

Two enzyme linked immunosorbent assays for detecting antibodies against meningococcal capsular polysaccharides A and C

O A O Akinwolere, D S Kumararatne, R Bartlett, D M Goodall, D Catty

Abstract

Aims—To evaluate two of the recent methods of coating microtitre plates in the enzyme linked immunosorbent assay (ELISA) for detecting human antibodies against meningococcal capsular polysaccharides A and C with a view to validating a specific meningococcal antibody assay for routine clinical use.

Methods—Two four-layer ELISA protocols were standardised: one method utilised meningococcal polysaccharides conjugated to poly-L-lysine polypeptide for coating the microtitre plates; another used polysaccharides mixed with methylated human serum albumin (mHSA). Titration curves were plotted for the ELISAs and the squared Pearson correlation coefficient (R^2) was used to determine the degree of accuracy of fit of the curves. Specificity tests were performed by inhibition and adsorption studies.

Results—Both methods gave good titration curves with a high R^2 of >0.98 , indicating a high degree of accuracy in forming the curves. The titration end point after vaccination, obtained by the mHSA method, was 20 times higher, however, than that obtained by the poly-L-lysine method. Specificity tests showed that in the ELISA using polysaccharide/poly-L-lysine, antibody activity of a pre-vaccination serum sample was inhibited by 37%, and of post-vaccination serum by 50% with 1000-fold excess antigen. Antibody activity (post-vaccination) was reduced by 51% and 59%, respectively, by adsorption with antigen-coated Sepharose beads or adsorption with suspensions of killed meningococci. In contrast, antibody activity of a pre-vaccination serum was inhibited by 60% and a post-vaccination serum by 90% in ELISA employing polysaccharides mixed with mHSA. Reproducibility was better with the use of methylated human serum albumin than with poly-L-lysine; the former showed intrabatch and interbatch coefficients of variation of 4% and 2%, respectively, compared with 43% (intrabatch) and 16% (interbatch) obtained with the poly-L-lysine.

Conclusion—It is concluded that the antibody assay using meningococcal polysaccharides groups A and C mixed with mHSA is much better than that using

polysaccharides coupled with poly-L-lysine.

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Neisseria meningitidis is the causative organism in meningococcal disease, including septicaemia and meningitis. Meningococcal meningitis has a worldwide distribution, either in the endemic form in industrialised countries or as epidemics in developing countries.¹ Epidemics are now rare in the temperate countries but the number of cases in parts of Europe increased suddenly in the late 1980s,² reaching epidemic levels in Finland, Norway, The Netherlands, Spain and the United Kingdom. *N meningitidis* groups A, B, and C account for more than 90% of the isolates from patients.³ In the United Kingdom, infections caused by group C are not uncommon, accounting for 40% of infections in 1987,⁴ and they still account for about a third of all meningococcal disease.⁵ Group A strains account for 2%; group B infections still predominate.

Vaccines are available for groups A and C (as well as groups Y and W135) but not group B.⁶⁻⁸ Antibodies against the meningococcal capsular polysaccharides (MCP) groups A and C are protective in adults and children above 2 years,^{6,8} and antibodies have been detected by indirect haemagglutination four years after vaccination.⁹ A more sensitive and specific method for quantitating antibody responses to vaccinations needs to be found. Although the enzyme linked immunosorbent assay (ELISA) has often been used for measuring antibodies to protein antigens, its use for measuring antibodies to purified cell surface carbohydrates is often hindered by the failure of polysaccharides to adhere effectively to the polystyrene solid phase of the ELISA. Negatively charged polysaccharides, such as meningococcal capsular polysaccharides A or C, do not adsorb easily to polystyrene commonly used as the solid phase in ELISA. Various methods have been developed to bind polysaccharides to microtitre plates. These include covalent binding to tyramine¹⁰ and to poly-L-lysine,¹¹ use of capture antibodies¹² and mixing with methylated human serum albumin (mHSA).¹³

This study evaluates the use of two ELISA methods for the detection of specific antibodies against meningococcal capsular polysaccharides groups A and C (MCP-A and MCP-C)

Department of Immunology, Medical School, University of Birmingham, Birmingham B15 2TT
O A O Akinwolere
D S Kumararatne
D M Goodall
D Catty

Department of Infection
R Bartlett

Correspondence to:
Dr O A O Akinwolere
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in which the microtitre plates were coated with MCP either covalently bound to poly-L-lysine or mixed with mHSA.

