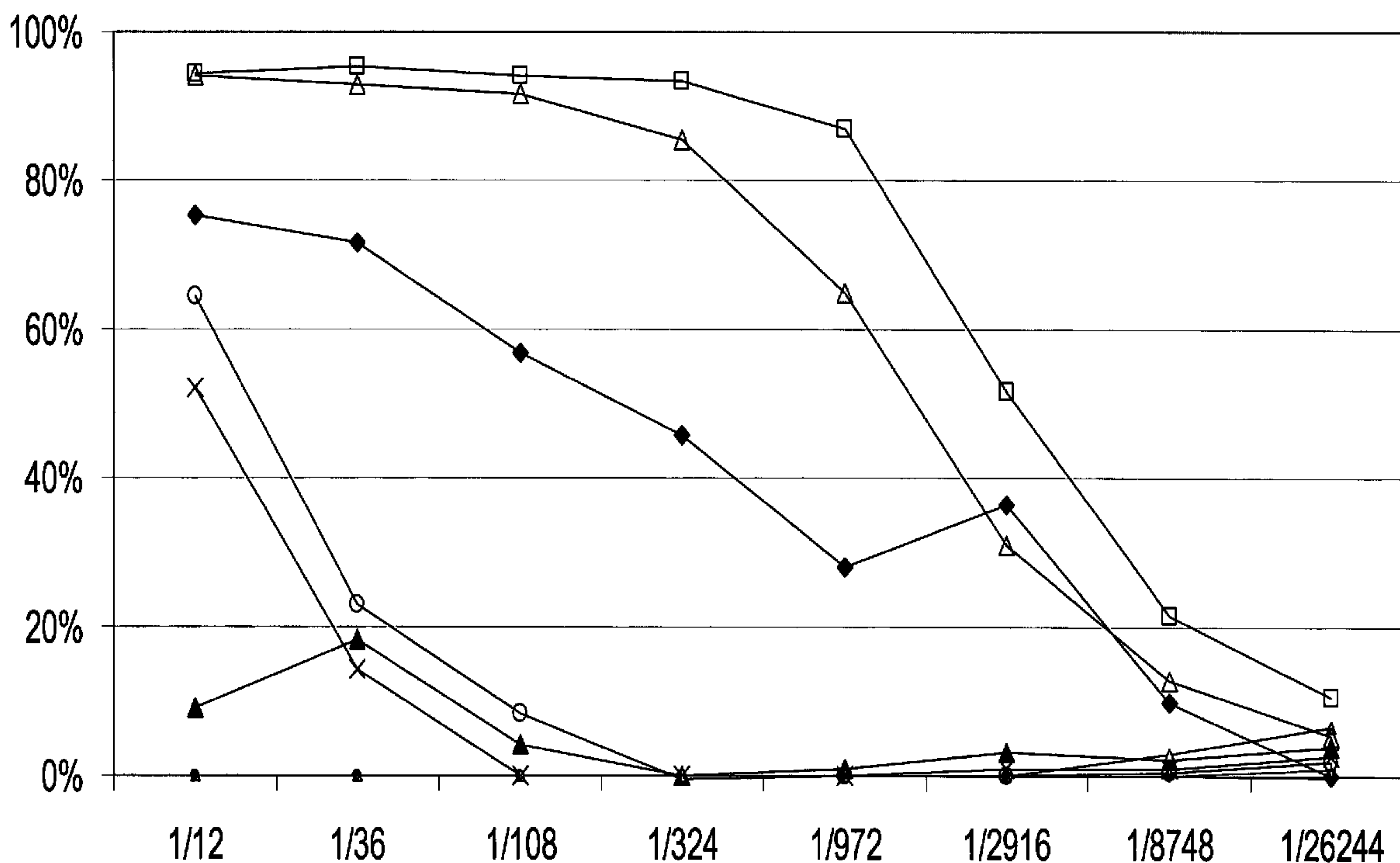




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SACCHARIDE DE PNEUMOCOQUE  
(54) Title: COMBINATIONS OF MENINGOCOCCAL FACTOR H BINDING PROTEIN AND PNEUMOCOCCAL  
SACCHARIDE CONJUGATES



**FIGURE 1**

(57) **Abrégé/Abstract:**

An immunogenic composition comprising: (i) a conjugated pneumococcal capsular saccharide; and (ii) a meningococcal factor H binding protein (fHBP) antigen, and not including meningococcal outer membrane vesicles, is useful for immunising a subject against bacterial meningitis.

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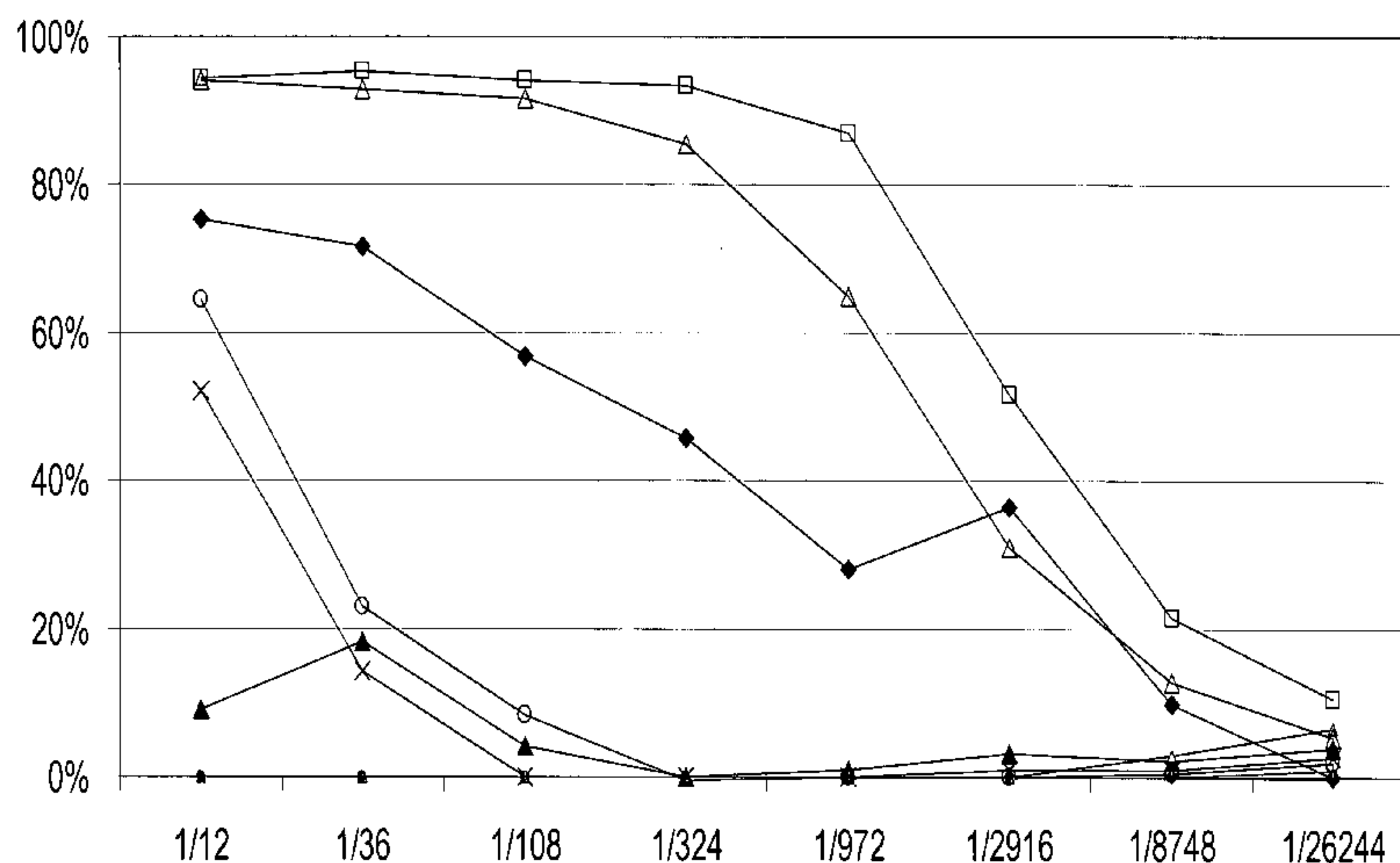


FIGURE 1

(57) Abstract: An immunogenic composition comprising: (i) a conjugated pneumococcal capsular saccharide; and (ii) a meningococcal factor H binding protein (fHBP) antigen, and not including meningococcal outer membrane vesicles, is useful for immunising a subject against bacterial meningitis.

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## COMBINATIONS OF MENINGOCOCCAL FACTOR H BINDING PROTEIN AND PNEUMOCOCCAL SACCHARIDE CONJUGATES

This application claims priority from US provisional applications 61/163,005 (filed 24th March 2009) and 61/270,407 (filed 7th July 2009), the complete contents of both of which are hereby  
5 incorporated herein by reference.

### TECHNICAL FIELD

This invention is in the field of combination vaccines, in particular those containing both a conjugated pneumococcal capsular saccharide and a meningococcal fHBP antigen.

### BACKGROUND ART

10 *Neisseria meningitidis* (meningococcus) is a Gram-negative spherical bacterium. Current meningococcal vaccines are also based on capsular saccharides. These include monovalent serogroup C conjugate vaccines (MENJUGATE™, MENINGITEC™ and NEISVAC-C™) and 4-valent conjugate mixtures for serogroups A, C, W135 and Y (MENACTRA™). There is currently no useful vaccine authorised for general use against serogroup B ('MenB'). Current research efforts for making  
15 a MenB vaccine are focusing on outer membrane vesicles (e.g. MENZB™, HEXAMEN™, NONAMEN™) or on purified components from the outer membrane, such as lipooligosaccharide and outer membrane proteins.

*Streptococcus pneumoniae* (pneumococcus) is a Gram-positive spherical bacterium. Current pneumococcal vaccines are based on capsular saccharides. The authorised pediatric vaccines are  
20 (a) PREVNAR™, which is a 7-valent mixture of conjugated saccharides from serotypes 4, 6B, 9V, 14, 18C, 19F & 23F, (b) SYNFLORIX™, a 10-valent conjugate mixture which also covers serotypes 1, 5 and 7F, and (c) PREVNAR 13™, a 13-valent conjugate mixture which also covers serotypes 3, 6A & 19A. Other 9-valent, 10-valent, 11-valent and 13-valent conjugate combinations are also known. The same 7 serotypes as PREVNAR™ have also been conjugated to an outer membrane  
25 vesicle complex ('OMPC') from a serogroup B strain of meningococcus [1].

Reference 2 discloses a composition for immunising against both pneumococcus and MenB, formed by combining a 13-valent pneumococcal conjugate vaccine ('13vPnC', Wyeth) with a 9-valent MenB outer membrane vesicle vaccine (NONAMEN™, NVI). A similar combination product is discussed in reference 3.

30 There remains a need for further and improved combination vaccines for protecting against both serogroup B meningococcus and pneumococcus.

### DISCLOSURE OF THE INVENTION

Unlike the compositions disclosed in reference 2, which used a combination of three different engineered outer membrane vesicles, the meningococcal serogroup B antigen in compositions of the  
35 present invention is based on a small number of purified antigens. The aim is to avoid the presence of complex or undefined mixtures of MenB antigens (e.g. outer membrane vesicles, as used in



references 1 and 2) in the composition. In particular, compositions of the invention include a purified meningococcal factor H binding protein (fHBP) antigen. It has been found that addition of pneumococcal conjugates to fHBP can enhance the anti-meningococcal response, and addition of fHBP to pneumococcal conjugates can enhance the anti-pneumococcus response.

5 Thus the invention provides an immunogenic composition comprising: (i) a conjugated pneumococcal capsular saccharide; and (ii) a meningococcal factor H binding protein (fHBP) antigen. The composition preferably does not include meningococcal outer membrane vesicles (including both naturally-occurring membrane vesicles that form spontaneously during bacterial growth and are released into culture medium, and artificial outer membrane vesicles that are formed  
10 *e.g.* by detergent treatment or sonication of meningococci).

A preferred composition includes: (i) an aluminium phosphate adjuvant; (ii) a conjugated pneumococcal capsular saccharide from each of at least pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, each of said saccharides being conjugated to a CRM197 carrier protein; and (iii) at  
15 least two different meningococcal factor H binding protein antigens, each of which is at least partially adsorbed to aluminium phosphate. A conjugated pneumococcal saccharide may also be adsorbed to aluminium phosphate. The composition may include sodium chloride and/or a buffer.

