44; Whitney *et al*., 2003, *N Engl J Med* 348:1737-46; Kyaw *et al*., 2006, *N Engl J Med* 354:1455-63; Hicks *et al*., 2007, *J Infect Dis* 196:1346-54; Traore *et al*., 2009, *Clin Infect Dis* 48:S181-S189.

U.S. Patent Application Publication No. US 2006/0228380 describes a 13-valent pneumococcal polysaccharide-protein conjugate vaccine including serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. Chinese Patent Application Publication No. CN 101590224 A describes a 14-valent pneumococcal polysaccharide-protein conjugate vaccine including serotypes 1, 2, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

Other PCVs have covered 7, 10, 11, or 13 of the serotypes contained in PCV-15 (U.S Pub. No. 2011/0195086), but immune interference has been observed for some serotypes (e.g. lower protection for serotype 3 in GSK's PCV-11) and lower response rates to serotype 6B in Pfizer's PCV-13 (PREVNAR® 13). See Prymula *et al*., 2006, Lancet 367:740-48 and Kieninger *et al*., Safety and Immunologic Non-inferiority of 13-valent Pneumococcal Conjugate Vaccine Compared to 7-valent Pneumococcal Conjugate Vaccine Given as a 4-Dose Series in Healthy Infants and Toddlers, presented at the 48th Annual ICAAC/ISDA 46th Annual Meeting, Washington DC, October 25-28, 2008.

The current multivalent pneumococcal vaccines have been effective in reducing the incidence of pneumococcal disease associated with those serotypes present in the vaccines. However, the prevalence of the pneumococci expressing serotypes not present in the currently available vaccines has been increasing. Accordingly, there is a need for additional pneumococcal vaccine compositions which provide protection against different sets of pneumococcal serotypes and which can provide complementary protection against pneumococcal serotypes not present in currently available vaccines.

SUMMARY

The disclosure provides multivalent immunogenic compositions comprising *S. pneumoniae* polysaccharide protein conjugates wherein each of the conjugates comprises a polysaccharide from an *S. pneumoniae* serotype conjugated to a carrier protein, wherein the serotypes of *S. pneumoniae* are as defined herein.

In an aspect, there is provided a multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, and wherein the *S. pneumoniae* serotypes are selected from the group consisting of:

I) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20;

II) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A;

III) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B;

IV) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20;

V) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A; and

VI) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B;

wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In another aspect, there is provided a multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In another aspect, there is provided a multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In another aspect, there is provided a multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In another aspect, there is provided a multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In another aspect, there is provided a multivalent immunogenic composition comprising 20 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In another aspect, there is provided a multivalent immunogenic composition comprising *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, and wherein the *S. pneumoniae* serotypes are selected from the group consisting of:

(1) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(2) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(3) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(4) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(5) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(6) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(7) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(8) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(9) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(10) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(11) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(12) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(13) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(14) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(15) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(16) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(17) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(18) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(19) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(20) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(21) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(22) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(23) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(24) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(25) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(26) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(27) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(28) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(29) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(30) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(31) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(32) 3, 7F, 19A, 22F, 33F,8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(33) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(34) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A,

(35) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(36) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(37) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A,

(38) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(39) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20,

(40) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20,

(41) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20, and

(42) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20,

wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

In particular examples of the disclosure, the serotypes of *S. pneumoniae* comprise a set of serotypes selected from the group consisting of:

a) 15A, 16F, 23A, 23B, 24F, 31 and 35B;

b) 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20; and

c) 3, 7F, 19A, 22F, 33F, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20.

In further particular examples of the disclosure, the serotypes of *S. pneumoniae* comprise a set of serotypes selected from the group consisting of:

a) 15A, 16F, 23A, 23B, 24F, 31 and 35B;

b) 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A; and

c) 3, 7F, 19A, 22F, 33F, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A.

In further particular examples of the disclosure, the serotypes of *S. pneumoniae* comprise a set of serotypes selected from the group consisting of:

a) 15A, 16F, 23A, 23B, 24F, 31 and 35B;

b) 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B; and

c) 3, 7F, 19A, 22F, 33F, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B.

In some examples, the set of serotypes of *S. pneumoniae* listed in a), b) or c) further comprises: serotype 6C, serotype 6A, or serotypes 6A and 6B.

