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Monoclonal Antibodies Identify Different Antigens in Proteins Eluted from Natural Rubber Latex Gloves Obtained from Different Sources

M J CARDOSA*, SHARIFAH HAMID**, S SAMUEL-VERGHESE***
AND H Y YEANG***

An indirect enzyme linked immunosorbent assay (ELISA) was used to show that proteins eluted from different samples of natural rubber latex gloves contained different relative proportions of various antigens as defined by monoclonal antibodies directed against B-serum and C-serum proteins. Thus some gloves with low total protein content were shown to have a high proportion of one of these antigens, but negligible amounts of other antigens, while some other gloves had high levels of more than one antigen tested These differences become important if some of these antigens are more allergenic than others, and this report highlights the inadequacy of total protein quantitation in the absence of specific immunoassays as a measure of the safety of a product

Concern about allergic responses to proteins associated with products manufactured from natural rubber latex[1] has generated interest in developing methods of assaying for the offending proteins associated with such products In the absence of specific knowledge of what the allergens may be, the measurement of total protein eluted from such products is being accepted as a means of indicating the level of disease-provoking allergens present This criterion is being applied in the manufacture of natural rubber latex examination gloves, for instance, but requires that we assume that all proteins are equally allergenic, an assumption that is clearly unlikely to be true Even in the well known arena of food allergies, there are particular foods which are notoriously allergy-inducing, while others are unknown to be a problem even in highly atopic individuals

Thus such short term measures cannot address the problem of whether a particular 'low-protein' glove may not in fact be a 'high-allergen' glove because of the possibility that whatever residual protein left in the product may happen to be highly allergenic This dilemma can only be resolved when the allergens of natural rubber latex are specifically identified

This report demonstrates with the use of monoclonal antibodies directed against proteins of natural rubber latex that latex examination gloves from different manufacturers do indeed have different amounts of different antigens An indirect ELISA was used to examine the presence of different monoclonal antibody-defined antigens in eluates from natural rubber latex examination gloves

*School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia
**Venture Technologies Sdn Bhd, Innovation and Consultancy Centre, Universiti Sains Malaysia, 11800 Penang, Malaysia
***Rubber Research Institute of Malaysia, P O Box 10150, Kuala Lumpur, Malaysia

MATERIALS AND METHODS

Glove proteins were eluted from pieces of latex examination gloves by immersing 4 g gloves in 10 ml distilled water for 3 h and powder from the gloves was removed by centrifugation. Natural rubber latex examination gloves used in this study were purchased from commercial outlets.

Total protein content of these eluates was determined using a micro-Lowry method described previously[2] using bovine serum albumin as the standard.

Latex sera comprising B-serum and C-serum were prepared from latex tapped from the rubber tree *Hevea brasiliensis* as previously described[3]. Briefly fresh latex was collected and centrifuged to give three major fractions: the rubber cream, the C-serum and the 'bottom fraction'. B-serum was derived from the 'bottom fraction' by repeated freeze-thaw cycles.

Mouse antiserum directed against B-serum was produced by immunising Balb/c strain mice with 0.5 ml B-serum intraperitoneally, followed by a second dose 3 weeks later. The mice were bled by cardiac puncture 2 weeks after the second dose and serum separated was stored in aliquots at –30°C.

Monoclonal antibodies were harvested from hybridoma[4,5] cell lines generated by fusion of spleen cells from a B-serum or C-serum immune Balb/c mouse with Sp2/0 mouse myeloma cells. The mice had been immunised intraperitoneally with 0.5 ml of B-serum or C-serum respectively. The fusion for hybridomas directed against C-serum was performed after a prefusion boost using 0.3 ml of C-serum 4 weeks after the first immunisation with C-serum. The fusion which generated the hybridomas directed against B-serum was performed after the B-serum immune mouse had been boosted with 0.5 ml of B-serum after 7 weeks. Hybridoma supernatants harvested from log phase cultures were used in all assays described.

Plasma from latex allergic patients were obtained from PlasmaLab International, Everett, WA, USA.

