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Immunoassays for Determination of Antigens and Allergens in Latex Products

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The quality control of latex products used in the medical setting has to take into account the presence of allergens which induce a Type I hypersensitivity response. Assays to measure the total protein eluted from these products does not address the problem of allergenicity of the proteins extracted. This paper introduces to rubber manufacturers the concept of enzyme immunoassay and describes various immunoassay formats by which antigens can be detected. The paper further describes a number of examples which show that gloves produced by different manufacturers contain different amounts of antigens and monoclonal antibodies are used to show that different antigens are found in different gloves.

The emergence of human allergic responses to proteins of natural rubber latex has raised the problem of designing new and specific assays for assessing the levels of antigens or more particularly, of allergens in latex products. This presupposes knowledge of the major antigens found in these latex products, and also of the proteins which elicit Type I hypersensitivity responses in humans. While the absence of definitive data on the allergenic proteins confounds the problem of designing highly specific assays, it is still necessary to address the problem of antigenic and allergenic proteins. It is sensible to begin with the premise that since allergens are a subset of antigens, the determination of antigenic content of extracts of latex products goes part of the way toward the design of more specific assays for quality control.

This paper will introduce the concept of the enzyme immunoassay in several formats and is aimed at providing some options to manufacturers interested in performing in-factory research into improving their product(s) with respect to antigen content; or to those who simply wish to expand their range of in-factory quality control procedures.

Antigen-antibody Reactions

The immune system evolved as a protective response to invasion by non-self agents such as bacteria and parasites and one of the best known molecules produced by the immune system is the immunoglobulin molecule or antibody. There are several classes of these and it is the IgE class which causes allergic responses. Antibody molecules are able to bind in a highly specific manner to particular sites on other molecules termed antigens. The antigen-antibody reaction which takes place can be exploited to design highly specific assays for the determination of either the antigen or the antibody and it is for this reason that such assays are called immunoassays.

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Enzyme Immunoassays

An example of a simple immunoassay is one in which a known antigen is used to detect the presence of antibodies which recognise the antigen. If an antibody recognises an antigen the antibody will bind to the antigen, thus forming an immune complex. The trick is to detect the formation of such immune complexes. In a very popular form of the immunoassay, labelled reagents are used to detect the presence of these complexes. When the label is an enzyme, the assay is called an enzyme immunoassay, and when it is a radioisotope, the assay is called a radio-immunoassay. In this paper we shall be discussing several enzyme immunoassays for the detection of antigens derived from natural rubber latex. The presence of bound enzyme is determined by using a colorigenic substrate for the enzyme. A common format used for enzyme immunoassays is the ELISA or enzyme linked immunosorbent assay which uses a 96 well microtitre plate in which all the reactions are performed. This allows batch processing of many samples and the intensity of colour generated can be measured in a spectro- photometer designed to read such microtitre plates, giving optical density readings for each well.

Indirect ELISA

An indirect ELISA is one in which the wells of an ELISA plate are coated with antigen and specific antibody is allowed to bind to this antigen. The presence of bound antigen is detected using an enzyme labelled antibody conjugate directed against the first antibody (these two antibodies are prepared in different species, such as rabbit and goat). A schematic diagram to illustrate the steps in this procedure is shown in *Figure 1.*

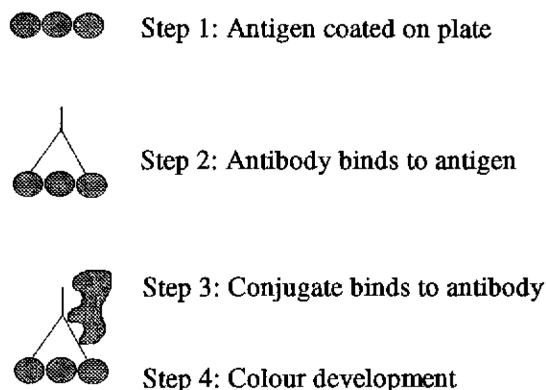

Figure 1. Indirect ELISA.

