a poor linear correlation of $r^2 = 0.27$ with the AgP content (*Figure 1b*). In this assay, majority of the gloves (19/21) with EP <60 μg/g corresponded to low to moderate allergen. With EP >60 μg/g, 62% of 66 gloves showed high allergen while the rest showed low to moderate allergen.

Standard Malaysian Gloves (SMG)[11] specified an EP (*ASTM 95*) limit of 200 μg/dm^2 for powdered and 50 μg/dm^2 for powder-free gloves. These values approximately equal to 260 μg/g and 60 μg/g, respectively. Based on the allergen assay used in the study, it could be expected that a certain proportion of powdered SMG with EP of 50 μg/dm^2 to 200 μg/dm^2 to contain high allergen while powder-free SMG contain low to moderate allergen. A survey on some SMG gloves supported this postulation (to be published).

EP determined by ASTM D5712-99. Unlike EP *(ASTM 95)*, the EP (*ASTM 99*) gave a better correlation ($r^2 = 0.76$) with the allergen content of 40 gloves (*Figure 1c*). This could be due to the smaller sample size used in the later study compared to the bigger sample size used in the earlier study. However, as observed with the *ASTM 95*, the *ASTM 99* also showed an EP limit of <60 μg/dm^2 to correspond to low to moderate allergen while EP of >60 μg/dm^2 corresponded to high allergen. This implied that NR gloves with EP (*ASTM 99*) values between 60 μg/dm^2 to the recommended limit of 200 μg/dm^2 could contain high AgP.

EP determined by EN 455-3:1999. The *EN 455* had a similar protocol as *ASTM 95* except in the extraction procedure, the extraction buffer and the standard curve (*Table 1*). Like EP (*ASTM 95*), the EP *(EN)* of 32 gloves also had a poor linear correlation of $r^2 = 0.37$ with the allergen values (*Figure 1d*). The EP *(EN)* exhibited a limit of <60 μg/g to correspond to low to moderate allergen content while EP >60 μg/g to correspond to high allergen.

Comparison Between Antigenic and Allergenic Protein Content

The correlation between AP and AgP of 55 gloves was observed to be poor with $r^2 = 0.57$ (*Figure 2a*). The data showed a high probability of gloves (36/41) with AP content below the recommended limit of 10 μg/dm^2 to correspond to low to moderate allergen. Above this limit the gloves generally had high allergen.

Comparison between Antigenic Protein and Total Extractable Protein as Determined by *ASTM D5712-99*

A plot of AP *versus* EP *(ASTM 99)* of 107 glove samples showed a non-linear correlation with $r^2 = 0.2$ (*Figure 2b*). Detail analysis showed all 37 gloves with EP <60 μg/dm^2 contained AP <10 μg/dm^2 although there was still a prominent number of gloves (71%) with EP values in the range of 60 μg/dm^2 – 100 μg/dm^2 having AP <10 μg/dm^2. Above 100 μg/dm^2, the AP content generally exceeded 10 μg/dm^2. The above results showed a higher probability of gloves with EP <100 μg/dm^2 meeting the recommended AP limit of 10 μg/dm^2 than gloves with EP of 100 μg/dm^2 – 200 μg/dm^2.

From the comparison between the AgP and EP or AP contents as summarized in *Table 4*, it could be deduced that gloves of low to moderate allergen corresponded to EP (*MS*) <100 μg/g, EP (*ASTM 95*) <60 μg/g, EP