Methods

N meningitidis group A and C capsular polysaccharides were kindly provided by Dr GM Carlone (Meningitis and Special Pathogens Branch, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia, USA). A divalent meningococcal polysaccharide vaccine [Mengivac (A + C), Institute Merieux, Lyon, France] was also investigated as a source of combined antigens. The single antigens were reconstituted with sterile water (1 mg/ml) and stored at 4°C before use, while lyophilised vaccine was stored at 4°C and used after reconstitution with proprietary diluent.

The method of Messina and colleagues¹¹ was modified to bind, covalently, poly-L-lysine polypeptide to meningococcal polysaccharide vaccine by using cyanuric chloride as the coupling agent. Briefly, 100 µl of the polysaccharides were made alkaline with 500 µl NaOH (0.01M), using 100 µl of phenolphthalein (0.001%) as an indicator, followed by activation of the polysaccharides by adding 0.5 mg cyanuric chloride for 10 seconds. Poly-L-lysine (0.1%) (100 µl) was then mixed with the activated polysaccharides and refrigerated at 4°C for two hours. The final concentration of the polysaccharides in the conjugate was 123.8 µg/ml while that of the poly-L-lysine was 133.3 µg/ml.

mHSA was prepared by using human serum albumin (HSA) (20% w/v) solution (Zenalb 20, BPL Bio Products Laboratory, UK) as the starting material. Briefly, 2.5 g (20 ml) HSA was mixed with four volumes of cold ethanol and left at -20°C for one hour. The solution was centrifuged at 3000 rpm with a microfuge for five minutes before washing the precipitate once with ethanol. The pellets were suspended in 250 ml methanol, and 2.1 ml of concentrated hydrochloric acid was added dropwise. The mixture was allowed to stand at room temperature in the dark for three days with slow stirring. The suspension was centrifuged at 3000 rpm for five minutes and the precipitate washed twice with methanol and twice with anhydrous ether. The mHSA was collected on filter paper, dried overnight over potassium hydroxide in a desiccator, and stored in an airtight container in a refrigerator (4°C). A stock solution of mHSA in distilled water (1 mg/ml) was stored at 4°C. Working solutions of mHSA were made freshly by diluting stock solution in phosphate buffered saline (PBS, 10 mM, pH 7.4). The working solution of mHSA was mixed dropwise with continuous rigorous stirring with MCP (A or C or A + C) in PBS (10 mM, pH 7.4) to give a final concentration of 5 µg/ml of either MCP or mHSA.

Lyophilised standard reference serum, CDC1992, provided by Dr GM Carlone, was reconstituted with sterile water while test serum samples were obtained from an adult volunteer (A) who was vaccinated with

Mengivac (A + C) vaccine. The pre-vaccination sample (Ao) was collected just before vaccination while the post-vaccination sample (A1) was obtained three months after vaccination. A post-tetanus toxoid and post-pneumococcal vaccination (but not post-meningococcal vaccination) pooled human serum sample (PP) was provided by the Department of Immunology, Dudley Road Hospital, Birmingham, UK, while serum from a hypogammaglobulinaemic patient (ADS) was provided by the Clinical Immunology Services, Medical School, Birmingham. All serum samples were stored at -20°C.

All monoclonal antibodies used in this study were produced at Recognition Sciences Ltd., University of Birmingham. These monoclonal antibodies were: R10Z8E9 for pan IgG; 2D7 for IgA; and CH6 for IgM. The conjugate was a polyclonal sheep anti-mouse immunoglobulin (WHO Collaborating Centre for Immunological Reagents, University of Birmingham) adsorbed against human immunoglobulin and conjugated to horseradish peroxidase.

ELISA PROCEDURES

ELISA with MCP/poly-L-lysine conjugate coating

Microtitre plates [Nunc Immunoplate I, Gibco Ltd., Inchinnan, UK] were coated overnight at 4°C with 90 µl of the MCP/poly-L-lysine conjugate at an optimal concentration of 0.1 µg/ml in carbonate/bicarbonate (0.05M) coating buffer, pH 9.5. The wells were then incubated sequentially with: (1) 90 µl reference or test serum diluted serially in bovine serum albumin (BSA) (1% w/v) in PBS (10 mM, pH 7.4) containing 0.05% (v/v) Tween 20 (PBS-T, Sigma Chemical Co Ltd, Poole) at 4°C for 48 hours; (2) 90 µl specific murine monoclonal antibodies to human immunoglobulin G (R10Z8E9, Recognition Sciences Ltd, Birmingham) diluted 1 in 250 in PBS-T containing 1% BSA, at 37°C for one hour; (3) 90 µl sheep anti-mouse immunoglobulin horseradish peroxidase conjugate (WHO Collaborating Centre for Immunological Reagents, University of Birmingham), diluted 1 in 1000 in PBS-T containing 1% BSA and normal sheep serum (NSS) (0.5% v/v).