In some examples, at least one of the polysaccharide protein conjugates is formed by a conjugation reaction comprising an aprotic solvent, e.g. dimethylsulfoxide (DMSO). In specific examples, each of the polysaccharide protein conjugates is formed by a conjugation reaction comprising an aprotic solvent. As shown herein, the use of DMSO as a solvent during reductive amination of polysaccharide-protein conjugates results in unexpectedly superior stability and enhanced immunogenicity for those serotypes relative to the same conjugates prepared under aqueous conditions.

Also provided are methods for inducing a protective immune response in a human patient comprising administering the multivalent immunogenic compositions of the disclosure to the patient. In some examples of the methods of the disclosure, the patient was previously treated with a multivalent pneumococcal vaccine.

A multivalent immunogenic composition of the disclosure may be used as part of a treatment regimen with a different, complementary pneumococcal vaccine. Accordingly, the disclosure provides a method of inducing a protective immune response in a human patient comprising administering a multivalent immunogenic composition of the disclosure to the patient, further comprising administering a multivalent pneumococcal vaccine to the patient in any order. In particular examples, the multivalent pneumococcal vaccine is comprised of multiple *S. pneumoniae* polysaccharide protein conjugates wherein each of the conjugates comprises polysaccharide from an *S. pneumoniae* serotype conjugated to a carrier protein. In other examples, the multivalent pneumococcal vaccine is comprised of unconjugated capsular polysaccharides.

In another aspect, there is provided use of the multivalent immunogenic composition as defined herein in the manufacture of a medicament for inducing a protective immune response in a human patient.

In another aspect, there is provided use of the multivalent immunogenic composition as defined herein in the manufacture of a medicament for the prevention of pneumococcal pneumoniae and invasive disease in adults 18 years of age and older.

In another aspect, there is provided use of the multivalent immunogenic composition as defined herein in the manufacture of a medicament for the prevention of pneumococcal pneumoniae and invasive disease caused by the 24 *Streptococcus pneumoniae* strains (3, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, and 35B).

In another aspect, there is provided a multivalent immunogenic composition as defined herein for use as a medicament.

Also provided are methods for preparing a serotype 8 *Streptococcus pneumoniae* polysaccharide-protein conjugate utilizing a conjugation reaction in an aprotic solvent, wherein the conjugation reaction does not use cyanoborohydride.

The disclosure also provides multivalent immunogenic compositions comprising *S. pneumoniae* polysaccharide protein conjugates wherein each of the conjugates comprises a polysaccharide from an *S. pneumoniae* serotype conjugated to a carrier protein, wherein select serotypes of *S. pneumoniae* provide cross-reactivity to other select serotypes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts the 600 MHz one-dimensional ^{1}H NMR spectrum of the capsular polysaccharide from *S. pneumonia* serotype 6C in deuterium oxide (D_2O) at 50°C. Signals arising from internal standards (DMSO and DSS-d_6) and residual water (HOD) are marked. Minor signals marked by * are due to S. *pneumonia* cell wall residuals such as C-polysaccharide and/or peptidoglycans.

FIGURE 2 depicts the 600 MHz one-dimensional ^{1}H NMR spectrum of the capsular polysaccharide from *S. pneumonia* serotype 15A in deuterium oxide (D_2O) at 50°C. Signals arising from internal standards (DMSO and DSS-d_6) and residual water (HOD) are marked. Minor signals marked by * are due to S. *pneumonia* cell wall residuals such as C-polysaccharide and/or peptidoglycans.

FIGURE 3 depicts the 600 MHz one-dimensional ^{1}H NMR spectrum of the capsular polysaccharide from *S. pneumonia* serotype de-O-acetylated 15B in deuterium oxide (D_2O) at 50°C. Signals arising from internal standards (DMSO and DSS-d_6) and residual water (HOD) are marked. Minor signals marked by * are due to S. *pneumonia* cell wall residuals such as C-polysaccharide and/or peptidoglycans.

FIGURE 4 depicts the 600 MHz one-dimensional ^{1}H NMR spectrum of the capsular polysaccharide from *S. pneumonia* serotype 35B in D_2O at 50°C. Signals arising from internal standards (DMSO and DSS-d_6) and residual water (HOD) are marked. Minor signals marked by * are due to S. *pneumonia* cell wall residuals such as C-polysaccharide and/or peptidoglycans.

FIGURE 5 depicts the ^{1}H NMR identity region useful for serotype identification of *S. pneumonia* serotype 6C. Signal positions of each anomeric proton of the repeating unit from each monosaccharide residue are marked.

FIGURE 6 depicts the ^{1}H NMR identity region useful for serotype identification of *S. pneumonia* serotype 15A. Signal positions of each anomeric proton of the repeating unit from each monosaccharide residue are marked.