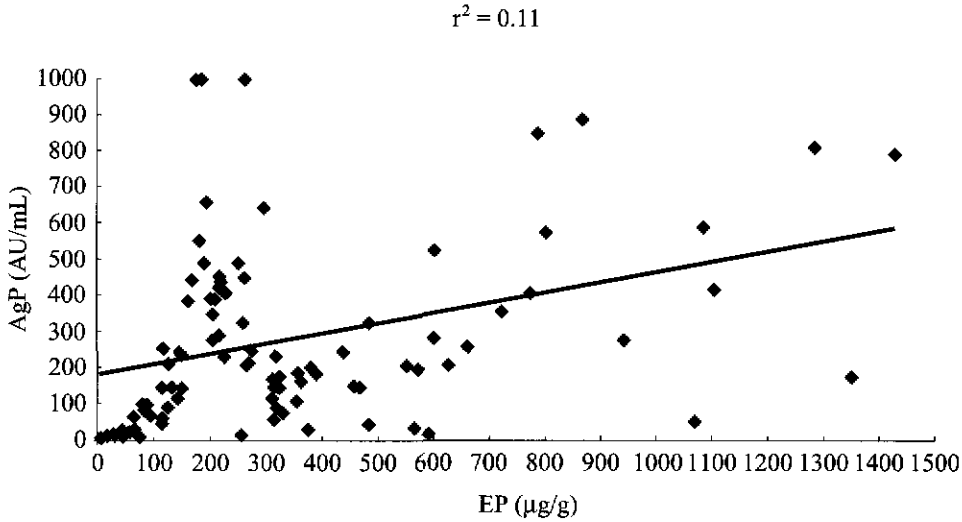

Figure 1a. Correlation between AgP and EP (MS).

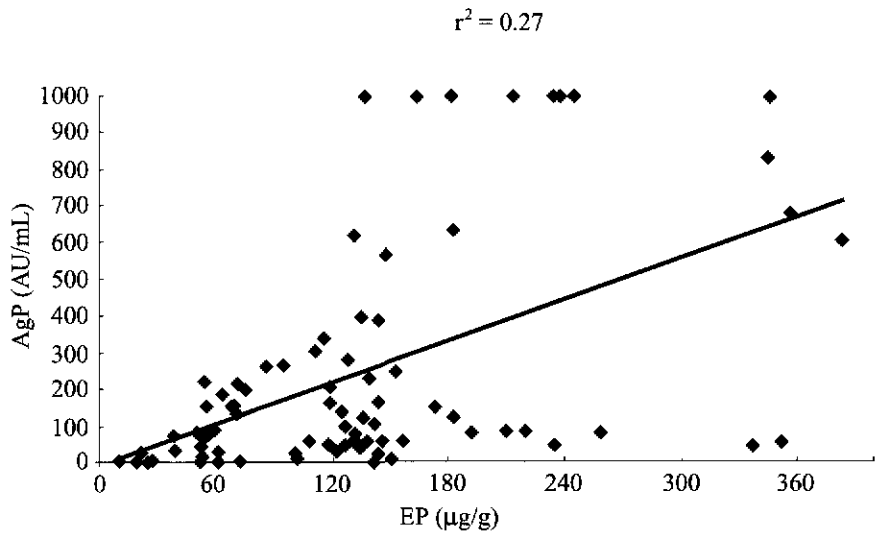

Figure 1b. Correlation between AgP and EP (ASTM 95).

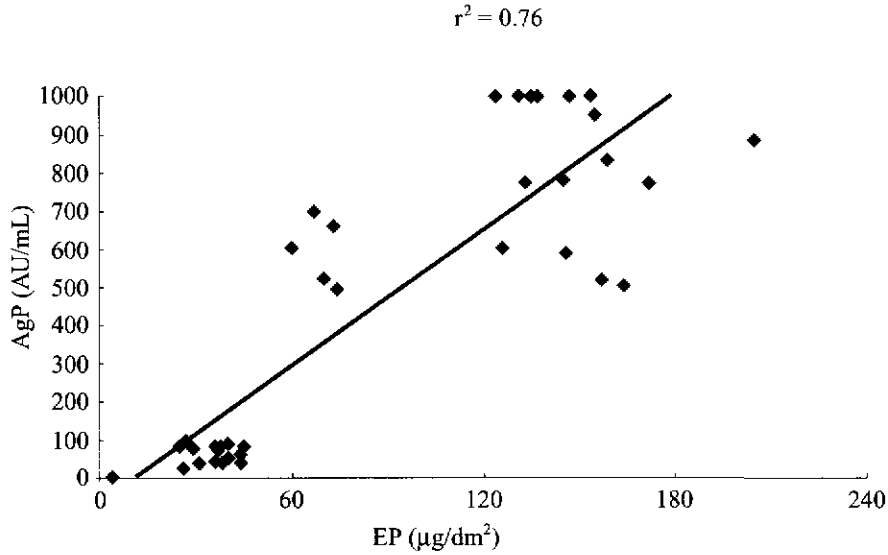

Figure 1c. Correlation between AgP and EP (ASTM 99).