Figure 2 shows a dose response curve of B-serum antigens detected by a rabbit antiserum prepared by immunising rabbits with proteins eluted from latex gloves[1]. Such curves may be used as standard curves against which to determine the amount of antigen present in samples such as glove extracts as suggested by Beezhold[2], or simply by comparing the optical density of different samples as shown in *Figure 3* where it is clear that some glove samples have higher antigenicity as measured by reactivity with rabbit antiserum to glove proteins. It is also possible to express the differences in the various samples by a relative value such as a ratio or by assigning units to a standard such as B-serum.

Although this is apparently a very straightforward application of the simple indirect enzyme immunoassay, there are two major limitations to this approach. This first of these is the assumption that all antigens will bind equally well to the microplate under the standard conditions used, an assumption which is not necessarily true, particularly when many of the antigens found in eluates from latex products are likely to be smaller cleavage or hydrolytic products of larger source proteins. The second problem is a logistic one in that the sample to be analysed must be bound to the

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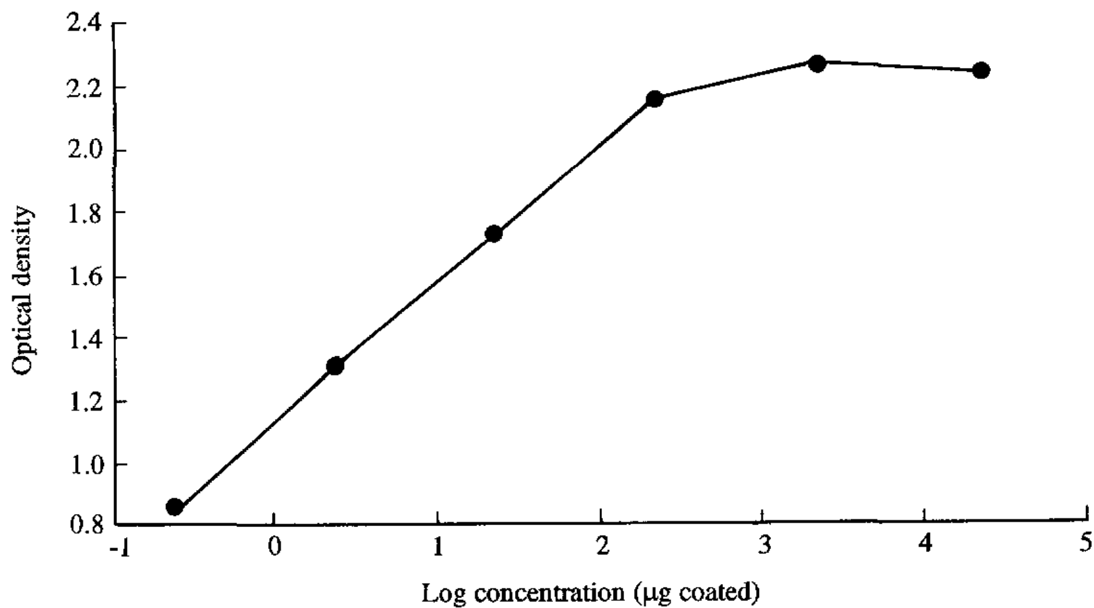

Figure 2. B-serum dose response (Tested against rabbit antiserum).