The invention also provides a process for preparing an immunogenic composition of the invention, comprising steps of: (i) mixing at least one conjugated pneumococcal capsular saccharide with an aluminium phosphate adjuvant to form a conjugate/adjuvant mixture; and (ii) mixing the  
20 conjugate/adjuvant mixture with at least one meningococcal factor H binding protein.

The invention also provides a process for preparing an immunogenic composition of the invention, comprising steps of: (i) mixing at least one conjugated pneumococcal capsular saccharide with at least one meningococcal factor H binding protein to form an antigen mixture; and (ii) mixing the antigen mixture with an aluminium phosphate adjuvant.

25 The invention also provides a process for preparing an immunogenic composition of the invention, comprising steps of: (i) mixing at least one meningococcal factor H binding protein with an aluminium phosphate adjuvant to form a protein/adjuvant mixture; and (ii) mixing the protein/adjuvant mixture with at least one conjugated pneumococcal capsular saccharide.

In a less preferred embodiment, the invention also provides a process for preparing an immunogenic  
30 composition of the invention, comprising steps of: (i) mixing at least one meningococcal factor H binding protein with an aluminium phosphate adjuvant to form a protein/adjuvant mixture; (ii) mixing at least one conjugated pneumococcal capsular saccharide with an aluminium phosphate adjuvant to form a conjugate/adjuvant mixture; and (iii) mixing the protein/adjuvant mixture and conjugate/adjuvant mixture.



Preferably a process of the invention does not include a step of mixing an aluminium hydroxide adjuvant with any of (i) a meningococcal factor H binding protein, (ii) an aluminium phosphate adjuvant, (iii) a conjugated pneumococcal capsular saccharide, (iv) a conjugate/adjuvant mixture, (v) an antigen mixture, or (vi) a protein/adjuvant mixture. Thus aluminium hydroxide is not added to  
5 be a component of the immunogenic composition.

***Conjugated pneumococcal capsular saccharide(s)***

Compositions of the invention include at least one pneumococcal capsular saccharide. The capsular saccharide is conjugated to a carrier protein.

The invention can include capsular saccharide from one or more different pneumococcal serotypes.

10 Where a composition includes saccharide antigens from more than one serotype, these are preferably prepared separately, conjugated separately, and then combined. Methods for purifying pneumococcal capsular saccharides are known in the art (*e.g.* see reference 4) and vaccines based on purified saccharides from 23 different serotypes have been known for many years. Improvements to these methods have also been described *e.g.* for serotype 3 as described in reference 5, or for serotypes 1,  
15 4, 5, 6A, 6B, 7F and 19A as described in reference 6.

Pneumococcal capsular saccharide(s) will typically be selected from the following serotypes: 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and/or 33F. Thus, in total, a composition may include a capsular saccharide from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or more different serotypes. Compositions which include  
20 at least serotype 6B saccharide are useful.

A useful combination of serotypes is a 7-valent combination *e.g.* including capsular saccharide from each of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Another useful combination is a 9-valent combination *e.g.* including capsular saccharide from each of serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F. Another useful combination is a 10-valent combination *e.g.* including capsular saccharide  
25 from each of serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. An 11-valent combination may further include saccharide from serotype 3. A 12-valent combination may add to the 10-valent mixture: serotypes 6A and 19A; 6A and 22F; 19A and 22F; 6A and 15B; 19A and 15B; or 22F and 15B. A 13-valent combination may add to the 11-valent mixture: serotypes 19A and 22F; 8 and 12F; 8 and 15B; 8 and 19A; 8 and 22F; 12F and 15B; 12F and 19A; 12F and 22F; 15B and 19A; 15B and  
30 22F; 6A and 19A, *etc.*

Thus a useful 13-valent combination includes capsular saccharide from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19 (or 19A), 19F and 23F *e.g.* prepared as disclosed in references 7 to 10. One such combination includes serotype 6B saccharide at about 8µg/ml and the other 12 saccharides at concentrations of about 4µg/ml each. Another such combination includes serotype 6A and 6B  
35 saccharides at about 8µg/ml each and the other 11 saccharides at about 4µg/ml each.

Suitable carrier proteins for conjugates include bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof. These are commonly used in conjugate vaccines. For example, the CRM197 diphtheria toxin mutant is useful [11]. Other suitable carrier proteins include synthetic peptides [12,13], heat shock proteins [14,15], pertussis proteins [16,17], cytokines [18], lymphokines [18], hormones [18], growth factors [18], artificial proteins comprising multiple human CD4<sup>+</sup> T cell epitopes from various pathogen-derived antigens [19] such as N19 [20], protein D from *H.influenzae* [21-23], pneumolysin [24] or its non-toxic derivatives [25], pneumococcal surface protein PspA [26], iron-uptake proteins [27], toxin A or B from *C.difficile* [28], recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) [29], *etc.* The OMPC used in reference 1 is excluded herein from possible carriers for pneumococcal saccharide because it is a meningococcal outer membrane vesicle.

Particularly useful carrier proteins for pneumococcal conjugate vaccines are CRM197, tetanus toxoid, diphtheria toxoid and *H.influenzae* protein D. CRM197 is used in PREVNAR™. A 13-valent mixture may use CRM197 as the carrier protein for each of the 13 conjugates, and CRM197 may be present at about 55-60µg/ml.

Where a composition includes conjugates from more than one pneumococcal serotype, it is possible to use the same carrier protein for each separate conjugate, or to use different carrier proteins. In both cases, though, a mixture of different conjugates will usually be formed by preparing each serotype conjugate separately, and then mixing them to form a mixture of separate conjugates. Reference 30 describes potential advantages when using different carrier proteins in multivalent pneumococcal conjugate vaccines, but the PREVNAR™ product successfully uses the same carrier for each of seven different serotypes.

A carrier protein may be covalently conjugated to a pneumococcal saccharide directly or via a linker. Various linkers are known. For example, attachment may be via a carbonyl, which may be formed by reaction of a free hydroxyl group of a modified saccharide with CDI [31,32] followed by reaction with a protein to form a carbamate linkage. Carbodiimide condensation can be used [33]. An adipic acid linker can be used, which may be formed by coupling a free -NH<sub>2</sub> group (*e.g.* introduced to a saccharide by amination) with adipic acid (using, for example, diimide activation), and then coupling a protein to the resulting saccharide-adipic acid intermediate [34,35]. Other linkers include β-propionamido [36], nitrophenyl-ethylamine [37], haloacyl halides [38], glycosidic linkages [39], 6-aminocaproic acid [40], N-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) [41], adipic acid dihydrazide ADH [42], C<sub>4</sub> to C<sub>12</sub> moieties [43], *etc.*

Conjugation via reductive amination can be used. The saccharide may first be oxidised with periodate to introduce an aldehyde group, which can then form a direct covalent linkage to a carrier protein via reductive amination *e.g.* to the ε-amino group of a lysine. If the saccharide includes multiple aldehyde groups per molecule then this linkage technique can lead to a cross-linked product,



where multiple aldehydes react with multiple carrier amines. This cross-linking conjugation technique is particularly useful for at least pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.

A pneumococcal saccharide may comprise a full-length intact saccharide as prepared from pneumococcus, and/or may comprise fragments of full-length saccharides *i.e.* the saccharides may be shorter than the native capsular saccharides seen in bacteria. The saccharides may thus be depolymerised, with depolymerisation occurring during or after saccharide purification but before conjugation. Depolymerisation reduces the chain length of the saccharides. Depolymerisation can be used in order to provide an optimum chain length for immunogenicity and/or to reduce chain length for physical manageability of the saccharides. Where more than one pneumococcal serotype is used then it is possible to use intact saccharides for each serotype, fragments for each serotype, or to use intact saccharides for some serotypes and fragments for other serotypes.

Where a composition includes saccharide from any of serotypes 4, 6B, 9V, 14, 19F and 23F, these saccharides are preferably intact. In contrast, where a composition includes saccharide from serotype 18C, this saccharide is preferably depolymerised.

A serotype 3 saccharide may also be depolymerised, For instance, a serotype 3 saccharide can be subjected to acid hydrolysis for depolymerisation [7] *e.g.* using acetic acid. The resulting fragments may then be oxidised for activation (*e.g.* periodate oxidation, maybe in the presence of bivalent cations *e.g.* with  $MgCl_2$ ), conjugated to a carrier (*e.g.* CRM197) under reducing conditions (*e.g.* using sodium cyanoborohydride), and then (optionally) any unreacted aldehydes in the saccharide can be capped (*e.g.* using sodium borohydride) [7]. Conjugation may be performed on lyophilized material *e.g.* after co-lyophilizing activated saccharide and carrier.

A serotype 1 saccharide may be at least partially de-O-acetylated *e.g.* achieved by alkaline pH buffer treatment [8] such as by using a bicarbonate/carbonate buffer. Such (partially) de-O-acetylated saccharides can be oxidised for activation (*e.g.* periodate oxidation), conjugated to a carrier (*e.g.* CRM197) under reducing conditions (*e.g.* using sodium cyanoborohydride), and then (optionally) any unreacted aldehydes in the saccharide can be capped (*e.g.* using sodium borohydride) [8]. Conjugation may be performed on lyophilized material *e.g.* after co-lyophilizing activated saccharide and carrier.

A serotype 19A saccharide may be oxidised for activation (*e.g.* periodate oxidation), conjugated to a carrier (*e.g.* CRM197) in DMSO under reducing conditions, and then (optionally) any unreacted aldehydes in the saccharide can be capped (*e.g.* using sodium borohydride) [44]. Conjugation may be performed on lyophilized material *e.g.* after co-lyophilizing activated saccharide and carrier.

One or more pneumococcal capsular saccharide conjugates may be present in lyophilised form.

Pneumococcal conjugates can ideally elicit anticapsular antibodies that bind to the relevant saccharide *e.g.* elicit an anti-saccharide antibody level  $\geq 0.20\mu g/mL$  [45]. The antibodies may be



evaluated by enzyme immunoassay (EIA) and/or measurement of opsonophagocytic activity (OPA). The EIA method has been extensively validated and there is a link between antibody concentration and vaccine efficacy.

***Meningococcal factor H binding protein(s)***

5 Compositions of the invention include at least one meningococcal factor H binding protein (fHBP).

The fHBP antigen has been characterised in detail. It has also been called protein '741' [SEQ IDs 2535 & 2536 in ref. 56], 'NMB1870', 'GNA1870' [refs. 46-48], 'P2086', 'LP2086' or 'ORF2086' [49-51]. It is naturally a lipoprotein and is expressed across all meningococcal serogroups. The structure of fHbp's C-terminal immunodominant domain ('fHbpC') has been determined by NMR  
10 [52]. This part of the protein forms an eight-stranded  $\beta$ -barrel, whose strands are connected by loops of variable lengths. The barrel is preceded by a short  $\alpha$ -helix and by a flexible N-terminal tail.

The fHBP antigen falls into three distinct variants [53] and it has been found that serum raised against a given family is bactericidal within the same family, but is not active against strains which express one of the other two families *i.e.* there is intra-family cross-protection, but not inter-family  
15 cross-protection. Compositions of the invention can include a single fHBP variant, but advantageously include fHBP from two or three of the variants.

Where a composition comprises a single fHBP variant, it may include one of the following:

- (a) a first polypeptide comprising a first amino acid sequence, where the first amino acid sequence comprises an amino acid sequence (i) having at least  $a\%$  sequence identity to SEQ ID NO: 1 and/or (ii) consisting of a fragment of at least  $x$  contiguous amino acids from SEQ ID NO: 1;  
20
- (b) a second polypeptide, comprising a second amino acid sequence, where the second amino acid sequence comprises an amino acid sequence (i) having at least  $b\%$  sequence identity to SEQ ID NO: 2 and/or (ii) consisting of a fragment of at least  $y$  contiguous amino acids from SEQ ID NO: 2;
- (c) a third polypeptide, comprising a third amino acid sequence, where the third amino acid  
25 sequence comprises an amino acid sequence (i) having at least  $c\%$  sequence identity to SEQ ID NO: 3 and/or (ii) consisting of a fragment of at least  $z$  contiguous amino acids from SEQ ID NO: 3.

Where a composition comprises two different meningococcal fHBP antigens, it may include a combination of: (i) a first and second polypeptide as defined above; (ii) a first and third polypeptide as defined above; or (iii) a second and third polypeptide as defined above. A combination of a first  
30 and third polypeptide is preferred.

Where a composition comprises two different meningococcal fHBP antigens, although these may share some sequences in common, the first, second and third polypeptides have different fHBP amino acid sequences.

A polypeptide comprising the first amino acid sequence will, when administered to a subject, elicit  
35 an antibody response comprising antibodies that bind to the wild-type meningococcus protein having



nascent amino acid sequence SEQ ID NO: 60 (MC58). In some embodiments some or all of these antibodies do not bind to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 61 or to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 62.