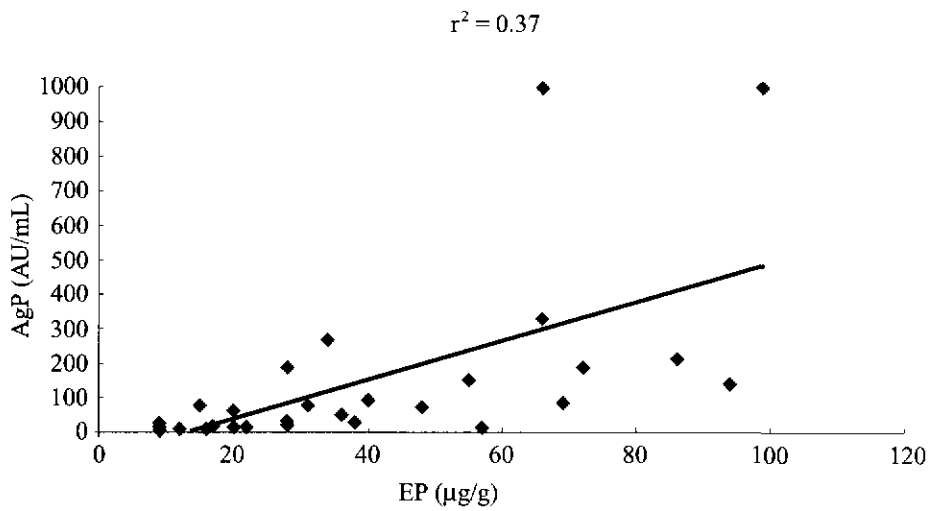

Figure 1d. Correlation between AgP and EP (EN).

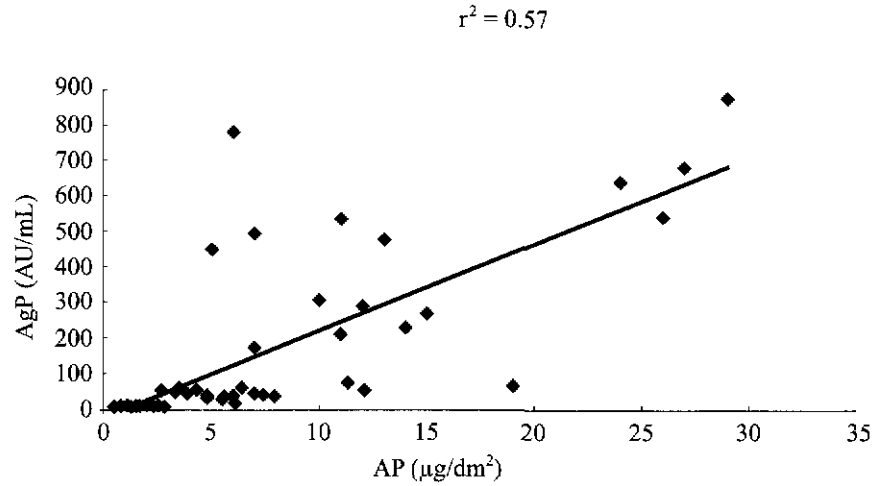

Figure 2a. Correlation between AgP and AP

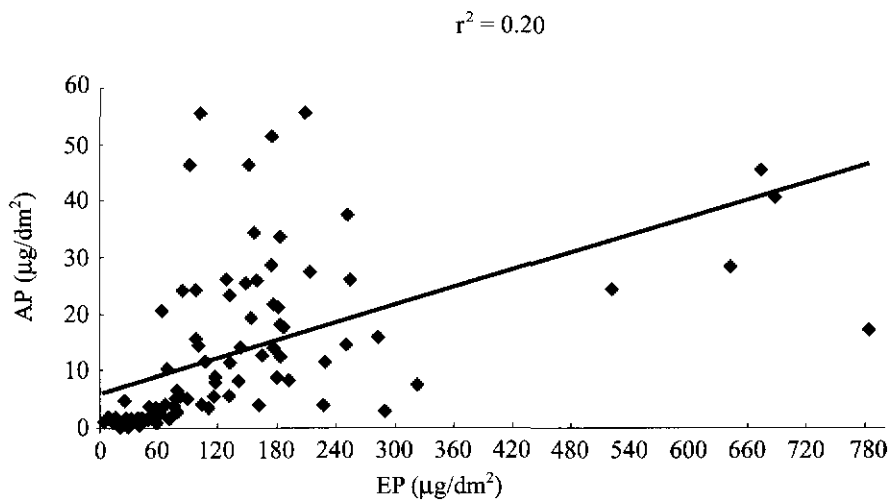

Figure 2b. Correlation between AP and EP (ASTM 99).