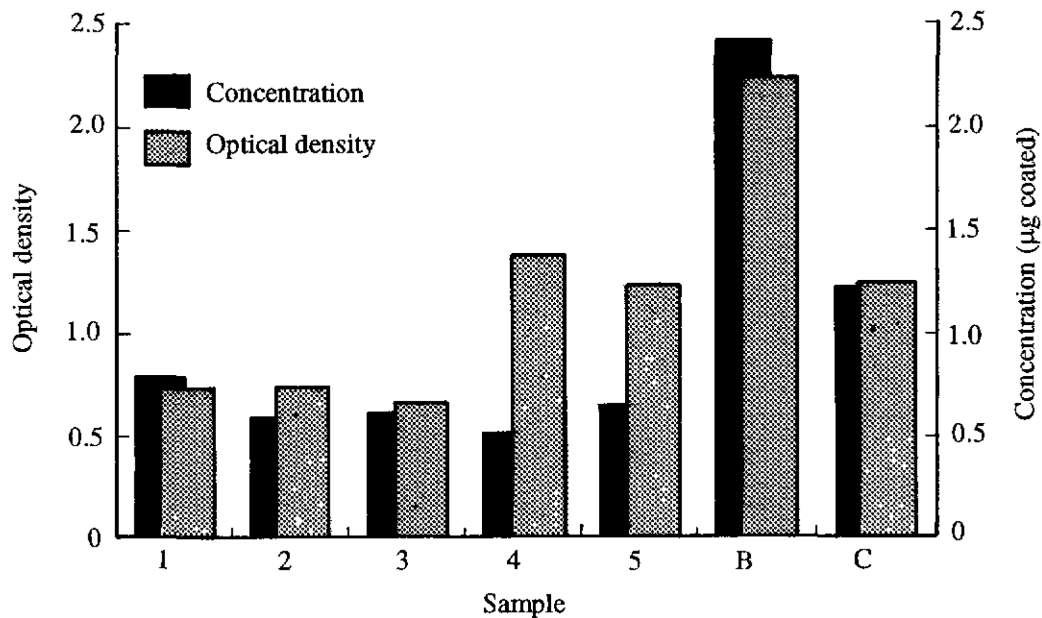

Figure 3. Indirect ELISA (Antigenicity of glove samples).

microplate by the end user, introducing a large element of variability and inconsistency especially when the end user may be inexperienced in handling immunoassays.

Sandwich ELISA

One solution to the problem of inconsistency in coating ELISA plates when the unknown is the antigen in an immunoassay is to turn the assay around and coat the microplate with a specific antibody instead. This antibody will bind the antigen of interest and the antigens may then be identified using another antibody directed against a second site on the antigen of interest. It is usual in this type of immunoassay (which is called a sandwich ELISA) to have the second antibody from a different species than the first, and the second antibody is normally labelled. A schematic diagram illustrating this type of immunoassay is shown in *Figure 4.* The disadvantages of such an immunoassay is the requirement for two different specific antibodies directed against the antigens of interest.

Competitive ELISA

Instead of detecting the presence of bound antigen with a second labelled antibody as described above, it is possible to bypass this requirement for a second antibody by using a labelled known antigen which will be recognised by the antibody coated onto the microplate. If, before adding the labelled known antigen, the unknown (the sample in this case) is also added to the well to compete with the labelled antigen for binding to the antibody on the plate, then it is possible to determine the presence of antigen in the sample, the degree of inhibition of labelled antigen being a measure of the amount of antigen present in the sample. This presupposes that the amount of labelled antigen added is not in excess. This ELISA format is represented in the diagram in *Figure 5.*

Results from competitive ELISAs can be expressed in several ways. *Figure 6* shows the results of a competitive ELISA in which the antigens eluted from nine glove samples from different manufacturers were determined in reference to B-serum and C-serum. The total protein concentration of each eluted sample is shown by the solid bars with the values on the left Y axis. The antigenicity of the samples was expressed as the percent by which each sample could inhibit the labelled antigen (prepared from B- and C-serum) from binding to one of two antibody preparations: (i) a rabbit antiserum directed against glove

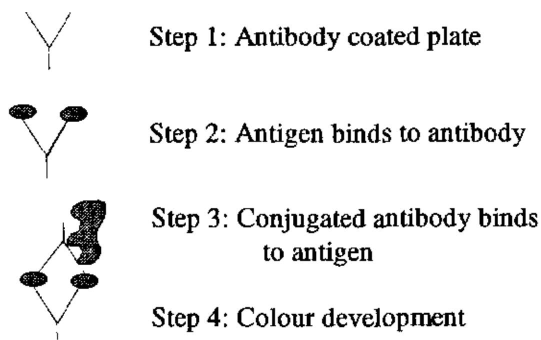

Figure 4.·Sandwich ELISA.