Wells were washed four times with PBS-T after each incubation. The assay was developed with 90 µl of OPD substrate per well (0.4 mg/ml o-phenylenediamine dihydrochloride; Sigma) in a 0.15M phosphate citrate buffer (pH 5.0) containing 0.012% hydrogen peroxide (H₂O₂; BDH Ltd, Poole, UK). Sulphuric acid (25 µl (20% v/v) per well) was used to stop the colour reaction after optimal development and the plates were read by absorbance at 492 nm using an automated ELISA reader (Anthos Reader 2001, Anthos Labtec Instruments Ltd, Basingstoke, UK).

ELISA WITH MCP/mHSA MIXTURE COATING

This procedure was modified from the method described by Arakere and Frasci¹³ and by Carlone *et al.*¹⁴ Briefly, *N meningitidis*

polysaccharide groups A or C or A + C, in coating buffer (10 mM PBS, pH 7.4) was added dropwise with continuous rigorous stirring to an equal volume of working solution of mHSA to give a final concentration of 5 µg/ml each (10 µg/ml of Mengivac (A + C)). This was used to coat microtitre plates (Immulon 2, Dynatech Laboratories Ltd, Sussex, UK) overnight at 4°C (100 µl/well). The plates were washed five times with wash buffer (PBS-T), then 200 µl of serum conjugate (S-C) buffer (PBS-T containing 10% v/v fetal calf serum) was added to each well. Plates were left to stand at room temperature for one hour after which the buffer was decanted and plates blotted vigorously on absorbent towels. Serum diluted in S-C buffer (200 µl) was added to the first well of the dilution series, after which nine duplicate serial dilutions of reference standard or test sera were made by well-to-well transfer in the microtitre plates using a multichannel pipette (100 µl was transferred and mixed five times in each well). The plates were covered and incubated overnight at 4°C. Plates were washed five times as described above and the wells were then incubated at 37°C for one hour sequentially with: (1) 100 µl of specific murine monoclonal antibodies to human immunoglobulins (G (R10Z8E9), A (2D7), or M (CH6), produced by Recognition Sciences Ltd, University of Birmingham), diluted appropriately in S-C buffer; (2) 100 µl sheep anti-mouse Ig horseradish peroxidase conjugate diluted in S-C buffer containing NSS (0.5% v/v).

The plates were washed five times after each step and the assay developed with 100 µl OPD substrate per well, as described before.

STATISTICAL ANALYSIS AND INTERPRETATION OF ASSAYS

Graphs of the absorbance against $\log_{10}x$ of the serum dilution were plotted using a standard computer program. These sigmoid scatter curves were expected to follow third order polynomial equations and therefore computerised methods for determining adequacy of curve fit were used. The squared Pearson correlation coefficient (R^2) was used to determine the degree of accuracy of fit between the experimental data and the model curves. A titration curve was obtained for each serum sample and, where appropriate, compared with the curve of a standard positive reference serum. The antibody titre for each individual was expressed as a reciprocal of the dilution of serum which gave an absorbance reading equivalent to one third the maximum value produced by the standard reference serum.

INHIBITION ASSAYS

Competitive inhibition was assessed using a single dilution of a serum sample. The dilution was chosen after comparing the inhibitions for serum dilutions between 1 in 20 and 1 in 160. Inhibition was at a maximum between 1 in 80 and 1 in 160 dilutions. The sera were diluted in PBS-T containing 10% polyethyl-

ene glycol (BDH Ltd) or in S-C buffer, and distributed into nine aliquots for each buffer. Eight two-fold dilutions of MCP(A + C) in PBS-T containing 10% polyethylene glycol or in S-C buffer were prepared starting at the highest concentration (100 µg/ml) and ending with the lowest concentration (0.78 µg/ml). An equal volume of a different serial dilution of MCP(A + C) was added to each of eight aliquots containing serially diluted serum; the same volume of buffer alone was added to the ninth serum aliquot as the 0% inhibition control. The serum and polysaccharide dilutions were allowed to react for one hour at 37°C and antibody activity of the mixture was then determined by two ELISA procedures, as described before.