Indirect ELISA were performed following standard protocols[6], coating the wells of 96-well microtitre plates with proteins eluted from gloves. The proteins were diluted in carbonate-bicarbonate buffer pH 9.6 and left to bind overnight at 4°C, after which the plates were blocked with 0.5% casein in phosphate buffered saline (PBS) pH 7.4 for 2 h at room temperature. The presence of bound antigen was detected using monoclonal antibodies and mouse polyclonal antisera as positive controls and a monoclonal antibody directed against dengue virus was used as a negative control. Incubation was at room temperature for 2 h. The secondary antibody used was a goat anti-mouse immunoglobulin HRP conjugate (Biorad, USA) at a dilution of 1:3000 for 1 h at room temperature. Colour development was achieved using hydrogen peroxide and o-phenylenediamine for 30 min at room temperature when the reaction was stopped with 2.5*M* sulphuric acid. Optical density was read at 492 nm wavelength. Washing between steps was performed using a multipin hand washer in a protocol involving 3 wash cycles with a 1 min soak time using a wash buffer containing phosphate buffered saline pH 7.4 and 0.05% Tween 20.

RESULTS

Figure 1 shows the optical density readings obtained in ELISAs performed with mouse antiserum directed against B-serum *(Panel A)* and 4 different monoclonal antibodies derived from the fusion of spleen cells from the B-serum immune mouse (*Panels B* to *E*). The