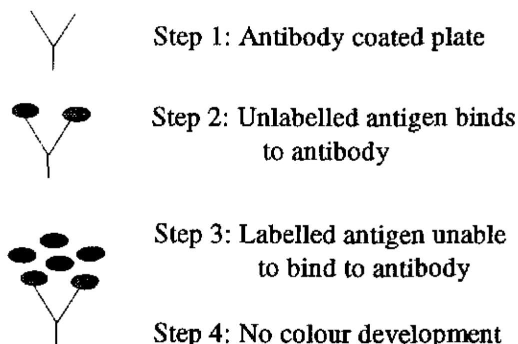

Figure 5. Competitive ELISA

proteins, and (ii) a monoclonal antibody we have named RB4 which is directed against one of the major allergens of natural rubber latex.

The histogram illustrates several points: (1) Different samples contain different amounts of total protein; (2) Much of this protein is probably antigenic and this is reflected by the general correlation which is seen between total protein and antigenicity as measured by the rabbit antiserum directed against glove proteins, this is to be expected since the rabbits producing this antiserum were immunised with proteins eluted from gloves; (3) The single allergen (RB4 protein) which was tested separately was found in all glove samples, but in varying amounts. For instance, *sample number 4* had very low amounts of RB4 protein, but the total protein content of this glove sample was also low. On the other hand, *sample number 5* had a high total protein content, with most of the antigen being accounted for by the allergen defined by monoclonal antibody RB4. In fact, this allergen accounted for varying proportions of the total antigen content as can be seen in *Figure 7* in which the inhibitory properties of the glove samples were converted to arbitrary units per microgram protein present. One example of the usefulness of this method of expressing the results is again illustrated by *samples number 4* and *5* where it is clear that although *sample number 4* has a relatively low concentration of RB4 allergen, the relative antigen concentration of the eluate is equivalent to the other glove samples. *Sample number 5,* on the other hand has relatively more antigen as well as RB4 allergen than any of the other samples.

Since different samples have different total protein content, and yet different relative concentrations of antigen and allergen, it is also important to find a way to express an overall index of antigenicity or allergenicity to avoid the dilemma of producing devices of a very low protein content, but in which all the protein is allergenic, as compared to producing a device which does not contain any allergenic proteins. One method of expressing such an index is shown in *Figure 8* where both the amount of protein and the antigenicity or allergenicity of the proteins eluted are taken into consideration and expressed as an index (units antigen/allergen × protein concentration). This clearly shows very large variations in the quality of the nine glove samples with respect to antigenicity and allergenicity (as represented by a single allergenic protein, RB4).

Dot Enzyme Immunoassays

One of the major obstacles to the implementation of ELISA as a routine quality control tool in the natural rubber latex industry would be the requirement for trained technical personnel and capital equipment for an immunochemistry laboratory. Although the ELISA format is very attractive because spectrophotometric readings allow sensitive quantitation of antigen and allergen levels, it is also possible to design immunoassays which are semi-quantitative or qualitative, allowing pass-fail decisions at the level of the factory floor. One such immunoassay is the dot enzyme immunoassay (DEIA) which uses sheets of nitrocellulose or nylon as the solid phase upon which the reaction takes place. All the different types of ELISAs described above can be performed as DEIAs and *Figure 9* shows an example of one such DEIA which shows the antigenicity of the nine different glove sample eluates when measured in an indirect DEIA against a mouse antiserum against B-serum proteins *(Panel A)* and against a monoclonal antibody to another allergen we have named RC2 *(Panel B)*. The third panel *(C)* shows the reactivity of the same samples against another monoclonal antibody which recognises a latex protein not found in the gloves.