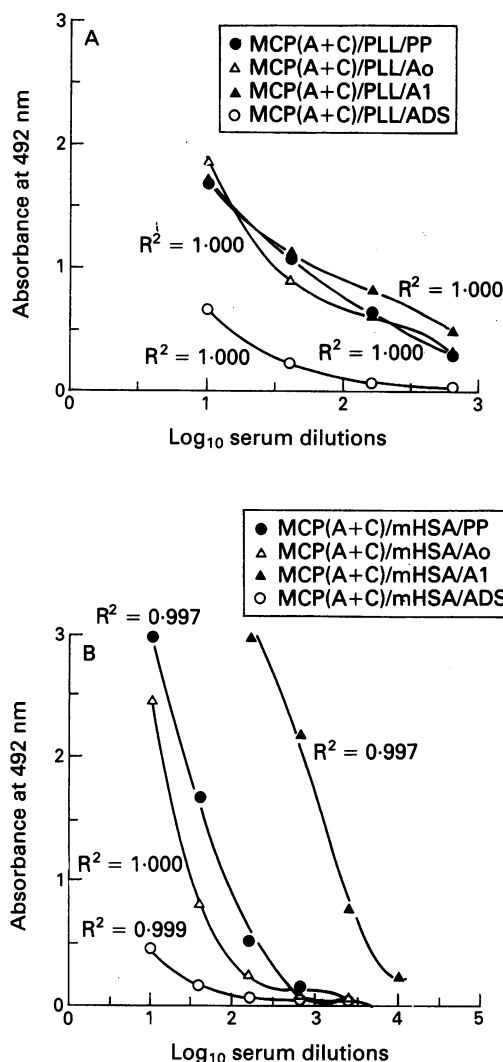
ADSORPTION ASSAYS

Adsorption of meningococcal antibodies was performed separately with: (1) MCP (A + C)-coated activated Sepharose beads (Sepharose 4B, Pharmacia Fine Chemicals, Herts, UK) using a method obtained from Pharmacia, as modified by the Immunodiagnostic Research Laboratory (IDRL), University of Birmingham; (2) suspensions of killed *N meningitidis* serogroups A and C organisms (Public Health Laboratory Service, National Collection of Type Cultures, London, UK).

Adsorption with antigens coated to activated Sepharose beads

Cyanogen bromide (0.01 g) activated Sepharose beads (CN-Br Sepharose 4B, Pharmacia) were preswollen with 500 µl HCl (1 mM) in each of five Eppendorf tubes and washed with 1 ml HCl (1 mM) for five minutes at 6500 rpm using a microfuge. The supernatant fluid was discarded and 1 ml of MCP(A + C)/poly-L-lysine conjugate antigen, dialysed overnight at 4°C against NaHCO₃ (0.1 M)/NaCl (0.5 M) buffer (pH 8.3), was added. Uncoated Sepharose beads with buffer in five other tubes were used as controls. The tubes were rotated and mixed for two hours at room temperature and washed twice with NaHCO₃/NaCl buffer (pH 8.3). The supernatant fluids were discarded and the antigen and controls were washed with three cycles of alternating pH. Each cycle consisted of a wash using sodium acetate (0.1 M)/sodium chloride (1 M) buffer (pH 4), followed by a wash using borate (0.1 M)/NaCl (1 M) buffer (pH 8). The supernatant fluid was discarded after the final wash. One milli-litre each of five serum dilutions (1 in 10, 1 in 40, 1 in 80, 1 in 160, 1 in 640 in PBS-T containing 1% BSA and 5% polyethylene glycol) was added to the antigen and control tubes; duplicate serum dilutions were stored at -20°C (as pre-adsorption sera). Adsorption was permitted at 37°C for two hours on a rotor and the tubes were then centrifuged at 6500 rpm for five minutes. The supernatant fluids were stored at -20°C (post-adsorption sera). The pre- and post-adsorption sera were tested by ELISA using MCP(A + C)/poly-L-lysine conjugate coating, as described before.