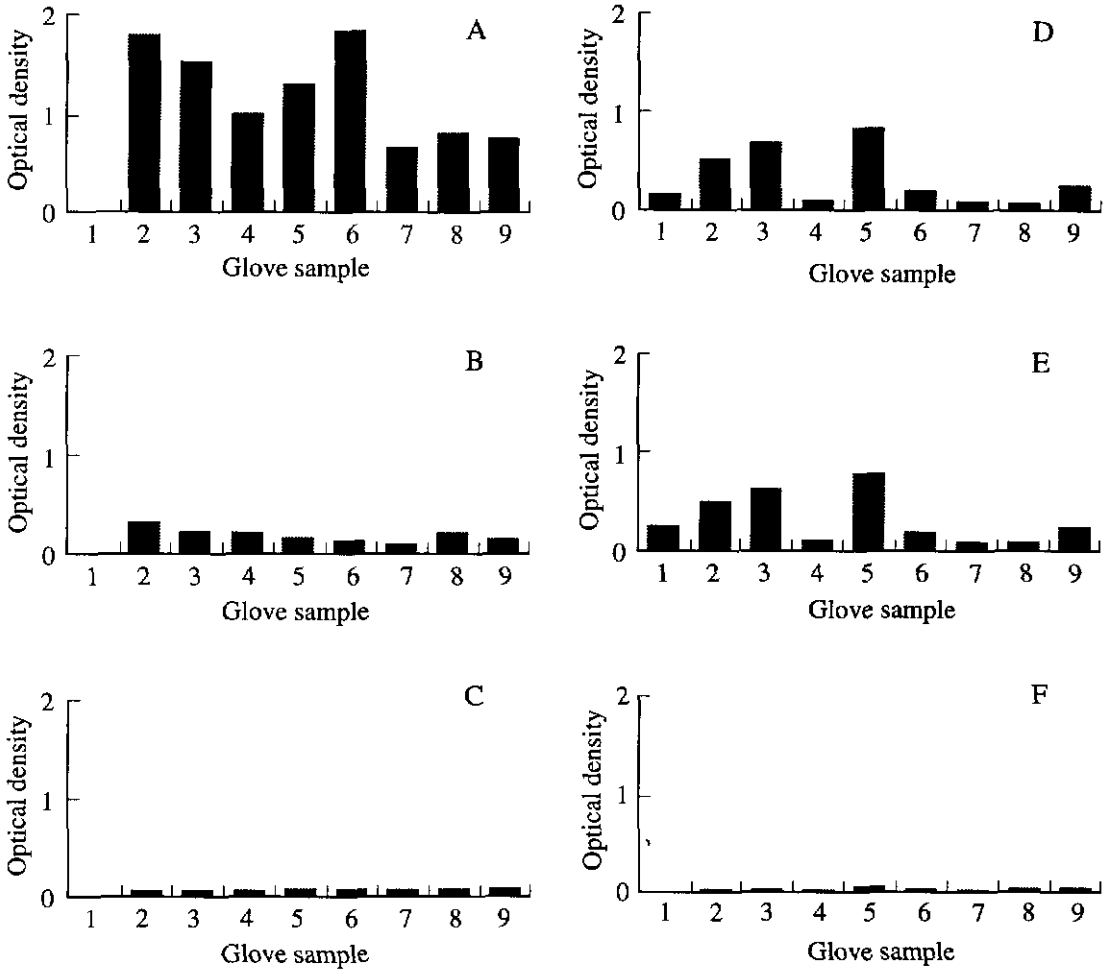

Figure 1. Indirect ELISA showing the reactivity of various glove protein eluates with a mouse antiserum directed against B-serum (A), monoclonal antibodies against B-serum proteins designated USM/RB3 (B), USM/RB6 (C), USM/RB4 (D), USM/RB10 (E) and the negative control from the parent myeloma Sp2/0 (F).

last panel *(F)* represents the results obtained when supernatants from the myeloma parent cells (Sp2/0) were used. Surprisingly one of the 9 glove samples tested *(Number 1)* did not have any antigens detectable by mouse antiserum directed against B-serum although this sample did contain proteins at a concentration similar to the other samples. This mouse polyclonal antibody detected differing amounts of B-serum antigens as represented by optical density.

One of the four monoclonal antibodies (USM/RB6) did not detect any antigens in any of the glove samples tested as can be seen when *Panel C* is compared with *Panel F* which is the negative control. The monoclonal antibody (USM/RB3) represented by *Panel B* detects low levels of antigens in all gloves with the exception of glove *Number 1*, a similar result to that obtained by using the mouse polyclonal antibody to B-serum, while the 2 monoclonal antibodies (USM/RM4 and USM/RB10) represented in *Panels D* and *E* show almost identical patterns of recognition of glove samples, with low recognition of certain gloves (*Numbers 1, 4, 7* and *8*) moderate recognition of *Numbers 6* and *9* and fairly strong reactions with gloves *Numbers 2, 3* and *5*. These 2 monoclonal antibodies appear to recognise the same polypeptide.

Using a monoclonal antibody (USM/RC2) derived from the C-serum fusion as a model, indirect ELISA was performed using serial dilutions of the hybridoma supernatant containing the monoclonal antibody USM/RC2. The histogram in *Figure 2* shows the results of this titration for each glove extract and for B-serum and C-serum (2.4 µg of each coated per well) or the control containing no antigen. One point clearly demonstrated in this histogram is that USM/RC2 recognises an antigen found in C-serum but not in B-serum. Furthermore, the dose response of hybridoma supernatant appears to show a prozone effect in some cases when used undiluted. It was thus decided that this particular supernatant would be used at a 1:5 dilution.

Figure 3 shows the optical density obtained in the indirect ELISA using USM/RC2 as the detecting antibody at a 1:5 dilution, comparing these data with the protein concentration of the sample eluates. It is clear that some gloves had a high protein content but relatively lower optical density readings (for example, glove samples *Numbers 3* and *9* compared to other with lower protein concentration, but with relatively high optical density readings (for example, glove samples *Numbers 4* and *8*).

When these optical density data were divided by the concentration of protein in each sample, thus showing the relative content of the antigen defined by USM/RC2 with respect to total protein concentration, it became dramatically clear that glove samples such as *Numbers 4* and *8* had a very high proportion of this particular antigen in their total protein eluted *(Figure 4)*. Thus the low amounts of protein extractable from these gloves appears to be mainly derived from a protein recognised by USM/RC2 and if this protein happens to be an allergen, the low total protein levels seen in these gloves would be very misleading from the point of view of safety. In another example, glove *Number 2* had a much higher proportion of protein recognised by USM/RC2 than did glove *Number 1* although these two samples had the same amount of extractable protein, thus again giving a misleading picture about the apparent equivalence of these two gloves.