TABLE 4 LIMITS OF TOTAL EXTRACTABLE AND ANTIGENIC PROTEINS FOR A LOW TO MODERATE ALLERGEN LEVEL

Test methods	Extractable protein and antigenic protein limit	Glove fraction[a]
MS 1392 1998	<100 μg/g	20/20 (100%)
ASTM D5712-95	<60 μg/g	19/21(90%)
EN 455-3 1999	<60 μg/g	20 23 (87%)
ASTM D5712-99	<60 μg/dm^2	19/19 (100%)
ASTM D 6499-00	<10 μg/dm^2	37/41 (90%)

[a] Number of gloves with low to moderate allergen level/number of gloves with corresponding EP or AP limits tested

(*ASTM 99*) <60 μg/dm^2, EP *(EN)* <60 μg/g and AP <10 μg/dm^2 Above these limits the AgP contents were generally high

Interference on the EP, Antigen and Allergen Assays

As observed in *Figures 1* and *2* there were instances when high EP gloves of >60 μg/dm^2 contained AP <10 μg/dm^2 and AgP <100 AU/mL Likewise there were also cases of low EP gloves of <60 μg/dm^2 giving high AP >10 μg/dm^2 and AgP >100 AU/mL The earlier instances could be due to the presence of substances extracted from the gloves which interfered with the Modified Lowry but not on the ELISA inhibition assay The later could indicate interference on ELISA inhibition but not on Modified Lowry assay Judging from the data obtained the prevalence of interference on Modified Lowry was higher than the interference on ELISA inhibition assays No attempt was made in the present study to identify these interfering materials However, the following chemicals were tested to verify the above cases

Interference on modified Lowry assay The EP determination by Modified Lowry assay is based on the reduction of the Folin reagent by the protein-copper complex The method, however, is susceptible to interference by a number of chemicals[12] The *ASTM D5712-99*[3] offered an optional correction method to check this interference by correcting the absorbance readings obtained in the presence of copper reagent against those obtained in its absence A low EP value obtained on correction compared to a high non-corrected EP value could indicate interferences by non-proteinaceous materials *Table 5* listed a few substances, which at the specified concentrations affected the EP (*ASTM 99*) determination but not the AgP or AP determination As expected casein and savinase, being proteins, would respond to the total protein assay giving high EP values with or without correction DPG®, which has been reported[13] to interfere with the Modified Lowry assay, increased the EP value to 318 μg/g On correction the EP value dropped to below detection level Similarly BKF®, ZDEC® and potassium laurate gave EP values of >200 μg/g which dropped to <60 μg/g (except ZDEC®) on correction It was observed (supported by assay on another six proteins) that proteins will induce EP reduction of <20% while interfering chemicals will bring about an EP reduction of >60%

Although casein, savinase, DPG® and BKF® increased the EP value to >60 μg/g, the AgP

and AP were maintained at <100 AU/mL and <10 μg/dm^2, respectively This implied that none of the reagents markedly affected the ELISA-inhibition assay, meaning they did not contain the relevant allergenic or antigenic proteins Some effect of savinase on the assay could be due to its enzymatic activity which could digest some of the IgG or IgE antibodies resulting in some lost of the antibodies This gave the false impression that the sample had higher antigen or allergen value than the control

ZDEC® of 2 5% and 0 5% potassium laurate, however, inflated the AgP value to >100 AU/mL but maintained the AP content to <10 μg/g The interference effects were diminished at 10-fold lower concentrations This reflected the sensitivity of the two assays the RRIM ELISA inhibition assay seemed to be more sensitive to chemical interference than the *ASTM D6499-00*