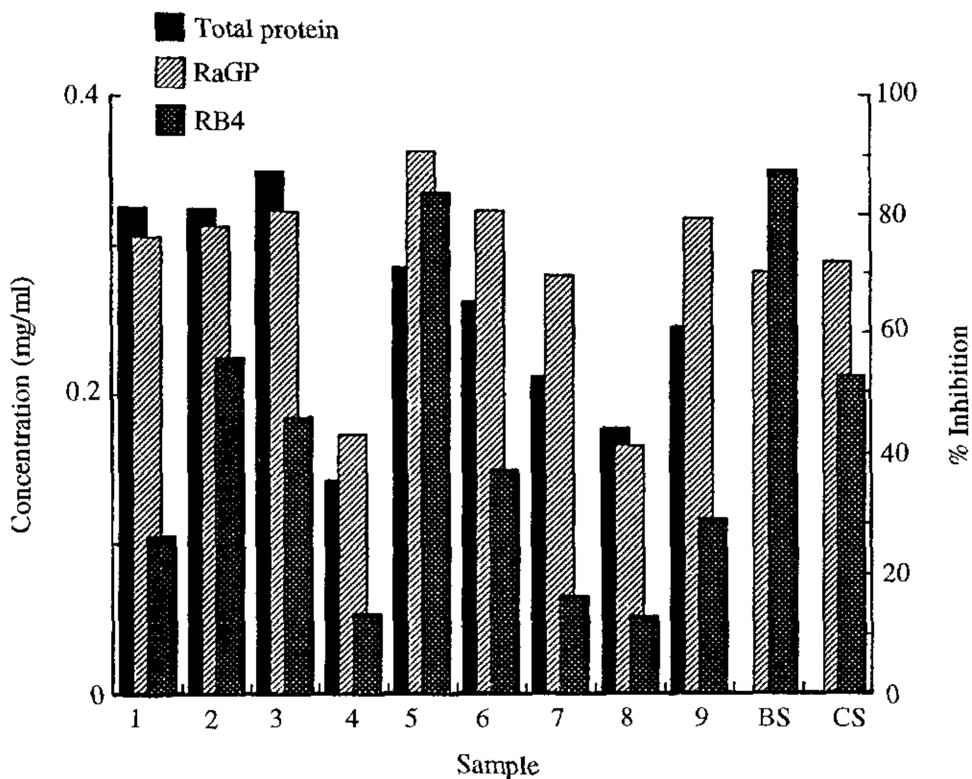

Figure 6. Competitive ELISA.