5 A polypeptide comprising the second amino acid sequence will, when administered to a subject, elicit an antibody response comprising antibodies that bind to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 61 (2996). In some embodiments some or all of these antibodies do not bind to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 60 or to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 62.

A polypeptide comprising the third amino acid sequence will, when administered to a subject, elicit an antibody response comprising antibodies that bind to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 62 (M1239). In some embodiments some or all of these antibodies do not bind to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 60 or to the wild-type meningococcus protein having nascent amino acid sequence SEQ ID NO: 61.

In some embodiments the fragment of at least  $x$  contiguous amino acids from SEQ ID NO: 1 is not also present within SEQ ID NO: 2 or within SEQ ID NO: 3. Similarly, the fragment of at least  $y$  contiguous amino acids from SEQ ID NO: 2 might not also be present within SEQ ID NO: 1 or within SEQ ID NO: 3. Similarly, the fragment of at least  $z$  contiguous amino acids from SEQ ID NO: 3 might not also be present within SEQ ID NO: 1 or within SEQ ID NO: 2. In some embodiments, when said fragment from one of SEQ ID NOs: 1 to 3 is aligned as a contiguous sequence against the other two SEQ ID NOs, the identity between the fragment and each of the other two SEQ ID NOs is less than 75% *e.g.* less than 70%, less than 65%, less than 60%, *etc.*

25 The value of  $a$  is at least 80 *e.g.* 82, 84, 86, 88, 90, 92, 94, 95, 96, 97, 98, 99 or more. The value of  $b$  is at least 80 *e.g.* 82, 84, 86, 88, 90, 92, 94, 95, 96, 97, 98, 99 or more. The value of  $c$  is at least 80 *e.g.* 82, 84, 86, 88, 90, 92, 94, 95, 96, 97, 98, 99 or more. The values of  $a$ ,  $b$  and  $c$  may be the same or different. In some embodiments,  $a$ ,  $b$  and  $c$  are identical.

The value of  $x$  is at least 7 *e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250). The value of  $y$  is at least 7 *e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250). The value of  $z$  is at least 7 *e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250). The values of  $x$ ,  $y$  and  $z$  may be the same or different. In some embodiments,  $x$ ,  $y$  and  $z$  are identical.



Fragments preferably comprise an epitope from the respective SEQ ID NO: sequence. Other useful fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of the respective SEQ ID NO: while retaining at least one epitope thereof.

5 Amino acid sequences used with the invention may, compared to SEQ ID NOs: 1 2 or 3, include one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) conservative amino acid replacements *i.e.* replacements of one amino acid with another which has a related side chain. Genetically-encoded amino acids are generally divided into four families: (1) acidic *i.e.* aspartate, glutamate; (2) basic *i.e.* lysine, arginine, histidine; (3) non-polar *i.e.* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, 10 tryptophan; and (4) uncharged polar *i.e.* glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In general, substitution of single amino acids within these families does not have a major effect on the biological activity. The polypeptides may have one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) single amino acid deletions relative to a reference sequence. The polypeptides may also include 15 one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) insertions (*e.g.* each of 1, 2, 3, 4 or 5 amino acids) relative to a reference sequence.

A useful first amino acid sequence has at least 85% identity (*e.g.* >95% or 100%) to SEQ ID NO: 1. Another useful first amino acid sequence has at least 95% identity (*e.g.* >98% or 100%) to SEQ ID NO: 66. Another useful first amino acid sequence has at least 95% identity (*e.g.* >98% or 100%) to 20 SEQ ID NO: 67.

A useful third amino acid sequence has at least 85% identity (*e.g.* >95% or 100%) to SEQ ID NO: 3. Another useful third amino acid sequence has at least 95% identity (*e.g.* >98% or 100%) to SEQ ID NO: 68.

Combinations comprising a mixture of first and third sequences based around SEQ ID NOs: 66 and 25 68 (or their close variants) are particularly useful. Another useful combination comprises a mixture of first and third sequences based around a mixture of SEQ ID NOs: 67 and 68 (or their close variants). Thus a composition may comprise a polypeptide comprising amino acid sequence SEQ ID NO: 63 and a polypeptide comprising amino acid sequence SEQ ID NO: 65.

In some embodiments fHBP polypeptide(s) are lipidated *e.g.* at a N-terminus cysteine. In other 30 embodiments, however, fHBP polypeptide(s) are not lipidated. For lipidated fHBPs, lipids attached to cysteines will usually include palmitoyl residues *e.g.* as tripalmitoyl-S-glyceryl-cysteine (Pam3Cys), dipalmitoyl-S-glyceryl cysteine (Pam2Cys), N-acetyl (dipalmitoyl-S-glyceryl cysteine), *etc.* Examples of mature lipidated fHBP sequences are SEQ ID NO: 63 (including SEQ ID NO: 66), SEQ ID NO: 64 (including SEQ ID NO: 67), and SEQ ID NO: 65 (including SEQ ID NO: 68).

35 Administration of a fHBP will preferably elicit antibodies which can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 1, 2 or 3. Advantageous fHBP antigens

for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

The total amount of a fHBP polypeptide will usually be between 1 and 500µg/dose *e.g.* between 60 and 200µg/dose or between 120 and 500µg/ml.

## 5 ***Further antigen(s)***

In addition to conjugated pneumococcal capsular saccharide(s) and meningococcal factor H binding protein(s), compositions of the invention can include further antigens from meningococcus, pneumococcus and/or further pathogen(s).

### *Meningococcal polypeptide antigens*

0 In addition to including meningococcal fHBP polypeptide antigen(s), a composition may include one or more further meningococcal polypeptide antigen(s). Thus a composition may include a polypeptide antigen selected from the group consisting of: 287, NadA, NspA, HmbR, NhhA, App, and/or Omp85. These antigens will usefully be present as purified polypeptides *e.g.* recombinant polypeptides. The antigen will preferably elicit bactericidal anti-meningococcal antibodies after  
5 administration to a subject. In some embodiments of the invention, immunogenic compositions either include only one meningococcal PorA serosubtype or, preferably, include no meningococcal PorA outer membrane protein.

A composition of the invention may include a 287 antigen. The 287 antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB2132  
10 (GenBank accession number GI:7227388; SEQ ID NO: 9 herein). The sequences of 287 antigen from many strains have been published since then. For example, allelic forms of 287 can be seen in Figures 5 and 15 of reference 55, and in example 13 and figure 21 of reference 56 (SEQ IDs 3179 to 3184 therein). Various immunogenic fragments of the 287 antigen have also been reported. Preferred 287 antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more  
15 identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 9, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. The most useful 287 antigens of the invention can elicit antibodies which, after  
20 administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 9. Advantageous 287 antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include a NadA antigen. The NadA antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB1994  
25 (GenBank accession number GI:7227256; SEQ ID NO: 10 herein). The sequences of NadA antigen from many strains have been published since then, and the protein's activity as a Neisserial adhesin



has been well documented. Various immunogenic fragments of NadA have also been reported. Preferred NadA antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 10; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 10, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 10. The most useful NadA antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 10. Advantageous NadA antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject. SEQ ID NO: 6 is one such fragment.

A composition of the invention may include a NspA antigen. The NspA antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB0663 (GenBank accession number GI:7225888; SEQ ID NO: 11 herein). The antigen was previously known from references 57 & 58. The sequences of NspA antigen from many strains have been published since then. Various immunogenic fragments of NspA have also been reported. Preferred NspA antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 11, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 11. The most useful NspA antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 11. Advantageous NspA antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

Compositions of the invention may include a meningococcal HmbR antigen. The full-length HmbR sequence was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB1668 (SEQ ID NO: 7 herein). Reference 59 reports a HmbR sequence from a different strain (SEQ ID NO: 8 herein). SEQ ID NOs: 7 and 8 differ in length by 1 amino acid and have 94.2% identity. The invention can use a polypeptide that comprises a full-length HmbR sequence, but it will often use a polypeptide that comprises a partial HmbR sequence. Thus in some embodiments a HmbR sequence used according to the invention may comprise an amino acid sequence having at least *i*% sequence identity to SEQ ID NO: 7, where the value of *i* is 50, 60, 70, 80, 90, 95, 99 or more. In other embodiments a HmbR sequence used according to the invention may comprise a fragment of at least *j* consecutive amino acids from SEQ ID NO: 7, where the value of *j* is 7, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more. In other embodiments a HmbR sequence used according to the invention may comprise an amino acid



sequence (i) having at least *i*% sequence identity to SEQ ID NO: 7 and/or (ii) comprising a fragment of at least *j* consecutive amino acids from SEQ ID NO: 7. Preferred fragments of *j* amino acids comprise an epitope from SEQ ID NO: 7. Such epitopes will usually comprise amino acids that are located on the surface of HmbR. Useful epitopes include those with amino acids involved in HmbR's binding to haemoglobin, as antibodies that bind to these epitopes can block the ability of a bacterium to bind to host haemoglobin. The topology of HmbR, and its critical functional residues, were investigated in reference 60. The most useful HmbR antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 7. Advantageous HmbR antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include a NhhA antigen. The NhhA antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB0992 (GenBank accession number GI:7226232; SEQ ID NO: 12 herein). The sequences of NhhA antigen from many strains have been published since *e.g.* refs 55 & 61, and various immunogenic fragments of NhhA have been reported. It is also known as Hsf. Preferred NhhA antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 12; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 12, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 12. The most useful NhhA antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 12. Advantageous NhhA antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include an App antigen. The App antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB1985 (GenBank accession number GI:7227246; SEQ ID NO: 13 herein). The sequences of App antigen from many strains have been published since then. Various immunogenic fragments of App have also been reported. Preferred App antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 13, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. The most useful App antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 13. Advantageous App antigens for use



with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include an Omp85 antigen. The Omp85 antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [54] as gene NMB0182 (GenBank accession number GI:7225401; SEQ ID NO: 14 herein). The sequences of Omp85 antigen from many strains have been published since then. Further information on Omp85 can be found in references 62 and 63. Various immunogenic fragments of Omp85 have also been reported. Preferred Omp85 antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 14; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 14, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 14. The most useful Omp85 antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 14. Advantageous Omp85 antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

#### Meningococcal lipooligosaccharide

In addition to including meningococcal fHBP polypeptide antigen(s), a composition may include one or more meningococcal lipooligosaccharide (LOS) antigen(s). Meningococcal LOS is a glucosamine-based phospholipid that is found in the outer monolayer of the outer membrane of the bacterium. It includes a lipid A portion and a core oligosaccharide region, with the lipid A portion acting as a hydrophobic anchor in the membrane. Heterogeneity within the oligosaccharide core generates structural and antigenic diversity among different meningococcal strains, which has been used to subdivide the strains into 12 immunotypes (L1 to L12). The invention may use LOS from any immunotype *e.g.* from L1, L2, L3, L4, L5, L6, L7 and/or L8.

The L2 and L3  $\alpha$ -chains naturally include lacto-N-neotetraose (LNnT). Where the invention uses LOS from a L2 or L3 immunotype this LNnT may be absent. This absence can be achieved conveniently by using mutant strains that are engineered to disrupt their ability to synthesise the LNnT tetrasaccharide within the  $\alpha$ -chain. It is known to achieve this goal by knockout of the enzymes that are responsible for the relevant biosynthetic additions [64,65]. For instance, knockout of the LgtB enzyme prevents addition of the terminal galactose of LNnT, as well as preventing downstream addition of the  $\alpha$ -chain's terminal sialic acid. Knockout of the LgtA enzyme prevents addition of the N-acetyl-glucosamine of LNnT, and also the downstream additions. LgtA knockout may be accompanied by LgtC knockout. Similarly, knockout of the LgtE and/or GalE enzyme prevents addition of internal galactose, and knockout of LgtF prevents addition of glucose to the Hep<sup>I</sup> residue. Any of these knockouts can be used, singly or in combination, to disrupt the LNnT

tetrasaccharide in a L2, L3, L4, L7 or L9 immunotype strain. Knockout of at least LgtB is preferred, as this provides a LOS that retains useful immunogenicity while removing the LNnT epitope.

In addition to, or in place of, mutations to disrupt the LNnT epitope, a knockout of the *galE* gene also provides a useful modified LOS, and a lipid A fatty transferase gene may similarly be knocked out [66]. At least one primary O-linked fatty acid may be removed from LOS [67]. LOS having a reduced number of secondary acyl chains per LOS molecule can also be used [68]. The LOS will typically include at least the GlcNAc-Hep<sub>2</sub>phosphoethanolamine-KDO<sub>2</sub>-Lipid A structure [69]. The LOS may include a GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc trisaccharide while lacking the LNnT tetrasaccharide.