As Modified Lowry is currently the preferred method to assess the protein content of gloves, caution has to be exercised in interpreting the values obtained The present study showed that high EP >60 μg/g (or μg/dm^2) could also be attributed to the presence of non-latex proteins (or non-allergenic or non-antigenic proteins) or to the interfering chemicals The latter could be checked by applying the correction method A low EP value on correction compared to high non-corrected EP value, together with EP reduction of >60% signify chemical interference However, the interference by non-allergenic or non-antigenic proteins could only be confirmed by carrying out additional test, preferably the antigen assay

Interference on IgG or IgE-ELISA assay
There were non-protein chemicals (*Table 6*) such as SDS, KOH and $Ca(NO_3)_2$ which did not inflate the EP values but unexpectedly affected the AP and AgP values In the IgG/IgE-ELISA inhibition assay high antigen/allergen values are obtained when there are few or no antibodies available to bind the standard antigen/allergen coated on the microtitre plate This is normally due to the binding of the antibodies to the antigenic/allergenic proteins in the test sample during the initial inhibition step leaving few to no antibodies left to bind the standard antigen/allergen coated on the microtitre plate in the later step This reasoning, however, could not explain the 'loss' of antibodies when they were incubated with SDS, KOH and $Ca(NO_3)_2$ The plausible explanation could be that these chemicals changed the structure or conformation of the antibodies (which are proteins) rendering them unable to bind to the standard antigens/allergens This gave a false impression that their 'loss' was due to their binding to the antigenic/allergenic proteins in the earlier inhibition step which consequently resulted in a high antigen/allergen content SDS and KOH are known to denature proteins while calcium ion has the potential to complex with proteins

Potassium oleate (0 5%) and 2 5% TiO_2 on the other hand affected only the IgE-ELISA inhibition assay but not the IgG-ELISA inhibition assay Their interference was also diminished at 10-fold lower concentrations This reaffirmed the earlier observation on the greater sensitivity of AgP assay to chemical interference than the AP assay

The presence of interfering materials in the glove extracts could result from post treatment of finished gloves or from insufficient/improper leaching of the gloves during production *Table 7* shows that with proper leaching all the extracts of HA latex films compounded with the common chemicals used in the manufacture

TABLE 5. CHEMICALS INTERFERING WITH THE EP (*ASTM 99*) DETERMINATION

Chemicals	Concentration (g/100 mL)[a]	EP_{NC} (μg/g)	EP_C (μg/g)	% EP reduction	AgP content (AU/mL)	AP content (μg/g)
Control-I		*<LOD*	*<LOD*		*<0.5*	2.2
Casein	0.005	165	131	21	2	1.3
Savinase	0.05	202	193	5	24	7.1
DPG	1.25	318	<LOD	>100	<0 5	1.1
Control-II		54	49	9	60	2.3
BKF®	1.67	*>800*	*<LOD*	*>100*	67	2.5
ZDEC®	2.50	218	80	63	316 (38)	1.4
Potassium laurate	0.50	>800	48	>94	856 (73)	2.0

[a]The amount of chemicals in 100 mL of the combined volume of the chemicals and the control.
EP_{NC} and EP_C: EP non-corrected and EP-corrected values, respectively
% EP reduction: $(EP_{NC} - EP_C) * 100 / EP_{NC}$
LOD: Limit of detection of 23.5 μg/g
The AgP values at 10-fold lower concentration in parentheses.

TABLE 6. CHEMICALS INTERFERING WITH THE *ASTM D6499-00* OR WITH RRIM ELISA INHIBITION METHOD

Chemicals	Concentration (g/100 mL)	Total EP content (μg/g)	AgP content (AU/mL)	AP content (μg/g)
Control-I		42	39	3.3
SDS	0.5	47	521	>10
Potassium oleate	0.5	46	232 (35)	1.0
TiO_2	2.5	46	296 (87)	2.5
Control-II		54	60	2.3
$Ca(NO_3)_2$	5.0	32	100	>10
KOH	0.5	<LOD	>1000	>10

The AgP value at 10-fold lower concentration in parentheses.

of gloves and added at the levels listed contained EP <60 μg/dm^2 , AgP <100 AU/mL and AP<10 μg/dm^2 . The exceptions were with the films containing ZnO and DPG, which contained higher EP values but similar levels of AgP and AP. This indicated the presence of materials in their extracts interfering with the Modified Lowry assay. The presence of DPG in the extract of film containing DPG was clearly indicated by low EP value on correction and an EP reduction of >100%. The interference by ZnO if present in the extract was not conclusive.