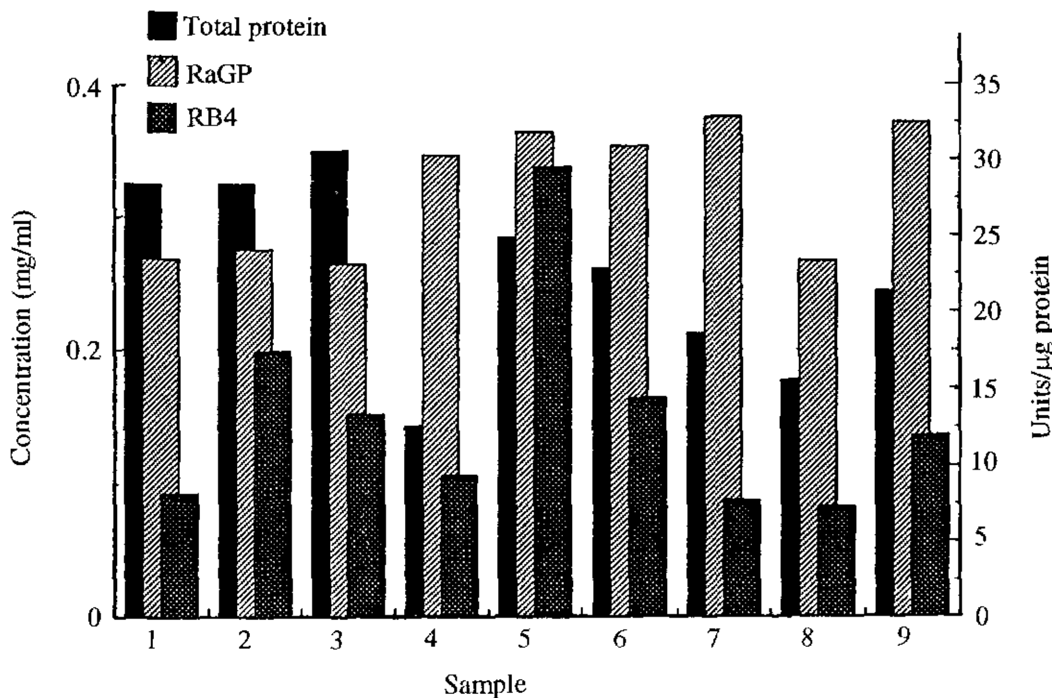

Figure 7. Antigens/allergens (Relative to protein concentration).

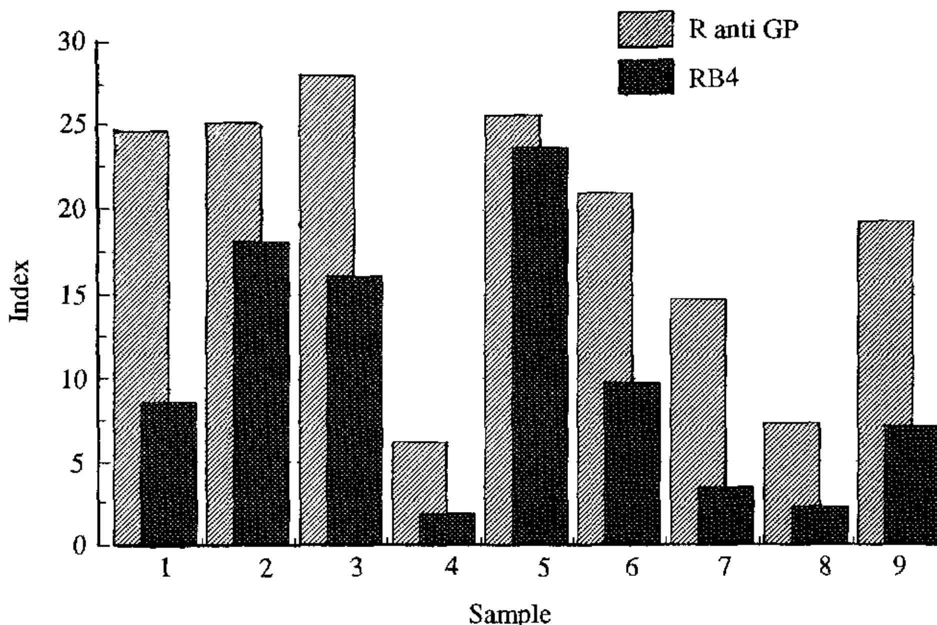

Figure 8. Antigen/allergen index.

CONCLUSION

The enzyme immunoassay is a useful tool for the determination of antigen and allergen levels found in natural rubber latex products and many different formats may be designed for different purposes. We have described the use of competitive ELISA in an application which may assist manufacturers of natural rubber latex devices in assuring the quality of their products with respect to the problem of allergenic responses. The ELISA format may be replaced also by a DEIA format for ease of use by manufacturers.

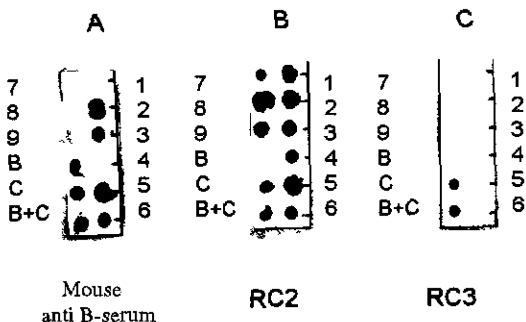

Figure 9. Dot enzyme immunoassay.

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