LOS may be included in compositions of the invention in various forms. It may be used in purified form on its own. It may be conjugated to a carrier protein. When LOS is conjugated, conjugation may be via a lipid A portion in the LOS or by any other suitable moiety *e.g.* its KDO residues. If the lipid A moiety of LOS is absent then such alternative linking is required. Conjugation techniques for LOS are known from *e.g.* references 67, 69, 70, 71, *etc.* Useful carrier proteins for these conjugates are discussed above *e.g.* bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof.

The LOS may be from a strain (*e.g.* a genetically-engineered meningococcal strain) which has a fixed (*i.e.* not phase variable) LOS immunotype as described in reference 72. For example, L2 and L3 LOS immunotypes may be fixed. Such strains may have a rate of switching between immunotypes that is reduced by more than 2-fold (even >50-fold) relative to the original wild-type strain. Reference 72 discloses how this result can be achieved by modification of the *lgtA* and/or *lgtG* gene products.

LOS may be O-acetylated on a GlcNAc residue attached to its Heptose II residue *e.g.* for L3 [73].

An immunogenic composition can include more than one type of LOS *e.g.* LOS from meningococcal immunotypes L2 and L3. For example, the LOS combinations disclosed in reference 74 may be used.

A LOS antigen can preferably elicit bactericidal anti-meningococcal antibodies after administration to a subject.

However, preferred compositions of the invention are free from meningococcal lipooligosaccharide.

#### Meningococcal capsular saccharide antigen(s)

In addition to including meningococcal fHBP polypeptide antigen(s), a composition may include one or more meningococcal capsular saccharide conjugates. A composition of the invention may include one or more conjugates of capsular saccharides from 1, 2, 3, or 4 of meningococcal serogroups A, C, W135 and Y *e.g.* A+C, A+W135, A+Y, C+W135, C+Y, W135+Y, A+C+W135, A+C+Y, A+W135+Y, A+C+W135+Y, *etc.* Compositions including a serogroup C saccharide or including saccharides from all four of serogroups A, C, W135 and Y are ideal.



The capsular saccharide of serogroup A meningococcus is a homopolymer of ( $\alpha 1 \rightarrow 6$ )-linked *N*-acetyl-D-mannosamine-1-phosphate, with partial O-acetylation in the C3 and C4 positions. Acetylation at the C-3 position can be 70-95%. Conditions used to purify the saccharide can result in de-O-acetylation (*e.g.* under basic conditions), but it is useful to retain OAc at this C-3 position. In  
 5 some embodiments, at least 50% (*e.g.* at least 60%, 70%, 80%, 90%, 95% or more) of the mannosamine residues in a serogroup A saccharides are O-acetylated at the C-3 position. Acetyl groups can be replaced with blocking groups to prevent hydrolysis [75], and such modified saccharides are still serogroup A saccharides within the meaning of the invention.

The serogroup C capsular saccharide is a homopolymer of ( $\alpha 2 \rightarrow 9$ )-linked sialic acid (*N*-acetyl  
 10 neuraminic acid, or 'NeuNAc'). The saccharide structure is written as  $\rightarrow 9$ -Neu *p* NAc 7/8 OAc- ( $\alpha 2 \rightarrow$  . Most serogroup C strains have O-acetyl groups at C-7 and/or C-8 of the sialic acid residues, but about 15% of clinical isolates lack these O-acetyl groups [76,77]. The presence or absence of OAc groups generates unique epitopes, and the specificity of antibody binding to the saccharide may affect its bactericidal activity against O-acetylated (OAc+) and de-O-acetylated (OAc-) strains [78-  
 15 80]. Serogroup C saccharides used with the invention may be prepared from either OAc+ or OAc- strains. Licensed MenC conjugate vaccines include both OAc- (NEISVAC-C™) and OAc+ (MENJUGATE™ & MENINGITEC™) saccharides. In some embodiments, strains for production of serogroup C conjugates are OAc+ strains, *e.g.* of serotype 16, serosubtype P1.7a,1, *etc.*. Thus C:16:P1.7a,1 OAc+ strains may be used. OAc+ strains in serosubtype P1.1 are also useful, such as  
 20 the C11 strain.

The serogroup W135 saccharide is a polymer of sialic acid-galactose disaccharide units. Like the serogroup C saccharide, it has variable O-acetylation, but at sialic acid 7 and 9 positions [81]. The structure is written as:  $\rightarrow 4$ )-D-Neup5Ac(7/9OAc)- $\alpha$ -(2 $\rightarrow$ 6)-D-Gal- $\alpha$ -(1 $\rightarrow$  .

The serogroup Y saccharide is similar to the serogroup W135 saccharide, except that the  
 25 disaccharide repeating unit includes glucose instead of galactose. Like serogroup W135, it has variable O-acetylation at sialic acid 7 and 9 positions [81]. The serogroup Y structure is written as:  $\rightarrow 4$ )-D-Neup5Ac(7/9OAc)- $\alpha$ -(2 $\rightarrow$ 6)-D-Glc- $\alpha$ -(1 $\rightarrow$  .

The saccharides used according to the invention may be O-acetylated as described above (*e.g.* with the same O-acetylation pattern as seen in native capsular saccharides), or they may be partially or  
 30 totally de-O-acetylated at one or more positions of the saccharide rings, or they may be hyper-O-acetylated relative to the native capsular saccharides.

The saccharide moieties in conjugates may comprise full-length saccharides as prepared from meningococci, and/or may comprise fragments of full-length saccharides *i.e.* the saccharides may be shorter than the native capsular saccharides seen in bacteria. The saccharides may thus be  
 35 depolymerised, with depolymerisation occurring during or after saccharide purification but before conjugation. Depolymerisation reduces the chain length of the saccharides. One depolymerisation

method involves the use of hydrogen peroxide. Hydrogen peroxide is added to a saccharide (*e.g.* to give a final H<sub>2</sub>O<sub>2</sub> concentration of 1%), and the mixture is then incubated (*e.g.* at about 55°C) until a desired chain length reduction has been achieved. Another depolymerisation method involves acid hydrolysis. Other depolymerisation methods are known in the art. The saccharides used to prepare  
 5 conjugates for use according to the invention may be obtainable by any of these depolymerisation methods. Depolymerisation can be used in order to provide an optimum chain length for immunogenicity and/or to reduce chain length for physical manageability of the saccharides. In some embodiments, saccharides have the following range of average degrees of polymerisation (Dp): A=10-20; C=12-22; W135=15-25; Y=15-25. In terms of molecular weight, rather than Dp, useful  
 10 ranges are, for all serogroups: <100kDa; 5kDa-75kDa; 7kDa-50kDa; 8kDa-35kDa; 12kDa-25kDa; 15kDa-22kDa.

In some embodiments, the average molecular weight for saccharides from each of meningococcal serogroups A, C, W135 and Y may be more than 50kDa *e.g.*  $\geq 75\text{kDa}$ ,  $\geq 100\text{kDa}$ ,  $\geq 110\text{kDa}$ ,  $\geq 120\text{kDa}$ ,  $\geq 130\text{kDa}$ , *etc.* [82], and even up to 1500kDa, in particular as determined by MALLS. For instance: a  
 15 MenA saccharide may be in the range 50-500kDa *e.g.* 60-80kDa; a MenC saccharide may be in the range 100-210kDa; a MenW135 saccharide may be in the range 60-190kDa *e.g.* 120-140kDa; and/or a MenY saccharide may be in the range 60-190kDa *e.g.* 150-160kDa.

The mass of meningococcal saccharide per serogroup in a composition will usually be between 1µg and 20µg *e.g.* between 2 and 10 µg per serogroup, or about 4µg or about 5µg or about 10µg. Where  
 20 conjugates from more than one serogroup are included then they may be present at substantially equal masses *e.g.* the mass of each serogroup's saccharide is within +10% of each other. As an alternative to an equal ratio, a double mass of serogroup A saccharide may be used. Thus a vaccine may include MenA saccharide at 10µg and MenC, W135 and Y saccharides at 5µg each.

Useful carrier proteins and linkage chemistries are discussed above. Useful carriers include  
 25 diphtheria toxoid, tetanus toxoid and CRM197.

Conjugates with a saccharide:protein ratio (w/w) of between 1:5 (*i.e.* excess protein) and 5:1 (*i.e.* excess saccharide) may be used *e.g.* ratios between 1:2 and 5:1 and ratios between 1:1.25 and 1:2.5. As described in reference 83, different meningococcal serogroup conjugates in a mixture can have different saccharide:protein ratios *e.g.* one may have a ratio of between 1:2 & 1:5, whereas another  
 30 has a ratio between 5:1 & 1:1.99.

As described in reference 84, a mixture can include one conjugate with direct saccharide/protein linkage and another conjugate with linkage via a linker. This arrangement applies particularly when using saccharide conjugates from different meningococcal serogroups *e.g.* MenA and MenC saccharides may be conjugated via a linker, whereas MenW135 and MenY saccharides may be  
 35 conjugated directly to a carrier protein.



*Pneumococcal polypeptide antigen(s)*

In addition to including conjugated pneumococcal capsular saccharide(s), a composition may include one or more pneumococcal polypeptide antigen(s). Thus a composition may include one or more of: (1) a spr0057 antigen; (2) a spr0286 antigen; (3) a spr0565 antigen; (4) a spr1098 antigen; (5) a spr1345 antigen; (6) a spr1416 antigen; (7) a spr1418 antigen; (8) a spr0867 antigen; (9) a spr1431 antigen; (10) a pneumolysin; (11) a spr2021 antigen; (12) a spr0096 antigen; (13) a spr1433 antigen; and/or (14) a spr1707 antigen.

A composition may include one or more of: (1) a PspA polypeptide; (2) a PsaA polypeptide; (3) a PspC polypeptide; (4) a LytA polypeptide; (5) a PhtA polypeptide; (6) a PhtA polypeptide; (7) a PhtA polypeptide; and/or (8) a PhtD polypeptide.

A composition may include a subunit of a pneumococcal pilus, such as RrgA, RrgB and/or RrgC.

A pneumococcal polypeptide antigen can preferably elicit protective antibodies after administration to a subject.

The original 'spr0057' sequence was annotated in reference 85 as 'Beta-N-acetyl-hexosaminidase precursor' (see GI:15902101). For reference purposes, the amino acid sequence of full length spr0057 as found in the R6 strain is given as SEQ ID NO: 18 herein. Preferred spr0057 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 18; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 18, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr0057 proteins include variants of SEQ ID NO: 18. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 18. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 18 while retaining at least one epitope of SEQ ID NO: 18. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 32, which omits the natural leader peptide and sortase recognition sequences.

The original 'spr0286' sequence was annotated in reference 85 as 'Hyaluronate lyase precursor' (see GI:15902330). For reference purposes, the amino acid sequence of full length spr0286 as found in the R6 strain is given as SEQ ID NO: 19 herein. Preferred spr0286 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 19; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 19, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr0286 proteins include variants of SEQ ID NO: 19. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 19. Other preferred fragments lack one or more amino

acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 19 while retaining at least one epitope of SEQ ID NO: 19. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 33, which omits the natural leader peptide and sortase recognition sequences. Other suitable fragments are SEQ ID NOs: 34 and 35.

The original 'spr0565' sequence was annotated in reference 85 as 'beta-galactosidase precursor' (see GI:15902609). For reference purposes, the amino acid sequence of full length spr0565 as found in the R6 strain is given as SEQ ID NO: 20 herein. Preferred spr0565 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 20; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 20, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr0565 proteins include variants of SEQ ID NO: 20 (*e.g.* SEQ ID NO: 66; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 20. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 20 while retaining at least one epitope of SEQ ID NO: 20. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 36, which omits the natural leader peptide and sortase recognition sequences. Other suitable fragments are SEQ ID NOs: 37 and 38.

A variant form of spr0565 is SEQ ID NO: 39 herein. The use of this variant form for immunisation is reported in reference 86 (SEQ ID NO: 178 therein). Useful spr0565 polypeptides may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 39, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These polypeptides include variants of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39 while retaining at least one epitope of SEQ ID NO: 39. Other fragments omit one or more protein domains. Immunogenic fragments of SEQ ID NO: 39 are identified in table 1 of reference 86.

Because spr0565 is naturally a long polypeptide (>2000 aa) it can be more convenient to express fragments. Thus a suitable form of spr0565 for use with the invention may be less than 1500 amino acids long (*e.g.* <1400, <1300, <1200, <1100, *etc.*). Such short forms of spr0565 include 'spr0565A' (SEQ ID NO: 37) and 'spr0565B' (SEQ ID NO: 38).