WHAT IS CLAIMED IS:

(1) A multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, and wherein the *S. pneumoniae* serotypes are selected from the group consisting of:

I) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20;

II) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A;

III) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B;

IV) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20;

V) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A; and

VI) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B;

wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(2) The multivalent immunogenic composition of claim 1 , wherein at least one of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent.

(3) The multivalent immunogenic composition of claim 1 or 2, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent.

(4) The multivalent immunogenic composition of any of claims 2 to 3, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(5) The multivalent immunogenic composition of any of claims 1 to 4, wherein the carrier protein is selected from the group consisting of Outer Membrane Protein Complex (OMPC), tetanus toxoid, diphtheria toxoid, protein D and CRM197.

(6) The multivalent immunogenic composition of any of claims 1 to 5, wherein the carrier protein is CRM197.

(7) The multivalent immunogenic composition of any of claims 1 to 6, wherein the composition further comprises an adjuvant.

(8) The multivalent immunogenic composition of any of claims 1 to 6, wherein the composition does not comprise an adjuvant.

(9) A multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(10) The multivalent immunogenic composition of claim 9, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(11) The multivalent immunogenic composition of claim 9, wherein the composition does not comprise an adjuvant.

(12) A multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(13) The multivalent immunogenic composition of claim 12, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(14) The multivalent immunogenic composition of claim 12, wherein the composition does not comprise an adjuvant.

(15) A multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(16) The multivalent immunogenic composition of claim 15, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(17) The multivalent immunogenic composition of claim 15, wherein the composition does not comprise an adjuvant.

(18) A multivalent immunogenic composition comprising 21 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(19) The multivalent immunogenic composition of claim 18, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(20) The multivalent immunogenic composition of claim 18, wherein the composition does not comprise an adjuvant.

(21) A multivalent immunogenic composition comprising 20 distinct *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, wherein the *S. pneumoniae* serotypes are 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A,

6C, 15A, 16F, 23F, 24F, 31 and 35B, and the carrier protein is CRM197, and wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(22) The multivalent immunogenic composition of claim 21, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(23) The multivalent immunogenic composition of claim 21, wherein the composition does not comprise an adjuvant.

(24) A multivalent immunogenic composition comprising *S. pneumoniae* polysaccharide carrier protein conjugates, wherein each of the conjugates comprises a polysaccharide of a particular *S. pneumoniae* serotype conjugated to a carrier protein, and wherein the *S. pneumoniae* serotypes are selected from the group consisting of:

(1) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(2) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(3) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(4) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(5) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(6) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(7) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(8) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(9) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(10) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(11) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(12) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(13) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(14) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(15) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(16) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23A, 23B, 24F, 31 and 35B,

(17) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(18) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(19) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(20) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6C, 15A, 16F, 23F, 24F, 31 and 35B,

(21) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(22) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(23) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(24) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(25) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(26) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15C, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(27) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(28) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(29) 3, 7F, 19A, 22F, 33F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(30) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 15A, 16F, 23F, 24F, 31 and 35B,

(31) 3, 7F, 19A, 22F, 33F, 8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 6B, 15A, 16F, 23F, 24F, 31 and 35B,

(32) 3, 7F, 19A, 22F, 33F,8, 9N, 39, 11A, 12F, 15B, 17F, 20A, 6A, 15A, 16F, 23F, 24F, 31 and 35B,

(33) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(34) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A,

(35) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(36) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(37) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20A,

(38) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20B,

(39) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20,

(40) 3, 7F, 19A, 22F, 33F, 6C, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20,

(41) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24F, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20, and

(42) 3, 7F, 19A, 22F, 33F, 6A, 15A, 16F, 23A, 23B, 24B, 31, 35B, 8, 9N, 10A, 11A, 12F, 15C, 17F, and 20,

wherein the composition does not comprise polysaccharide carrier protein conjugates containing polysaccharides of any other *S. pneumoniae* serotype.

(25) The multivalent immunogenic composition of claim 24, wherein at least one of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent.

(26) The multivalent immunogenic composition of claim 24, wherein each of the polysaccharide carrier protein conjugates is formed by a conjugation reaction comprising an aprotic solvent.

(27) The multivalent immunogenic composition of claim 25 or 26, wherein the aprotic solvent is dimethylsulfoxide (DMSO).

(28) The multivalent immunogenic composition of claim 24, wherein the carrier protein is selected from the group consisting of Outer Membrane Protein Complex (OMPC), tetanus toxoid, diphtheria toxoid, protein D and CRM197.