CONCLUSION

All the monoclonal antibodies used in this study were produced from different hybridoma clones and recognised entirely different proteins except for USM/RB4 and USM/RB10

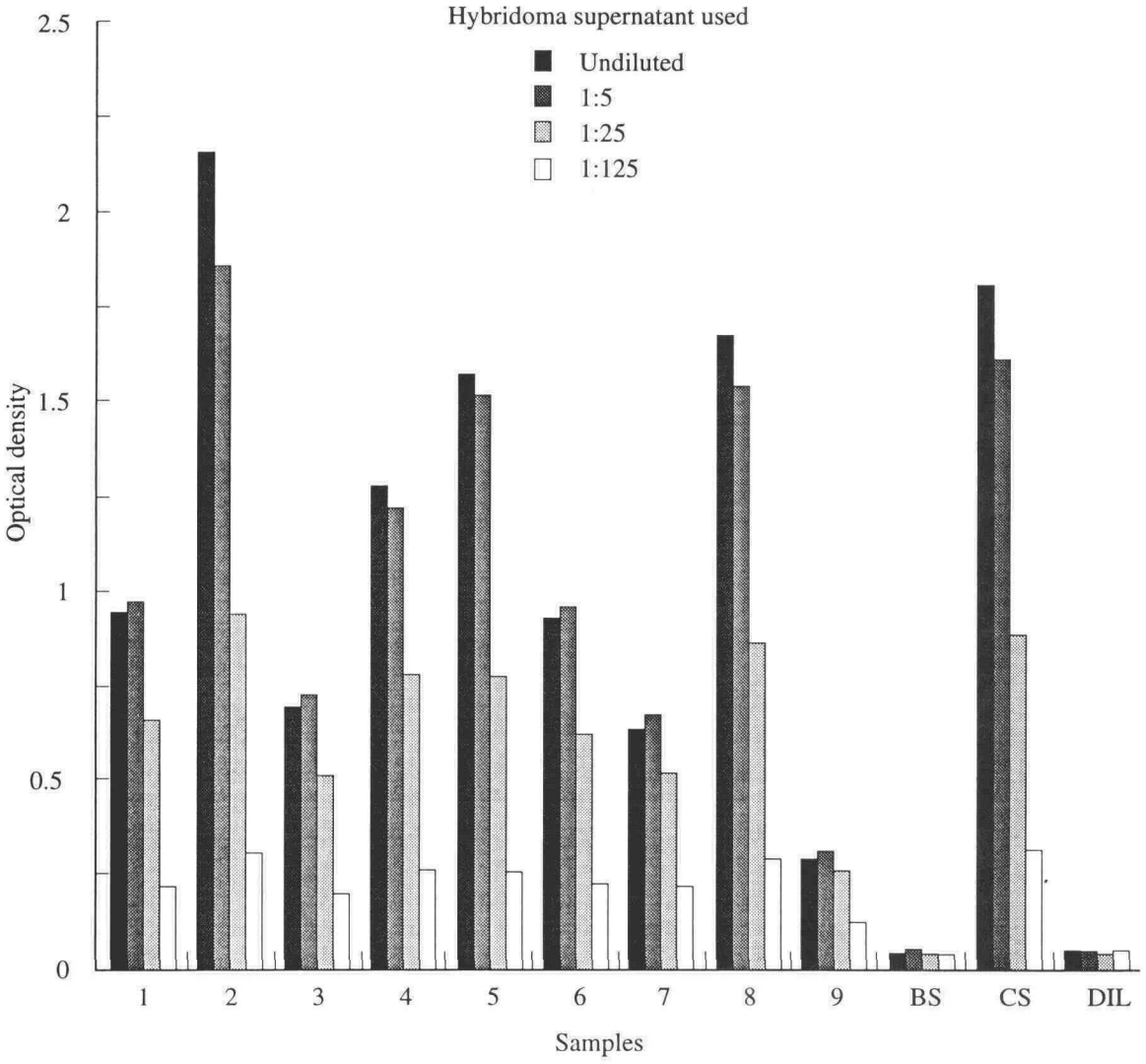

Figure 2. Indirect ELISA showing a titration of monoclonal antibody against C-serum designated USM/RC2 against glove protein eluates coated onto a 96-well ELISA plate. Control wells containing B-serum (BS) and C-serum (CS) are included with a negative control containing no protein in the initial coating buffer (DIL).