The original 'spr1098' sequence was annotated in reference 85 as 'Sortase' (see GI:15903141). For reference purposes, the amino acid sequence of full length spr1098 as found in the R6 strain is given as SEQ ID NO: 21 herein. Preferred spr1098 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 21; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 21, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1098 proteins include variants of SEQ ID NO: 21. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 21. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 21 while retaining at least one epitope of SEQ ID NO: 21. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 40, which omits the natural leader peptide sequence.

The original 'spr1345' sequence was annotated in reference 85 as 'hypothetical protein' (see GI:15903388). For reference purposes, the amino acid sequence of full length spr1345 as found in the R6 strain is given as SEQ ID NO: 22 herein. Preferred spr1345 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 22; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 22, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1345 proteins include variants of SEQ ID NO: 22. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 22. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 22 while retaining at least one epitope of SEQ ID NO: 22. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 41, which omits the natural leader peptide and sortase recognition sequences.

The original 'spr1416' sequence was annotated in reference 85 as 'hypothetical protein' (see GI:15903459). For reference purposes, the amino acid sequence of full length spr1416 as found in the R6 strain is given as SEQ ID NO: 23 herein. Preferred spr1416 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 23; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 23, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1416 proteins include variants of SEQ ID NO: 23. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 23. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more

amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 23 while retaining at least one epitope of SEQ ID NO: 23. Other fragments omit one or more protein domains.

The original 'spr1418' sequence was annotated in reference 85 as 'hypothetical protein' (see  
 5 GI:15903461). For reference purposes, the amino acid sequence of full length spr1418 as found in the R6 strain is given as SEQ ID NO: 24 herein. Preferred spr1418 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 24; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO:  
 10 24, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1418 proteins include variants of SEQ ID NO: 24. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 24. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO:  
 15 24 while retaining at least one epitope of SEQ ID NO: 24. Other fragments omit one or more protein domains.

The original 'spr0867' sequence was annotated in reference 85 as 'Endo-beta-N-acetylglucosaminidase' (see GI:15902911). For reference purposes, the amino acid sequence of full length spr0867 as found in the R6 strain is given as SEQ ID NO: 25 herein. Preferred spr0867  
 20 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 25; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 25, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr0867 proteins include variants of SEQ ID  
 25 NO: 25. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 25. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 25 while retaining at least one epitope of SEQ ID NO: 25. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 42, which omits  
 30 the natural leader peptide sequence.

The original 'spr1431' sequence was annotated in reference 85 as '1,4-beta-N-acetylmuramidase' (see GI:15903474). It is also known as 'LytC', and its use for immunisation is reported in reference 100. For reference purposes, the amino acid sequence of full length spr1431 as found in the R6 strain is given as SEQ ID NO: 26 herein. Preferred spr1431 polypeptides for use with the invention comprise  
 35 an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 26; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 26, wherein 'n' is 7



or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1431 proteins include variants of SEQ ID NO: 26. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 26. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 26 while retaining at least one epitope of SEQ ID NO: 26. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 43, which omits the natural leader peptide sequence.

The amino acid sequence of full length pneumolysin as found in the R6 strain is given as SEQ ID NO: 27 herein. Preferred pneumolysin polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 27; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 27, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These pneumolysin proteins include variants of SEQ ID NO: 27. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 27. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 27 while retaining at least one epitope of SEQ ID NO: 27. Other fragments omit one or more protein domains. Mutant forms of pneumolysin for vaccination use are known in the art [25, 87-92], and these mutant forms may be used with the invention. Detoxification can be achieved by C-terminal truncation (*e.g.* see ref. 93) *e.g.* deleting 34 amino acids, 45 amino acids, 7 amino acids [94], *etc.* Further mutations, numbered according to SEQ ID NO: 27, include Pro325→Leu (*e.g.* SEQ ID NO: 44) and/or Trp433→Phe (*e.g.* SEQ ID NO: 45). These mutations may be combined with C-terminal truncations *e.g.* to combine a Pro325→Leu mutation with a 7-mer truncation (*e.g.* SEQ ID NO: 46).

The original 'spr2021' sequence was annotated in reference 85 as 'General stress protein GSP-781' (see GI:15904062). For reference purposes, the amino acid sequence of full length spr2021 as found in the R6 strain is given as SEQ ID NO: 28 herein. Preferred spr2021 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 28; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 28, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr2021 proteins include variants of SEQ ID NO: 28. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 28. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 28 while retaining at least one epitope of SEQ ID NO: 28. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 47, which omits the natural leader peptide sequence.



Reference 86 annotates spr2021 as a secreted 45kDa protein with homology to GbpB and discloses its use as an immunogen (SEQ ID NO: 243 therein; SP2216). Immunogenic fragments of spr2021 are identified in table 1 of reference 86 (page 73). Another useful fragment of spr2021 is disclosed as SEQ ID NO: 1 of reference 95 (amino acids 28-278 of SEQ ID NO: 28 herein).

5 The original 'spr0096' sequence was annotated in reference 85 as 'hypothetical protein' (see GI:15902140). For reference purposes, the amino acid sequence of full length spr0096 as found in the R6 strain is given as SEQ ID NO: 29 herein. Preferred spr0096 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ  
10 ID NO: 29; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 29, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr0096 proteins include variants of SEQ ID NO: 29 (*e.g.* SEQ ID NO: 40; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 29. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the  
15 C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 29 while retaining at least one epitope of SEQ ID NO: 29. Other fragments omit one or more protein domains.

A variant form of spr0096, with an insert near its C-terminus relative to SEQ ID NO: 29, is SEQ ID NO: 48 herein. The use of this variant for immunisation is reported in reference 86 (SEQ ID NO: 150  
20 therein), where it is annotated as a LysM domain protein. Thus a spr0096 for use with the invention may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 48; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 48, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150,  
25 200, 250 or more). These polypeptides include variants of SEQ ID NO: 48. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 48. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 48 while retaining at least one epitope of SEQ ID NO: 48. Other fragments omit one or more protein  
30 domains. Immunogenic fragments of SEQ ID NO: 48 are identified in table 1 of reference 86.

A spr0096 polypeptide may be used in the form of a dimer *e.g.* a homodimer.

The original 'spr1433' sequence was annotated in reference 85 as 'hypothetical protein' (see GI:15903476). For reference purposes, the amino acid sequence of full length spr1433 as found in the R6 strain is given as SEQ ID NO: 30 herein. Preferred spr1433 polypeptides for use with the  
35 invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ



ID NO: 30; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 30, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1433 proteins include variants of SEQ ID NO: 30. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 30. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 30 while retaining at least one epitope of SEQ ID NO: 30. Other fragments omit one or more protein domains.

The original 'spr1707' sequence was annotated in reference 85 as 'ABC transporter substrate-binding protein - oligopeptide transport' (see GI:15903749). For reference purposes, the amino acid sequence of full length spr1707 as found in the R6 strain is given as SEQ ID NO: 31 herein. Preferred spr1707 polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 31; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 31, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1707 proteins include variants of SEQ ID NO: 31 (*e.g.* SEQ ID NO: 100; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 31. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 31 while retaining at least one epitope of SEQ ID NO: 31. Other fragments omit one or more protein domains.

A variant form of spr1707, differing from SEQ ID NO: 31 by 4 amino acids, is SEQ ID NO: 49 herein. The use of SEQ ID NO: 49 for immunisation is reported in reference 86 (SEQ ID NO: 220 therein). Thus a spr1707 polypeptide for use with the invention may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 49; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 49, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These polypeptides include variants of SEQ ID NO: 49. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 49. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 49 while retaining at least one epitope of SEQ ID NO: 49. Other fragments omit one or more protein domains. Immunogenic fragments of SEQ ID NO: 49 are identified in table 1 of reference 86.

PspA is the Pneumococcal surface protein A. For reference purposes, the amino acid sequence of full length PspA is SEQ ID NO: 50 herein. In the R6 genome PspA is spr0121 [85]. Preferred PspA polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more

identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 50; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 50, wherein 'n' is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PspA proteins include variants of SEQ ID NO: 50. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 50. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 50 while retaining at least one epitope of SEQ ID NO: 50. Other fragments omit one or more protein domains. The use of PspA for immunisation is reported *inter alia* in reference 96. It can advantageously be administered in combination with PspC.

PsaA is the Pneumococcal surface adhesin. For reference purposes, the amino acid sequence of full length PsaA is SEQ ID NO: 51 herein. Preferred PsaA polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 51; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 51, wherein 'n' is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PsaA proteins include variants of SEQ ID NO: 51. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 51. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 51 while retaining at least one epitope of SEQ ID NO: 51. Other fragments omit one or more protein domains. A useful fragment of PsaA is disclosed as SEQ ID NO: 3 in reference 95 (corresponding to amino acids 21-519 of SEQ ID NO: 51 herein). The use of PsaA for immunisation is reported in reference 97. It can be used in combination with PspA and/or PspC.

PspC is the pneumococcal surface protein C [98] and is also known as choline-binding protein A (CbpA). Its use for immunisation is reported in references 99 and 100. In the R6 strain it is spr1995 and, for reference, the amino acid sequence of full length spr1995 is SEQ ID NO: 52 herein. Preferred PspC polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 52; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 52, wherein 'n' is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spr1995 proteins include variants of SEQ ID NO: 52 (e.g. SEQ ID NO: 27; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 52. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 52



while retaining at least one epitope of SEQ ID NO: 52. Other fragments omit one or more protein domains.

A variant of PspC is known as 'Hic'. It is similar to PspC, as shown in Figure 1 of reference 101, where it is reported to bind to factor H (fH). For reference purposes, the amino acid sequence of full length Hic is SEQ ID NO: 53 herein. A Hic protein may be used with the invention in addition to or in place of a PspC polypeptide. Preferred Hic polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 53; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 53, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hic proteins include variants of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53 while retaining at least one epitope of SEQ ID NO: 53. Other fragments omit one or more protein domains.

PspC and/or Hic can advantageously be used in combination with PspA and/or PsaA.

LytA is the N-acetylmuramoyl-L-alanine amidase (autolysin). For reference purposes, the amino acid sequence of full length LytA is SEQ ID NO: 54 herein. In the R6 genome LytA is spr1754 [85]. Preferred LytA polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 54; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 54, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These LytA proteins include variants of SEQ ID NO: 54 (*e.g.* GI:18568354). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 54. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 54 while retaining at least one epitope of SEQ ID NO: 54. Other fragments omit one or more protein domains. The use of LytA for immunisation is reported in reference 102, particularly in a form comprising the LytA choline binding domain fused to a heterologous promiscuous T helper epitope.

PhtA is the Pneumococcal histidine triad protein A. For reference purposes, the amino acid sequence of full length PhtA precursor is SEQ ID NO: 55 herein. In the R6 genome PhtA is spr1061 [85]. Preferred PhtA polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 55; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 55, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16,



18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PhtA proteins include variants of SEQ ID NO: 55. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 55. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 55 while retaining at least one epitope of SEQ ID NO: 55. Other fragments omit one or more protein domains. The use of PhtA for immunisation is reported in references 103 and 104.

PhtB is the pneumococcal histidine triad protein B. For reference purposes, the amino acid sequence of full length PhtB precursor is SEQ ID NO: 56 herein. Xaa at residue 578 can be Lysine. Preferred PhtB polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 56; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 56, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PhtB proteins include variants of SEQ ID NO: 56. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 56. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 56 while retaining at least one epitope of SEQ ID NO: 56. Other fragments omit one or more protein domains. The use of PhtB for immunisation is reported in references 103, 104 and 105.

PhtD is the Pneumococcal histidine triad protein D. For reference purposes, the amino acid sequence of full length PhtD precursor is SEQ ID NO: 57 herein. In the R6 genome PhtD is spr0907 [85]. Preferred PhtD polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 57; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 57, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PhtD proteins include variants of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 57 while retaining at least one epitope of SEQ ID NO: 57. Other fragments omit one or more protein domains. The use of PhtD for immunisation is reported in references 103, 104 and 106.

PhtE is the Pneumococcal histidine triad protein E. For reference purposes, the amino acid sequence of full length PhtE precursor is SEQ ID NO: 58 herein. In the R6 genome PhtE is spr0908 [85]. Preferred PhtE polypeptides for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,



96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 58; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 58, wherein 'n' is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PhtE proteins include variants of SEQ ID NO: 58. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 58.

5 Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 58 while retaining at least one epitope of SEQ ID NO: 58. Other fragments omit one or more protein domains. The use of PhtE for immunisation is reported in references 103 and 104.