Figure 1 (A) ELISA curves of serum samples obtained from post-pool reference (PP), pre-vaccination (Ao), post-vaccination (A1) and antibody deficient (ADS) subjects showing the squared Pearson correlation coefficient (R^2) for each curve. Meningococcal capsular polysaccharide groups A and C vaccine (MCP(A+C)), conjugated to poly-L-lysine (PLL) were used to coat microtitre plates. The absorbance at 492 nm (ordinate) was plotted against the \log_{10} of serum dilutions (abscissa). (B) ELISA curves of serum samples (PP, Ao, A1, ADS) using MCP(A+C) mixed with methylated human serum albumin (mHSA) to coat microtitre plates, for comparison with fig 1A.



Adsorption with killed *N meningitidis*, serogroups A and C

Cultures of *N meningitidis* (serogroup A (NCTC) number 10025, strain M1027) and serogroup C (NCTC number 8554, strain 1628) were made in Todd Hewitt Broth and colony counts were performed using a Miles and Misra surface viable count.²⁷ The bacteria were killed by heating at 80°C for 20 minutes. Serial fourfold dilutions of serum (1 in 5, 1 in 20, 1 in 80, 1 in 320 in PBS-T containing 1% BSA and 5% polyethylene glycol) were made in bijou bottles and 500 μ l of each dilution was adsorbed, first with 500 μ l (2×10^9 bacteria) suspension of killed *N meningitidis*

Meningococcal antibody titres by ELISA methods

Serum	(PLL)		(mHSA)					
	MCP (A+C)	MCP (A+C)	(A)	MCP	MCP (C)	IgG	IgA	IgM
PP	66	86	204	10	281	16	5	13
Ao	39	34	42	16	174	8	<1	16
A1	105	2270	728	79	304	3565	728	304
ADS	6	2	<1	<1	<1	1	<1	<1
CDC 1992	ND	5297	4898	530	1888	1000	221	239

Meningococcal antibody titres (reciprocals) showing different immunoglobulin isotypes (IgG, IgA, and IgM) of serum samples (PP, Ao, A1, ADS, CDC 1992), using two ELISA methods (MCP/mHSA and MCP/PLL).

MCP group A or C was used to coat microtitre plates in the MCP/mHSA ELISA methods. Antibody titres (IgG) for the MCP/poly-L-lysine and MCP/mHSA methods using divalent meningococcal vaccine (A+C) are also shown. Titre of CDC 1992 was not determined (ND) using the divalent vaccine antigen.

serogroup A and then with 500 μ l (1.8×10^9 bacteria) suspension of killed *N meningitidis* serogroup C, for 30 minutes each at 37°C on a rotor. The Eppendorf tubes were centrifuged at 13 000 rpm for five minutes after adsorption with each bacterial strain and the supernatant fluids stored at -20°C. The pre- and post-adsorption sera were tested by ELISA using MCP(A+C)/poly-L-lysine conjugate coating, as described before.

Results

MENINGOCOCCAL ANTIBODY TITRES

The MCP/poly-L-lysine ELISA curves for various human sera (PP, Ao, A1, and ADS) using divalent antigens (A+C) are shown in fig 1A; MCP/mHSA ELISA curves for the same sera are shown for comparison in fig 1B. All the curves achieved a high squared Pearson correlation coefficient ($R^2 > 0.98$), indicating a high degree of accuracy in forming the curves. The table shows the meningococcal antibody titres (IgG) determined for the sera using the MCP/poly-L-lysine and MCP/mHSA assay methods; the titre of the post-vaccination serum, as measured by the MCP/mHSA assay method, is 20 times higher than that measured by the MCP/poly-L-lysine method. The table also shows the antibody titres of the immunoglobulin isotypes using the monovalent antigens (A or C) with MCP/mHSA ELISA.