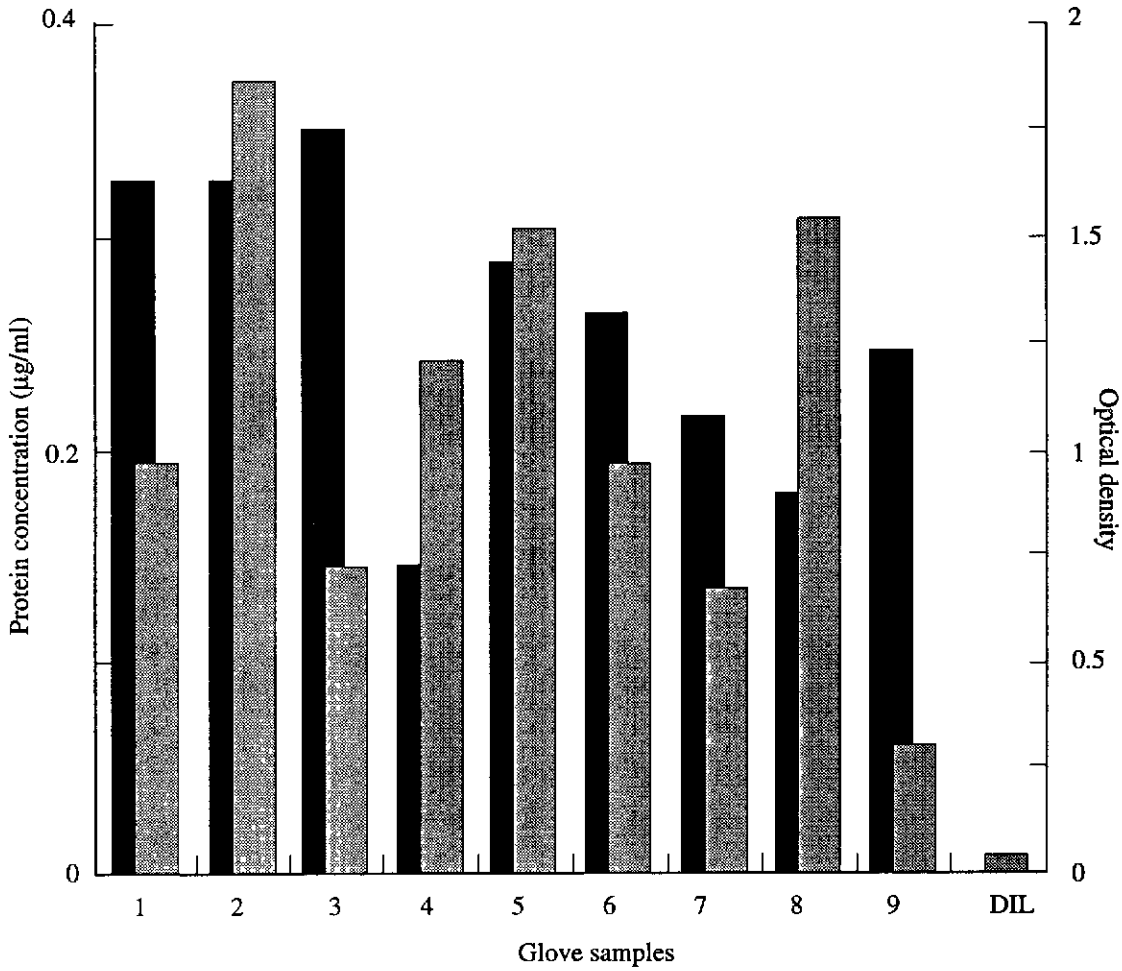

Figure 3. Indirect ELISA showing the reactivity of protein eluates from various glove samples to monoclonal antibody USM/RC2 in relation to the total protein concentration of the eluates. The black bars represent protein concentration in µg per ml and is shown on the Y axis on the left. The grey bars represent optical density measured at 492 nm and is shown on the Y axis on the right.