#### 10 Further antigens from other pathogen(s)

In addition to conjugated pneumococcal capsular saccharide(s) and meningococcal factor H binding protein(s), compositions of the invention can include antigen(s) from further pathogen(s).

For example, the composition may comprise one or more of the following further antigen(s):

- an antigen from hepatitis B virus, such as the surface antigen HBsAg.
- 15 – an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3.
- a diphtheria antigen, such as a diphtheria toxoid.
- a tetanus antigen, such as a tetanus toxoid.
- 20 – a saccharide antigen from *Haemophilus influenzae* B (Hib), typically conjugated.
- inactivated poliovirus antigen(s).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. DTP combinations are thus preferred.

#### **Hybrid polypeptides**

A meningococcal factor H binding protein may be present in the composition as an individual separate polypeptide, or it may be present as part of a 'hybrid' polypeptide *i.e.* where at least two (e.g. 2, 3, 4, 5, or more) antigens are expressed as a single polypeptide chain, as disclosed for  
30 meningococcal antigens in reference 107. This hybrid polypeptide approach can also be used for any additional meningococcal polypeptide(s) and/or pneumococcal polypeptides.

Hybrid polypeptides offer two main advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be  
35 employed in order to produce two polypeptides which are both antigenically useful.

A hybrid polypeptide may comprise two or more meningococcal and/or pneumococcal polypeptide sequences as disclosed above. Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten antigens are useful. In particular, hybrids consisting of amino acid sequences from two, three, four, or five antigens are preferred, such as two or three antigens.

- 5 Hybrid polypeptides can be represented by the formula  $\text{NH}_2\text{-A-}\{-\text{X-L-}\}_n\text{-B-COOH}$ , wherein: X is an amino acid sequence of an alternative meningococcal or pneumococcal antigen, as described above; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence;  $n$  is an integer of 2 or more (*e.g.* 2, 3, 4, 5, 6, *etc.*). Usually  $n$  is 2 or 3.
- 10 In some embodiments at least one -X- moiety is a fHBP. In other embodiments, at least two -X- moieties are a fHBP *e.g.* different fHBP variants. In other embodiments a fHBP may be present as an individual polypeptide and at least one -X- moiety in a hybrid polypeptide is a non-fHBP meningococcal polypeptide and/or is a pneumococcal polypeptide.

15 If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of  $X_1$  will be retained, but the leader peptides of  $X_2 \dots X_n$  will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of  $X_1$  as moiety -A-.

For each  $n$  instances of  $\{-\text{X-L-}\}$ , linker amino acid sequence -L- may be present or absent. For  
 20 instance, when  $n=2$  the hybrid may be  $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-L}_2\text{-COOH}$ ,  $\text{NH}_2\text{-X}_1\text{-X}_2\text{-COOH}$ ,  $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-COOH}$ ,  $\text{NH}_2\text{-X}_1\text{-X}_2\text{-L}_2\text{-COOH}$ , *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising  $\text{Gly}_n$  where  $n = 2, 3, 4, 5, 6, 7, 8, 9, 10$  or more), and histidine tags (*i.e.*  $\text{His}_n$  where  $n = 3, 4, 5, 6, 7, 8, 9, 10$  or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A  
 25 useful linker is GSGGGG (SEQ ID NO:15) or GSGSGGGG (SEQ ID NO:16), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the  $(\text{Gly})_4$  tetrapeptide being a typical poly-glycine linker. Other suitable linkers, particularly for use as the final  $L_n$  are a Leu-Glu dipeptide or SEQ ID NO: 59.

30 -A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.*  $\text{His}_n$  where  $n = 3, 4, 5, 6, 7, 8, 9, 10$  or more). Other suitable N-terminal amino acid  
 35 sequences will be apparent to those skilled in the art. If  $X_1$  lacks its own N-terminus methionine, -A-



is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine *e.g.* Met-Ala-Ser, or a single Met residue.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His<sub>*n*</sub> where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more, such as SEQ ID NO: 17), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

- 10 A particularly useful combination of meningococcal polypeptide antigens, including a fHBP antigen, is disclosed in references 107 and 108, and a composition of the invention may thus include a conjugated pneumococcal capsular saccharide, a fHBP, and 1, 2, 3 or 4 of: (1) a 'NadA' protein; (2) a '936' protein; (3) a '953' protein; and (4) a '287' protein. For instance, a composition may include a conjugated pneumococcal capsular saccharide and: (i) a first polypeptide having amino acid  
15 sequence SEQ ID NO: 4; (ii) a second polypeptide having amino acid sequence SEQ ID NO: 5; and (iii) a third polypeptide having amino acid sequence SEQ ID NO: 6.

### ***Adjuvant(s)***

Compositions of the invention may include an immunological adjuvant. Thus, for example, they may include an aluminium salt adjuvant or an oil-in-water emulsion (*e.g.* a squalene-in-water emulsion).

- 20 Other adjuvants may also be used.

Suitable aluminium salts include hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), (*e.g.* see chapters 8 & 9 of ref. 109), or mixtures thereof. The salts can take any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*). The concentration of Al<sup>+++</sup> in a composition for administration to a patient is preferably less than 5mg/ml *e.g.* ≤4 mg/ml, ≤3 mg/ml, ≤2 mg/ml, ≤1 mg/ml, *etc.* A preferred range is between 0.3 and 1mg/ml. A maximum of  
25 0.85mg/dose is preferred.

A preferred aluminium salt adjuvant for use with the invention is an aluminium phosphate. This adjuvant is compatible with both fHBP and the PREVNAR™ and SYNFLORIX™ pneumococcal conjugate vaccines. The adjuvants known as "aluminium phosphate" are typically aluminium  
30 hydroxyphosphates, often also containing a small amount of sulfate (*i.e.* aluminium hydroxyphosphate sulfate). They may be obtained by precipitation, and the reaction conditions and concentrations during precipitation influence the degree of substitution of phosphate for hydroxyl in the salt. Hydroxyphosphates generally have a PO<sub>4</sub>/Al molar ratio between 0.3 and 1.2. Hydroxyphosphates can be distinguished from strict AlPO<sub>4</sub> by the presence of hydroxyl groups. For  
35 example, an IR spectrum band at 3164cm<sup>-1</sup> (*e.g.* when heated to 200°C) indicates the presence of structural hydroxyls.



The P/Al molar ratio of an aluminium phosphate adjuvant will generally be between 0.3 and 1.2, preferably between 0.8 and 1.2, or between 0.85 and 1.0, and more preferably about 0.9. A P/Al molar ratio of at least 0.5 can provide an adjuvant with better aging properties.

The aluminium phosphate adjuvant will generally be amorphous (*i.e.* amorphous to X-rays). It will generally be particulate (*e.g.* plate-like morphology as seen in transmission electron micrographs). Typical diameters of the plates are 10-100nm, and these form aggregates sized 0.5-20 $\mu$ m (*e.g.* about 1-10 $\mu$ m). Adsorptive capacities of between 0.7-1.5 mg protein per mg Al<sup>+++</sup> at pH 7.4 have been reported for aluminium phosphate adjuvants.

A typical adjuvant is amorphous aluminium phosphate with P/Al molar ratio between 0.84 and 0.92, and this adjuvant may be included at 0.6mg Al<sup>3+</sup>/ml.

The concentration of Al<sup>+++</sup> in a composition for administration to a patient is preferably less than 5mg/ml *e.g.*  $\leq 4$  mg/ml,  $\leq 3$  mg/ml,  $\leq 2$  mg/ml,  $\leq 1$  mg/ml, *etc.* A preferred range is between 0.2 and 1mg/ml. A maximum Al<sup>+++</sup> concentration of 0.85mg/dose is preferred.

The point of zero charge (PZC) of aluminium phosphate is inversely related to the degree of substitution of phosphate for hydroxyl, and this degree of substitution can vary depending on reaction conditions and concentration of reactants used for preparing the salt by precipitation. PZC is also altered by changing the concentration of free phosphate ions in solution (more phosphate = more acidic PZC) or by adding a buffer such as a histidine buffer (makes PZC more basic). Aluminium phosphates used according to the invention will generally have a PZC of between 4.0 and 7.0, more preferably between 5.0 and 6.5 *e.g.* about 5.7.

The adjuvants known as "aluminium hydroxide" are typically aluminium oxyhydroxide salts, which are usually at least partially crystalline. Aluminium oxyhydroxide, which can be represented by the formula AlO(OH), can be distinguished from other aluminium compounds by infrared (IR) spectroscopy, in particular by the presence of an adsorption band at 1070cm<sup>-1</sup> and a strong shoulder at 3090-3100cm<sup>-1</sup> [chapter 9 of ref. 109]. Aluminium hydroxide adjuvants generally have a PZC of about 11.4.

In one embodiment of the invention, an adjuvant component includes a mixture of both an aluminium hydroxide and an aluminium phosphate. In this case there may be more aluminium phosphate than hydroxide *e.g.* a weight ratio of at least 2:1 *e.g.*  $\geq 5:1$ ,  $\geq 6:1$ ,  $\geq 7:1$ ,  $\geq 8:1$ ,  $\geq 9:1$ , *etc.* Most preferably, however, an adjuvant component does not include aluminium hydroxide. Thus a composition may include aluminium hydroxyphosphate but no aluminium oxyhydroxides.

In preferred compositions of the invention, fHBP is adsorbed to an aluminium phosphate adjuvant. At least 50% of total fHBP in the composition may be adsorbed *e.g.*  $>50\%$ ,  $>60\%$ ,  $>70\%$ ,  $>80\%$ ,  $>90\%$ ,  $>95\%$  or substantially 100%. The proportion of adsorbed fHBP can be controlled by altering salt concentration and/or pH during formulation *e.g.* in general, a higher NaCl concentration can



decrease fHBP's adsorption. To facilitate stable adsorption of fHBP three useful techniques may be used: (i) adsorption may take place at a pH which is equal to or below the adjuvant's PZC; (ii) a fHBP and adjuvant are selected such that the fHBP's pI and the adjuvant's PZC are both within the range of 5.0 to 7.0; and (iii) if the fHBP has an isoelectric point above the adjuvant's PZC then a buffer is added to bring the pH to within 1.2 pH units of the PZC.

Suspensions of aluminium salt(s) used to prepare compositions of the invention may contain a buffer (*e.g.* a phosphate or a histidine or a Tris buffer), but this is not always necessary. The suspensions are preferably sterile and pyrogen-free. A suspension may include free aqueous phosphate ions *e.g.* present at a concentration between 1.0 and 20 mM, preferably between 5 and 15 mM, and more preferably about 10 mM. The suspensions may also comprise sodium chloride.

As an alternative to aluminium salt(s), various oil-in-water emulsion adjuvants are known. These typically include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5µm in diameter, and may even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220nm are preferred as they can be subjected to filter sterilization.

The invention can be used with oils such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils include nuts, seeds and grains. Peanut oil, soybean oil, coconut oil, and olive oil, the most commonly available, exemplify the nut oils. Jojoba oil can be used *e.g.* obtained from the jojoba bean. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil and the like. In the grain group, corn oil is the most readily available, but the oil of other cereal grains such as wheat, oats, rye, rice, teff, triticale and the like may also be used. 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, while not occurring naturally in seed oils, may be prepared by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils. Fats and oils from mammalian milk are metabolizable and may therefore be used in the practice of this invention. The procedures for separation, purification, saponification and other means necessary for obtaining pure oils from animal sources are well known in the art. Most fish contain metabolizable oils which may be readily recovered. For example, cod liver oil, shark liver oils, and whale oil such as spermaceti exemplify several of the fish oils which may be used herein. A number of branched chain oils are synthesized biochemically in 5-carbon isoprene units and are generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoids known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, which is particularly preferred herein. Squalane, the saturated analog to squalene, is also a preferred oil. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Other preferred oils are the tocopherols. Mixtures of oils can be used.



Where a composition includes a tocopherol, any of the  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$  or  $\xi$  tocopherols can be used, but  $\alpha$ -tocopherols are preferred. The tocopherol can take several forms *e.g.* different salts and/or isomers. Salts include organic salts, such as succinate, acetate, nicotinate, *etc.* D- $\alpha$ -tocopherol and DL- $\alpha$ -tocopherol can both be used. A preferred  $\alpha$ -tocopherol is DL- $\alpha$ -tocopherol, and the preferred  
 5 salt of this tocopherol is the succinate.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Preferred surfactants of the invention have a HLB of at least 10, preferably at least 15, and more preferably at least 16. The invention can be used with surfactants including, but not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate  
 10 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); polyoxyethylene  
 15 fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. Preferred surfactants for including in the emulsion are Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100.