SPECIFICITY ASSAYS

Competitive inhibition assays, as described for the two methods, showed less than 40% inhibition of antibody activity for the pre-vaccination serum (Ao) with the MCP/poly-L-lysine ELISA, compared with 60% with the MCP/mHSA ELISA. However, the antibody activity of the post-vaccination serum (A1) was inhibited by 50% using the MCP/poly-L-lysine ELISA, and by 90% using the

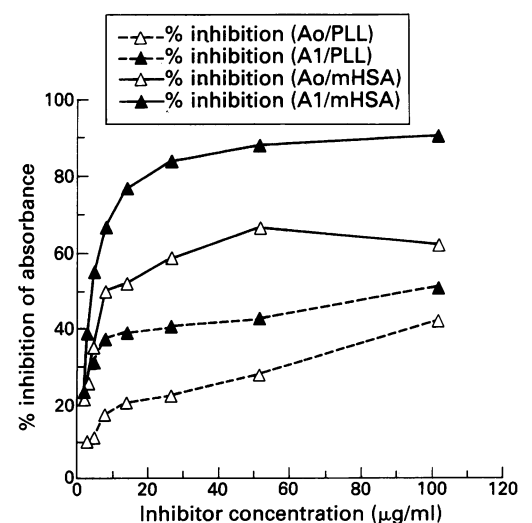


Figure 2 Shows ability to competitively inhibit antibody activity of pre- and post-vaccination samples with free meningococcal polysaccharides (A and C). The open symbols indicate the degree of inhibition achieved using pre-vaccination serum (Ao) and the closed symbols indicate the degree of inhibition with the post-vaccination serum (A1). The dotted lines represent the curves for the poly-L-lysine assay; the continuous lines represent the curves for the methylated human serum albumin assay.

MCP/mHSA ELISA (fig 2). Antibody activity in the post-vaccination serum was reduced by 51% after adsorption with antigen-coated Sepharose beads and maximally by 59% after adsorption with suspensions of killed meningococci, confirming the reduced specificity of the MCP/poly-L-lysine ELISA.

REPRODUCIBILITY

The MCP/poly-L-lysine ELISA gave antibody titre results that were less reproducible than the results obtained from the MCP/mHSA ELISA. The intrabatch coefficient of variation (CV) using the MCP/poly-L-lysine ELISA was 43%, while the interbatch CV was 16%. In contrast, the CV of the MCP/mHSA was 4% (intrabatch) and 2% (interbatch).

Discussion

Immunisation with meningococcal polysaccharide vaccines results in induction of protective antibodies in human kind. In adults these antibodies belong to the three main immunoglobulin isotypes IgG, IgM, and IgA.¹⁵ It is not only important to measure antibody responses to vaccinations but also necessary to identify people with low numbers of antibodies who are at risk of meningococcal infections. Antibodies against meningococcal polysaccharides have been measured by various methods, including bacterial antibody assay and immunofluorescence,¹⁶ opsonisation activity,¹⁷ passive haemagglutination,¹⁸ latex agglutination,¹⁹ radioantigen binding assay (RABA)²⁰ and ELISA.²¹ Of these, ELISA lends itself best to a routine and simple protocol which can sensitively measure the amount of antibodies in sera. The ELISA can also separately measure the antibody titre of the three main immunoglobulin isotypes.²² Other advantages of the ELISA include its relative simplicity, reagent stability, small sample volumes, and use of unsophisticated equipment. There is no universally standardised ELISA, however, for measuring antibodies against meningococcal capsular polysaccharides (MCP). We have standardised two four-layer ELISA protocols for measuring specific antibodies against MCP groups A and C. Serum IgG antibodies were detected using the divalent antigens (MCP-A + C) and monovalent antigens (MCP-A or MCP-C). The method of coating microtitre plates with MCP covalently bound to poly-L-lysine was compared with MCP mixed with mHSA. Although both methods gave good titration curves, the antibody titre obtained with the latter method using the post-vaccination serum was 20 times higher than that obtained with the poly-L-lysine method. The mHSA assay seems to reveal much better the critical dominant epitope site for antibody response to vaccination while the poly-L-lysine conjugate does not. Therefore, poly-L-lysine might either have destroyed this critical epitope on the polysaccharide or the covalent bonding of the two might have resulted in the loss of expression of the epitope or the epitope was unavailable for antibody binding in the conjugate when adsorbed to the solid phase.