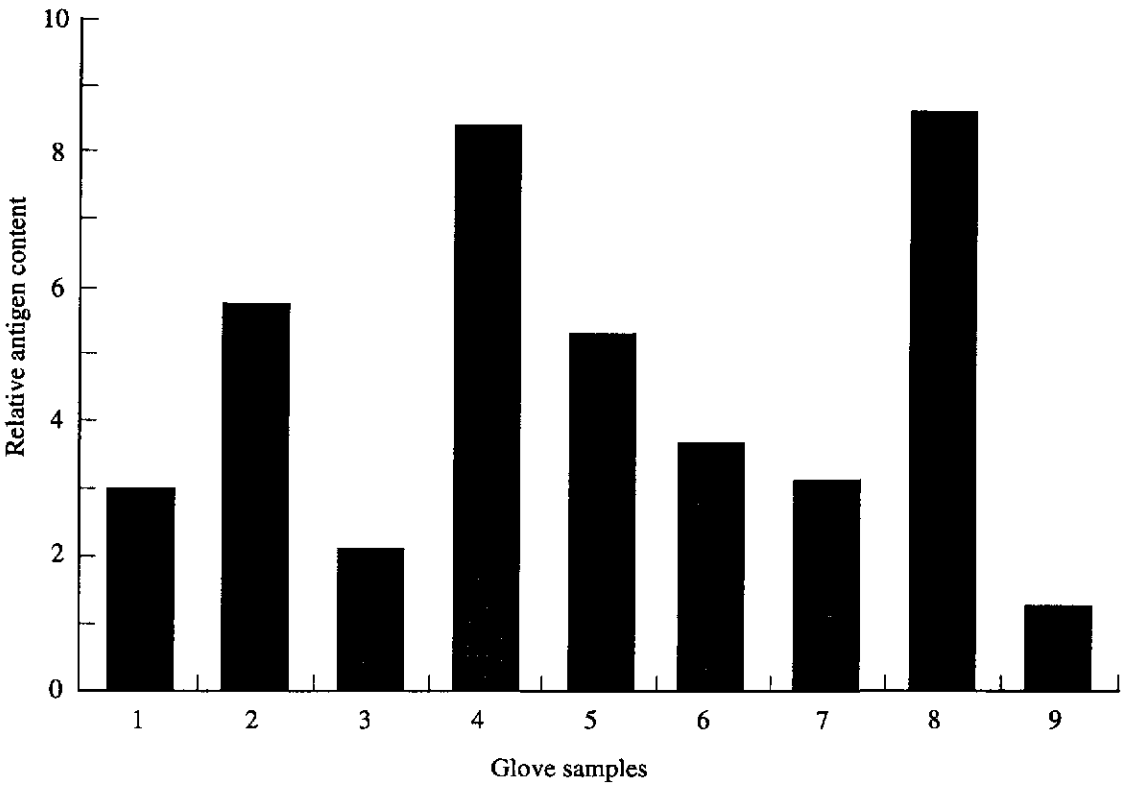

Figure 4. The relative proportion of antigen identified by monoclonal antibody USM/RC2 in glove protein eluates.

which appear to recognise the same protein as determined by Western blot analysis (data not shown). These two monoclonal antibodies also showed nearly identical profiles on the various gloves tested, thus showing that consistent results can be obtained using the techniques described here. The point however, is that different gloves not only contain different amounts of residual protein, but that the antigen content of these gloves are also different both in a quantitative as well as in a qualitative sense, as demonstrated particularly well with the data from the monoclonal antibody USM/RC2 which recognises a series of polypeptides derived from C-serum. This also illustrates how a sample with a very low protein content may have a high proportion of a particular antigen present. If this antigen represents an important allergen, the low protein content may not adequately reflect the quality of the product from the point of view of allergenicity.

It is thus imperative that specific knowledge about the offending allergens in natural rubber products be used to assist in the design and development of immunoassays which may be used in quality assurance programs.

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