20 Mixtures of surfactants can be used *e.g.* Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another useful combination comprises laureth-9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween  
 25 80) 0.01 to 1%, in particular about 0.1 %; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1 %, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20 %, preferably 0.1 to 10 % and in particular 0.1 to 1 % or about 0.5%.

Specific oil-in-water emulsion adjuvants useful with the invention include, but are not limited to:

- 30 • A sub-micron emulsion of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. This adjuvant is known as 'MF59' [110-112], as described in more detail in Chapter 10 of ref. 109 and chapter 12 of ref. 113. The MF59 emulsion may include citrate ions *e.g.* 10mM  
 35 sodium citrate buffer.



- 5      • An emulsion of squalene, a tocopherol, and polysorbate 80 (Tween 80). The emulsion may include phosphate buffered saline. It may also include Span 85 (*e.g.* at 1%) and/or lecithin. These emulsions may have from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% Tween 80, and the weight ratio of squalene:tocopherol is preferably  $\leq 1$  as this provides a more stable emulsion. Squalene and Tween 80 may be present volume ratio of about 5:2 or at a weight ratio of about 11:5. One such emulsion can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90ml of this solution with a mixture of (5g of DL- $\alpha$ -tocopherol and 5ml squalene), then microfluidising the mixture. The resulting emulsion may have submicron oil droplets *e.g.* with an average diameter of between 100 and 250nm, preferably about 180nm. The emulsion may also include a 3-de-O-acylated monophosphoryl lipid A (3d-MPL). Another useful emulsion of this type may comprise, per human dose, 0.5-10 mg squalene, 0.5-11 mg tocopherol, and 0.1-4 mg polysorbate 80 [114].
- 10      • An emulsion of squalene, a tocopherol, and a Triton detergent (*e.g.* Triton X-100). The emulsion may also include a 3d-MPL. The emulsion may contain a phosphate buffer.
- 15      • An emulsion comprising a polysorbate (*e.g.* polysorbate 80), a Triton detergent (*e.g.* Triton X-100) and a tocopherol (*e.g.* an  $\alpha$ -tocopherol succinate). The emulsion may include these three components at a mass ratio of about 75:11:10 (*e.g.* 750 $\mu$ g/ml polysorbate 80, 110 $\mu$ g/ml Triton X-100 and 100 $\mu$ g/ml  $\alpha$ -tocopherol succinate), and these concentrations should include any contribution of these components from antigens. The emulsion may also include squalene. The emulsion may also include a 3d-MPL. The aqueous phase may contain a phosphate buffer.
- 20      • An emulsion of squalane, polysorbate 80 and poloxamer 401 (“Pluronic™ L121”). The emulsion can be formulated in phosphate buffered saline, pH 7.4. This emulsion is a useful delivery vehicle for muramyl dipeptides, and has been used with threonyl-MDP in the “SAF-1” adjuvant [115] (0.05-1% Thr-MDP, 5% squalane, 2.5% Pluronic L121 and 0.2% polysorbate 80). It can also be used without the Thr-MDP, as in the “AF” adjuvant [116] (5% squalane, 1.25% Pluronic L121 and 0.2% polysorbate 80). Microfluidisation is preferred.
- 25      • An emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (*e.g.* polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (*e.g.* a sorbitan ester or mannide ester, such as sorbitan monoleate or ‘Span 80’). The emulsion is preferably thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm [117]. The emulsion may also include one or more of: alditol; a cryoprotective agent (*e.g.* a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. Such emulsions may be lyophilized.
- 30      • An emulsion having from 0.5-50% of an oil, 0.1-10% of a phospholipid, and 0.05-5% of a non-ionic surfactant. As described in reference 118, preferred phospholipid components are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin and cardiolipin. Sub-micron droplet sizes are advantageous.

35



- A sub-micron oil-in-water emulsion of a non-metabolisable oil (such as light mineral oil) and at least one surfactant (such as lecithin, Tween 80 or Span 80). Additives may be included, such as QuilA saponin, cholesterol, a saponin-lipophile conjugate (such as GPI-0100, described in reference 119, produced by addition of aliphatic amine to desacylsaponin via the carboxyl group of glucuronic acid), dimethyldioctadecylammonium bromide and/or N,N-dioctadecyl-N,N-bis (2-hydroxyethyl)propanediamine.
  - An emulsion comprising a mineral oil, a non-ionic lipophilic ethoxylated fatty alcohol, and a non-ionic hydrophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [120].
  - An emulsion comprising a mineral oil, a non-ionic hydrophilic ethoxylated fatty alcohol, and a non-ionic lipophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [120].
  - An emulsion in which a saponin (*e.g.* QuilA or QS21) and a sterol (*e.g.* a cholesterol) are associated as helical micelles [121].
- Oil-in-water emulsions can be used as adjuvants on their own, or as carriers for further immunostimulatory compounds *e.g.* immunostimulatory oligonucleotides, 3d-MPL, *etc.*

### ***Pharmaceutical compositions***

The invention is concerned with pharmaceutical compositions for administration to a patient. These will typically include a pharmaceutically acceptable carrier. A thorough discussion of pharmaceutically acceptable carriers is available in reference 122.

Effective dosage volumes can be routinely established, but a typical human dose of the composition has a volume of about 0.5ml *e.g.* for intramuscular injection. This is the dosage volume for the PREVNAR™ product, the RIVM OMV-based vaccine and MeNZB™. These dosage volumes are typical for intramuscular injection, but similar doses may be used for other delivery routes *e.g.* an intranasal OMV-based vaccine for atomisation may have a volume of about 100µl or about 130µl per spray, with four sprays administered to give a total dose of about 0.5ml.

The pH of a composition of the invention is usually between 6 and 8, and more preferably between 6.5 and 7.5 (*e.g.* about 7). Compositions may include a buffer *e.g.* a Tris buffer, a citrate buffer, phosphate buffer, a sodium succinate buffer, or a histidine buffer.

The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions of the invention for administration to patients are immunogenic, and are more preferably vaccine compositions. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Immunogenic compositions used as vaccines comprise an immunologically effective amount of



antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (e.g. non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. The antigen content of compositions of the invention will generally be expressed in terms of the amount of protein per dose.

Meningococci and pneumococci affect various areas of the body and so the compositions of the invention may be prepared in various liquid forms. For example, the compositions may be prepared as injectables, either as solutions or suspensions. The composition may be prepared for pulmonary administration e.g. by an inhaler, using a fine spray. The composition may be prepared for nasal, aural or ocular administration e.g. as spray or drops. Injectables for intramuscular administration are typical.

Compositions of the invention may include an antimicrobial, particularly when packaged in multiple dose format. Antimicrobials such as thiomersal and 2-phenoxyethanol are commonly found in vaccines, but it is preferred to use either a mercury-free preservative or no preservative at all.

Compositions of the invention may comprise detergent e.g. a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels e.g. <0.01%.

Compositions of the invention may include sodium salts (e.g. sodium chloride) to give tonicity. A concentration of  $10 \pm 2$  mg/ml NaCl is typical e.g. about 9 mg/ml.

### ***Methods of treatment***

The invention also provides a method for raising an immune response in a mammal, comprising administering a composition of the invention to the mammal. The immune response is preferably protective against both meningococcus and pneumococcus (for at least the meningococcal serogroups and pneumococcal serotypes represented in the composition) and preferably involves antibodies. The method may raise a booster response in a patient that has already been primed.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (e.g. a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults e.g. to assess safety, dosage, immunogenicity, *etc.*

The invention also provides compositions of the invention for use as a medicament. The medicament is preferably used, as described above, to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of: (i) at least one conjugated pneumococcal capsular saccharide; and (ii) a meningococcal factor H binding protein (fHBP) antigen, in the manufacture of a medicament for raising an immune response, as described above, in a mammal.

These uses and methods are preferably for the prevention and/or treatment of a disease caused by  
 5 *N.meningitidis* and/or *S.pneumoniae* e.g. bacterial (or, more specifically, meningococcal and/or pneumococcal) meningitis, or septicemia.

One way of checking efficacy of therapeutic treatment involves monitoring meningococcal and/or pneumococcal infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against antigens  
 10 after administration of the composition. Immunogenicity of compositions of the invention can be determined by administering them to test subjects (e.g. children 12-16 months age, or animal models [123]) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT) for meningococcus. These immune responses will generally be determined around 4 weeks after administration of the composition, and compared to values determined before  
 15 administration of the composition. A SBA increase of at least 4-fold or 8-fold is preferred. Where more than one dose of the composition is administered, more than one post-administration determination may be made.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (e.g. subcutaneously, intraperitoneally, intravenously,  
 20 intramuscularly, or to the interstitial space of a tissue), or by any other suitable route. The invention may be used to elicit systemic and/or mucosal immunity. Intramuscular administration to the thigh or the upper arm is preferred. Injection may be via a needle (e.g. a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be  
 25 used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Suitable timing between priming doses (e.g. between 4-16 weeks), and between priming and boosting, can be routinely determined. Multiple doses (e.g. 2 or 3 doses) are typical for establishing immunity against both pneumococcus and meningococcus.

30 In some embodiments of the invention, the pneumococcal and meningococcal antigens may be co-administered but separately i.e. the two antigens may be for simultaneous, separate or sequential administration. Typically, however, the two antigens will be admixed for simultaneous combined administration.



**General**

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references 124-130, *etc.*

- 5 The term “comprising” encompasses “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term “about” in relation to a numerical value *x* is optional and means, for example,  $x \pm 10\%$ .

- Where the invention concerns an “epitope”, this epitope may be a B-cell epitope and/or a T-cell epitope, but will usually be a B-cell epitope. Such epitopes can be identified empirically (*e.g.* using  
10 PEPSCAN [131,132] or similar methods), or they can be predicted (*e.g.* using the Jameson-Wolf antigenic index [133], matrix-based approaches [134], MAPITOPE [135], TEPITOPE [136,137], neural networks [138], OptiMer & EpiMer [139,140], ADEPT [141], Tsites [142], hydrophilicity [143], antigenic index [144] or the methods disclosed in references 145-149, *etc.*). Epitopes are the parts of an antigen that are recognised by and bind to the antigen binding sites of antibodies or T-cell  
15 receptors, and they may also be referred to as “antigenic determinants”.

- Where the invention uses a “purified” antigen, this antigen is separated from its naturally occurring environment. For example, the antigen will be substantially free from other meningococcal components, other than from any other purified antigens that are present. A mixture of purified antigens will typically be prepared by purifying each antigen separately and then re-combining them,  
20 even if the two antigens are naturally present in admixture.

- References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. 150. A preferred alignment is determined  
25 by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. 151.

- The word “substantially” does not exclude “completely” *e.g.* a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be  
30 omitted from the definition of the invention.

**BRIEF DESCRIPTION OF DRAWINGS**

- Figure 1 shows results of an opsonophagocytic activity assay against 6B Finland pneumococcus. The graph shows OPA killing % against serum dilution. Symbols are explained below for mouse groups 1 to 7, except for the filled diamonds (◆) which show data from a positive control anti-6B serum and  
35 filled triangles (▲) which show data using pre-immunisation serum. Some lines are not visible because they run along the X-axis (*i.e.* 0% activity).

**MODES FOR CARRYING OUT THE INVENTION**

In seeking a vaccine for protecting against both serogroup B meningococcus and pneumococcus, meningococcal fHBP antigen (strain MC58; 50µg/ml) was combined with a 7-valent pneumococcal capsular saccharide conjugate mixture (serotypes 4, 9V, 14, 18C, 19F and 23F at 4µg/ml; 6B at 8µg/ml). Compositions were intraperitoneally administered to seven groups of CD1 mice (8 mice per group) on a two-dose schedule (days 0 and 21). An aluminium phosphate adjuvant was used. None of the compositions included meningococcal outer membrane vesicles.

Five different compositions (A to E) were administered to mice:

	<b>fHBP</b>	<b>PCV7</b>	<b>Adjuvant</b>	<b>pH</b>	<b>Dosage volume</b>
<b>A</b>	-	+	100µg	6.01	100 µl
<b>B</b>	+	-	-	7.05	200 µl
<b>C</b>	+	-	100µg	6.94	200 µl
<b>D</b>	+	+	100µg	6.93	200 µl
<b>E</b>	-	-	100µg	-	200 µl

Seven groups of mice (1 to 7) were used and they received compositions A to E as follows:

	<b>Day 0</b>	<b>Day 21</b>	<b>Symbol in Figure 1</b>
<b>1</b>	A	-	×
<b>2</b>	A	A	Δ
<b>3</b>	B	B	▪
<b>4</b>	C	C	◇
<b>5</b>	D	B	○
<b>6</b>	D	D	□
<b>7</b>	E	E	●

Blood was taken at days 16 and 35 for evaluation of immune responses. Pneumococcal immunogenicity was assessed by an opsonophagocytosis assay and by the antibody titer against the saccharides. Meningococcal immunogenicity was assessed by serum bactericidal assay against two different strains (MC58 and H44/76).