Carbohydrates, coupled with poly-L-lysine,

were used as antigens for coating microtitre plates and were found to be sensitive and reproducible.¹¹ This was attributed partly to the maintenance of a constant alkaline pH in the conjugation process. In our experience, the use of poly-L-lysine is sensitive but less reproducible, although an alkaline pH was also maintained during conjugation of MCP to poly-L-lysine; we found a high intrabatch coefficient of variation (43%) and an interbatch coefficient of variation of 16%. The type of microtitre plate used in the assay may also contribute to the high intrabatch variation; this was confirmed by maximal absorbance of control wells coated with the coating buffer alone. The competitive inhibition assay with the poly-L-lysine method showed 50% inhibition of antibody activity of the post-vaccination serum, indicating the presence of non-specific binding to poly-L-lysine. This was also confirmed when poly-L-lysine was used as antigen to coat microtitre plate wells directly for the assay or to coat Sepharose beads for adsorption before the assay. In both cases maximal binding of antibodies was observed over the entire range of serum dilutions used in the assay and up to 42% adsorption of antibody activity was achieved with poly-L-lysine (data not shown). These observations suggest that the use of meningococcal polysaccharides, coupled with poly-L-lysine, for coating microtitre plates, can produce non-specific binding of antibodies to the polypeptides and may not be ideal in ELISA for measuring antimeningococcal antibodies. Other workers also found that poly-L-lysine produced non-specific absorbance with some sera in their meningococcal ELISA.¹³

mHSA mixed with meningococcal polysaccharides, on the other hand, showed 90% inhibition of antibody activity of post-vaccination serum. This shows minimal non-specific binding to the mHSA. We also found the use of mHSA to be more reproducible with low coefficients of variation of 4% (intrabatch) and 2% (interbatch). These figures compare with less than 10% variations reported for other validated ELISAs for measuring antibodies against encapsulated bacteria.²³ Our study has shown that the use of mHSA is better than that of poly-L-lysine for coating microtitre plates in meningococcal ELISA because it is more specific and more reproducible. We were also able to measure the different immunoglobulin isotypes of human antibodies against meningococcal polysaccharides groups A and C with this method.

It has been observed that standard ELISAs measure high avidity antibodies whereas amplified ELISAs can detect low avidity antibodies.²⁴ In our study the prolonged incubation of serum for 48 hours with the poly-L-lysine method, and for 24 hours with the mHSA method, suggests that low avidity antibodies were being detected. It was possible to reduce this serum incubation time to one hour in the poly-L-lysine method, and obtain a similar absorbance value, in the presence of 10% polyethylene glycol.²⁵ Detailed affinity studies are needed to confirm this observation.

A Centers for Disease Control standard reference serum was used for standardising the meningococcal assay as a primary step for measuring meningococcal antibodies, but there is no comparable standard reference serum available from the United Kingdom or from Europe. When such a serum is readily available it should be possible to determine the exact antibody concentration of test samples when such a reference is included on each plate of an assay.

Meningococcal antibody titres measured with divalent vaccine antigens reflect the higher antibody response to one of the component antigens. Although divalent (or tetravalent) vaccines are more readily available commercially than monovalent ones, their use as a source of antigen for coating microtitre plates in meningococcal ELISA is likely to be less than ideal as the antibodies to predominant epitopes may mask or inhibit the binding of antibodies to other antigens in the coating mixture. Therefore, we agree with the use of monovalent meningococcal polysaccharide vaccines or purified meningococcal capsular polysaccharides as recommended by the World Health Organisation as this will give a more meaningful measure of antibody responses of the individual to each of the vaccine component antigens. At present there are no easily available commercial sources of single meningococcal polysaccharides, but standardised polysaccharides for these assays may be available through agencies like the WHO. The advantage of using monovalent polysaccharide antigen over a mixture of different types of meningococcal polysaccharides is that each test gives a full measure of all antibodies capable of binding the single antigen irrespective of any antibody cross-reactions that might be present in the sera. Similarities exist between the residues of the polysaccharides from meningococci groups B and C and *Escherichia coli* types K1 and K92, on the one hand, and of the polysaccharides of meningococci of groups W135 and Y on the other. We did not expect any cross-reactions between groups A and B or C meningococcal polysaccharide because the chemical structure of group A polysaccharide is distinctly different from that of groups B or C; moreover the possibility of cross-reactions between these groups and other polysaccharides have been studied in detail before.^{21,26} They showed that cross-reactions only exist between group B meningococcal polysaccharide and *E coli* type K1, but only minor reactions occur between group C polysaccharide and *E coli* type K92.²¹ No cross-reactions have so far been found between the meningococcal groups except a slight cross-reaction reported between almost identical polysaccharides like groups W135 and Y polysaccharides.²⁶ Therefore, the ELISA using mHSA to coat microtitre plates will be useful in defining those at risk of meningococcal infections (groups A or C) and in quantitating human antibodies of different isotypes against meningococcal capsular polysaccharide A or C antigens.

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