Based on the study by Palosuo *et al.*[8] the low, moderate and high allergen level correspond

TABLE 7 PROTEIN CONTENT IN EXTRACTS OF NR LATEX FILMS CONTAINING VARIOUS CURING INGREDIENTS

Ingredients	Levels (p p h r)	EP content ($\mu g/dm^2$)	AgP content (AU/mL)	AP content ($\mu g/dm^2$)
Control-I		40	38	4 0
S	1 5	49	59	6 6
ZnO	0 2	82 (66)	90	4 8
ZDEC®	0 5	52	53	3 9
ZDBC®	0 5	45	49	3 8
Control-II		42	35	3 0
KOH	0 2	43	51	6 5
Potassium oleate	0 2	31	55	3 0
ZMBT®	0 2	43	48	5 5
BKF®	0 5	38	58	4 5
Control-III		39	66	3 6
Potassium oleate	2 0	45	50	4 5
SDS	0 5	26	52	4 4
DPG	0 9	257 (<LOD)	49	2 6
Wingstay L®	1 0	50	69	5 7

EP corrected value in parentheses

to SPT histamine ratio of <1, 1 0 and >1, respectively As the SPT histamine ratio is the median diameter of wheal (mm) produced by glove extract compared to diameter of wheal produced by histamine, a histamine ratio of greater than 1 indicates high allergenicity of the glove extracts Thus gloves with low to moderate allergen content may have a lower probability of eliciting allergic reaction to sensitized individuals compared to gloves with high allergen content Yip and Sussman[14] showed that 60% of negative allergic responses are consistently observed at EP (*MS*) <100 µg/g and EP (*ASTM 95*) <50 µg/g and these values corresponded to low to moderate allergen content, as demonstrated by the present study

In line with the general agreement, the present study also showed that the total extractable protein content determined by Modified Lowry methods and the IgE-binding capacity of certain allergen preparations may show no correlation Although there was no linear correlation between the EP and AP with AgP content, there were some indications as to the limits of EP and AP values which correspond to certain allergen levels According to Palosuo *et al* [8], a protein content of 10 ng/mL was assigned an allergen value of 0 1 AU/mL Thus to have a low allergen level of <10 AU/mL, the amount of EP should be <5 µg/g glove (1 µg/mL extract) For moderate level of 10 AU/mL to 100 AU/mL the EP would be 5 µg/g to 50 µg/g This means for low to moderate allergen level, the total protein should be <50 µg/g These data derived from Palosuo *et al* [8] corresponded well with results of the present study, which had an extended limit of 60 µg/g

At an EP content of <50 μg/g the AP content was <10 μg/dm^2 Thus for low to moderate allergen level the AP value should be <10 μg/dm^2 which was the value observed in the present study

CONCLUSION

The total EP values as determined by *MS 1392 1998*, *ASTM D5712-95* and *ASTM D5712-99* and *EN 455 3-1999* and the AP values of *ASTM D6499-00* generally showed poor linear correlation with the allergen content as determined by the IgE-ELISA inhibition method

However, the values obtained gave a good estimation of the level of allergenic proteins in the NR examination gloves It was observed (*Table 4*) that more than 87% of gloves with EP (*MS*) <100 μg/g, EP (*ASTM 95*) <60 μg/g, EP (*ASTM 99*) <60 μg/dm^2, EP (*EN*) <60 μg/g and AP <10 μg/dm^2 contained low to moderate allergen levels Above these limits the allergen values were normally high

The recommended AP limit of 10 μg/dm^2 generally corresponded to EP (*ASTM 99*) <60 μg/dm^2 However, there were still a high proportion of gloves with EP values of 60 μg/dm^2 – 100 μg/dm^2 containing AP below its limit Above this, the AP content exceeded its limit

Generally, EP <60 μg/dm^2 corresponded with AP <10 μg/dm^2 and AgP <100 AU/mL Deviations from these could indicate interferences on the protein assays Chemical interference on EP determination could be ascertained by carrying out the optional EP correction The interference on AP or AgP test could only be verified by carrying out the Modified Lowry assay

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