Meningococcal SBA titers were as follows:

	<b>MC58</b>		<b>H44/76</b>	
	<b>Day 16</b>	<b>Day 35</b>	<b>Day 16</b>	<b>Day 35</b>
<b>1</b>	-	<16	-	<16
<b>2</b>	-	<16	-	<16
<b>3</b>	<16	2048	<16	4096
<b>4</b>	<16	2048	<16	2048
<b>5</b>	16	4096	64	8192
<b>6</b>	<16	1024	<16	1024
<b>7</b>	-	<16	-	<16



Titers were acceptable in all of groups 3 to 6, but the highest titers after 35 days were seen in group 5 for both strains. These data indicate that the addition of pneumococcal conjugates to fHBP can enhance the anti-fHBP response.

OPA activity was assessed against the 6B Finland strain of pneumococcus using the UAB-MOPA method of reference 152 with baby rabbit complement. Results are shown in Figure 1. The best response is in group 6 (□) and then group 2 (Δ). The difference between groups 2 and 6 was that the immunising composition for group 6 included meningococcal fHBP in addition to the pneumococcal conjugates. Thus these data indicate that the addition of fHBP to pneumococcal conjugates can enhance the anti-pneumococcus response, at least against serotype 6B.

Sera were also assessed against the TIGR4 strain. OPA responses were similar in groups 2 and 6. Sera were also tested against pneumococcus serotype 35B, which is not covered by the 7-valent conjugates. As expected, the sera were not very effective in the OPA assay.

The OPA assay results can be expressed as an OPA titer, which is the reciprocal serum dilution yielding 50% bacterial killing. If the 50% of killing threshold lies between two dilutions, the titer is expressed as a range. By this measure, titers against the serotype 6B and 4 strains were as follows after the day 21 immunization:

	<b>6B Finland</b>	<b>TIGR4</b>
<b>1</b>	12	108-324
<b>2</b>	972-2916	2916-8748
<b>3</b>	<12	-
<b>4</b>	<12	-
<b>5</b>	12-36	324-972
<b>6</b>	2916	2916-8748
<b>7</b>	<12	<12

Overall, therefore, these data show that conjugated pneumococcal capsular saccharides can enhance the immunogenicity of meningococcal fHBP, and *vice versa*.

In separate experiments, mice were immunised with (i) 10µg of outer membrane vesicles prepared from strain MC58 of serogroup B meningococcus, (ii) 0.4µg of 7-valent pneumococcal capsular saccharide conjugate mixture, and (iii) a mixture of (i) and (ii). All compositions included aluminium hydroxide adjuvant. IgG titres (GMT) against the vesicles and against the serotype 14 saccharide were measured by Luminex assay. On day 34 (after immunization on days 1 and 21) titres were as follows in the three groups, measured in relative units (RLU/ml):

	<b>(i)</b>	<b>(ii)</b>	<b>(iii)</b>
<b>Anti-OMV</b>	0.14	5.61	2.65
<b>Anti-CP14</b>	76.1	1.7	81.7

Although these data are incomplete and not fully conclusive, the decrease in anti-OMV responses in group (iii) compared to group (ii) suggests that the advantageous interaction between pneumococcal saccharides and fHBP is not universal to all meningococcal antigens.

5 It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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**CLAIMS**

1. An immunogenic composition comprising: (i) a conjugated pneumococcal capsular saccharide; and (ii) a meningococcal factor H binding protein (fHBP) antigen, but not including meningococcal outer membrane vesicles.
- 5 2. The composition of claim 1, including capsular saccharide from two or more different pneumococcal serotypes.
3. The composition of claim 2, wherein capsular saccharides are selected from the following pneumococcal serotypes: 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.
- 10 4. The composition of claim 2 or claim 3, comprising capsular saccharide from each of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.
5. The composition of claim 2 or claim 3, comprising capsular saccharide from each of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19, 19F and 23F.
- 15 6. The composition of any one of claims 2 to 5, wherein each serotype's saccharide is separately conjugated to a carrier protein selected from the group consisting of CRM197, tetanus toxoid, diphtheria toxoid and *H.influenzae* protein D, and wherein the carrier protein for different serotypes may be the same or different.
7. The composition of any preceding claim, wherein the composition includes a single fHBP polypeptide comprising: (a) an amino acid sequence having at least 85% sequence identity to  
20 SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 1; or (b) an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 2; or (c) an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 3 and/or comprising an amino acid  
25 sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 3.
8. The composition of any one of claims 1 to 6, wherein the composition includes two different fHBP polypeptides: (a) a first polypeptide, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 1; (b) a second polypeptide,  
30 comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 3.
9. The composition of any preceding claim, including a polypeptide comprising an amino acid sequence having at least 93% sequence identity to SEQ ID NO: 3 and/or comprising an amino  
35 acid sequence consisting of a fragment of at least 40 contiguous amino acids from SEQ ID NO: 3

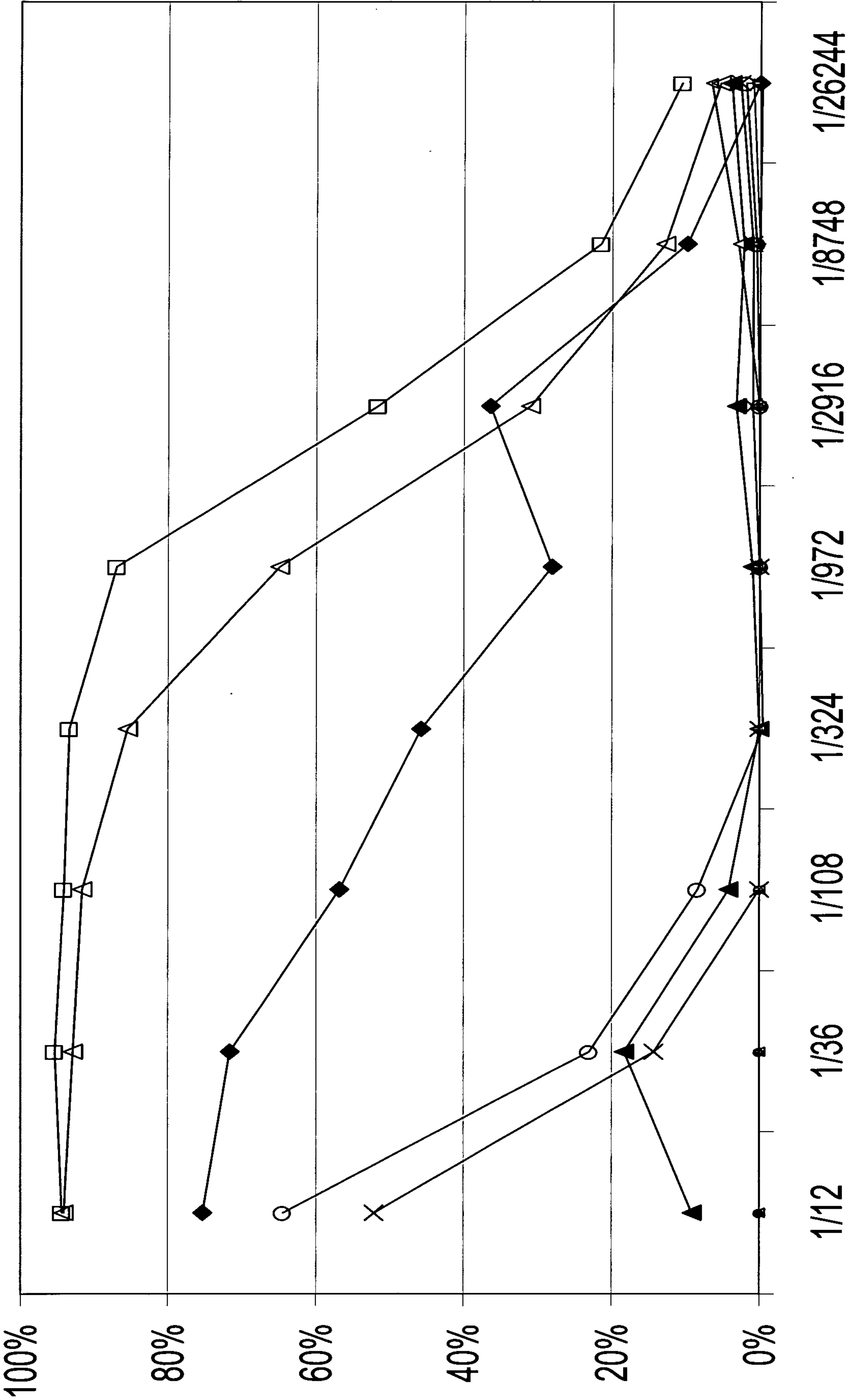


10. The composition of any one of claims 1 to 6, wherein the composition includes two different fHBP polypeptides: (a) a first polypeptide, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 1; and (b) a second polypeptide, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 2.
11. The composition of any preceding claim, comprising an aluminium phosphate adjuvant.
12. The composition of claim 11, comprising: (i) an aluminium phosphate adjuvant; (ii) a conjugated pneumococcal capsular saccharide from each of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, each of said saccharides being conjugated to a CRM197 carrier protein; and (iii) at least two different meningococcal factor H binding protein antigens, each of which is at least partially adsorbed to an aluminium phosphate adjuvant.
13. The composition of claim 12, wherein at least one of the conjugated pneumococcal saccharides is adsorbed to an aluminium phosphate adjuvant.
14. The composition of claim 12 or claim 13, wherein the composition includes sodium chloride and/or a buffer.
15. The composition of any one of claims 1 to 6, wherein the composition includes three different fHBP polypeptides: (a) a first polypeptide, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 1; (b) a second polypeptide, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 2; and (c) a third polypeptide, comprising an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 3.
16. The composition of any preceding claim, wherein a fHBP polypeptide therein is lipidated at a N-terminus cysteine.
17. The composition of any preceding claim, wherein the total amount of fHBP polypeptide(s) in the composition is less than 600µg.
18. The composition of any preceding claim, wherein the total amount of fHBP polypeptide(s) in the composition is less than 200µg.
19. The composition of any preceding claim, wherein the total amount of fHBP polypeptide(s) in the composition is less than 60µg.

20. The composition of any preceding claim, further including one or more conjugate(s) of capsular saccharides from 1, 2, 3, or 4 of meningococcal serogroups A, C, W135 and Y (*e.g.* including a conjugate of a serogroup C meningococcal capsular saccharide).
21. The composition of any preceding claim, including an aluminium hydroxyphosphate adjuvant.
- 5 22. The composition of any preceding claim, wherein the composition is free from aluminium hydroxide.
23. A method for raising an immune response in a mammal, comprising administering a composition of any preceding claim to the mammal.
24. The composition of any one of claims 1 to 22, for use as a medicament.
- 10 25. A process for preparing an immunogenic composition, comprising steps of: (i) mixing at least one conjugated pneumococcal capsular saccharide with an aluminium phosphate adjuvant to form a conjugate/adjuvant mixture; and (ii) mixing the conjugate/adjuvant mixture with at least one meningococcal factor H binding protein.
- 15 26. A process for preparing an immunogenic composition, comprising steps of: (i) mixing at least one conjugated pneumococcal capsular saccharide with at least one meningococcal factor H binding protein to form an antigen mixture; and (ii) mixing the antigen mixture with an aluminium phosphate adjuvant.
- 20 27. A process for preparing an immunogenic composition, comprising steps of: (i) mixing at least one meningococcal factor H binding protein with an aluminium phosphate adjuvant to form a protein/adjuvant mixture; and (ii) mixing the protein/adjuvant mixture with at least one conjugated pneumococcal capsular saccharide.
- 25 28. A process for preparing an immunogenic composition, comprising steps of: (i) mixing at least one meningococcal factor H binding protein with an aluminium phosphate adjuvant to form a protein/adjuvant mixture; (ii) mixing at least one conjugated pneumococcal capsular saccharide with an aluminium phosphate adjuvant to form a conjugate/adjuvant mixture; and (iii) mixing the protein/adjuvant mixture and conjugate/adjuvant mixture.
- 30 29. The process of any one of claims 25 to 28, which does not include a step of mixing an aluminium hydroxide adjuvant with any of (i) a meningococcal factor H binding protein, (ii) an aluminium phosphate adjuvant, (iii) a conjugated pneumococcal capsular saccharide; (iv) a conjugate/adjuvant mixture; (v) an antigen mixture; or (vi) a protein/adjuvant mixture.



FIGURE 1



**FIGURE 1**