(29) The multivalent immunogenic composition of claim 28, wherein the carrier protein is CRM197.

(30) The multivalent immunogenic composition of any of claims 24 to 29, wherein the composition further comprises an adjuvant.

(31) The multivalent immunogenic composition of any of claims 24 to 29, wherein the composition does not comprise an adjuvant.

(32) The multivalent immunogenic composition of any of claims 24 to 31, wherein the *S. pneumoniae* serotype 15C polysaccharide is a de-O-acetylated *S. pneumoniae* serotype 15B polysaccharide.

(33) The multivalent immunogenic composition of claims 1-8, wherein the *S. pneumoniae* serotype 15C polysaccharide is a de-O-acetylated *S. pneumoniae* serotype 15B polysaccharide.

(34) The multivalent immunogenic composition of any of claims 9 to 11, wherein the *S. pneumoniae* serotype 15C polysaccharide is a de-O-acetylated *S. pneumoniae* serotype 15B polysaccharide.

(35) The multivalent immunogenic composition of claims 15-17, wherein the *S. pneumoniae* serotype 15C polysaccharide is a de-O-acetylated *S. pneumoniae* serotype 15B polysaccharide.

(36) The multivalent immunogenic composition of claims 18-20, wherein the *S. pneumoniae* serotype 15C polysaccharide is a de-O-acetylated *S. pneumoniae* serotype 15B polysaccharide.

(37) Use of the multivalent immunogenic composition of any of claims 1 to 8 and 12 to 36 in the manufacture of a medicament for inducing a protective immune response in a human patient.

(38) The use of claim 37, wherein the patient was previously treated with a multivalent pneumococcal vaccine.

(39) The use of any of claims 37 to 38, wherein two or more doses of the multivalent immunogenic composition is to be administered to the patient.

(40) The use of claim 39, wherein the amount of time between doses is from about 4 weeks to about 1 year.

(41) The use of any of claims 37 to 40, wherein three doses are to be administered to the patient and the patient is immunocompromised.

(42) The use of any of claims 37 to 41, wherein a multivalent pneumococcal vaccine is to be administered to the patient in any order, and the multivalent immunogenic composition and the multivalent pneumococcal vaccine are not the same.

(43) The use of claim 42, wherein the multivalent pneumococcal vaccine is comprised of multiple *S. pneumonia* polysaccharide carrier protein conjugates wherein each of the conjugates comprises polysaccharide from a *S. pneumoniae* serotype conjugated to a carrier protein.

(44) The use of any of claims 42 to 43, wherein the multivalent immunogenic composition and the multivalent pneumococcal vaccine are to be administered concurrently.

(45) The use of any of claims 42 to 43, wherein the amount of time between administration of the multivalent immunogenic composition and the multivalent pneumococcal vaccine is from about 4 weeks to about 1 year.

(46) The use of any of claims 42, 43 or 45, wherein the multivalent immunogenic composition is to be administered before the multivalent pneumococcal vaccine.

(47) The use of any of claims 42, 43 or 45, wherein the multivalent pneumococcal vaccine is to be administered before the multivalent immunogenic composition.

(48) The use of any of claims 37 to 47, wherein the patient is 50 years of age or older.

(49) The use of any of claims 37 to 47, wherein the patient is between 2 and 18 years of age.

(50) The use of any of claims 37 to 47, wherein the patient is 18 years of age or older.

(51) The use of any of claims 37 to 47, wherein the patient is 65 years of age or older.

(52) The use of any of claims 37 to 51, wherein the patient is immunocompromised.

(53) The use of any of claims 37 to 52, wherein the multivalent immunogenic composition is to be administered by subcutaneous or intramuscular injection.

(54) The use of any of claims 37 to 53, wherein the multivalent immunogenic composition is to be administered concomitantly with a vaccine against influenza.

(55) Use of the multivalent immunogenic composition of claims 1, 9, 12, 15, 18, 21 or 24 in the manufacture of a medicament for the prevention of pneumococcal pneumoniae and invasive disease in adults 18 years of age and older.

(56) Use of the multivalent immunogenic composition of claims 1, 9, 12, 15, 18, 21 or 24 in the manufacture of a medicament for the prevention of pneumococcal pneumoniae and invasive disease caused by the 24 *Streptococcus pneumoniae* strains (3, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, and 35B).

(57) The multivalent immunogenic composition of any of claims 1 to 8 and 12 to 36 for use as a medicament.