

WHAT IS CLAIMED IS:

1. A multivalent pneumococcal conjugate composition, comprising 22-27 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, and 35B.
2. The multivalent pneumococcal conjugate composition of claim 1, comprising 27 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 18C, 19A, 19F, 23A, 23B, 22F, 23F, 24F, 33F, and 35B.
3. The multivalent pneumococcal conjugate composition of claim 1, comprising 26 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, and 35B and four serotypes selected from 15A, 15C, 23A, 23B, and 24F.
4. The multivalent pneumococcal conjugate composition of claim 1, comprising 25 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, and 35B and three serotypes selected from 15A, 15C, 23A, 23B, and 24F.

5. The multivalent pneumococcal conjugate composition of claim 1, comprising 24 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, and 35B and two serotypes selected from 15A, 15C, 23A, 23B, and 24F.

6. The multivalent pneumococcal conjugate composition of claim 1, comprising 23 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, and 35B and one serotype selected from 15A, 15C, 23A, 23B, and 24F.

7. The multivalent pneumococcal conjugate composition of claim 1, comprising 22 different pneumococcal capsular polysaccharide-protein conjugates, wherein each pneumococcal capsular polysaccharide-protein conjugate comprises a protein carrier conjugated to a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae*, wherein the *Streptococcus pneumoniae* serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, and 35B.

8. The multivalent pneumococcal conjugate composition of any of the preceding claims, wherein the protein carrier comprises CRM₁₉₇ and/or tetanus toxoid.

9. The multivalent pneumococcal conjugate composition of claim 8, wherein at least two of the capsular polysaccharides are conjugated to tetanus toxoid and the remaining capsular polysaccharides are conjugated to CRM₁₉₇, wherein the at least two capsular polysaccharides that are conjugated to tetanus toxoid are selected from the group consisting of serotypes 1, 3, 5, 15B, and 22F.

10. The multivalent pneumococcal conjugate composition of claim 2, wherein the capsular polysaccharides from serotypes 1 and 5 are conjugated to the tetanus toxoid, and the capsular

polysaccharides from serotypes 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, and 35B are conjugated to CRM₁₉₇.

11. The multivalent pneumococcal conjugate composition of claim 2, wherein the capsular polysaccharides from serotypes 1 and 3 are conjugated to the tetanus toxoid, and the capsular polysaccharides from serotypes 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, and 35B are conjugated to CRM₁₉₇.

12. The multivalent pneumococcal conjugate composition of claim 2, wherein the capsular polysaccharides from serotypes 3 and 5 are conjugated to the tetanus toxoid, and the capsular polysaccharides from serotypes 1, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, and 35B are conjugated to CRM₁₉₇.

13. The multivalent pneumococcal conjugate composition of claim 2, wherein the capsular polysaccharides from serotypes 1, 5, 15B, and 22F are conjugated to the tetanus toxoid, and the capsular polysaccharides from serotypes 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15C, 18C, 19A, 19F, 23A, 23B, 23F, 24F, 33F, and 35B are conjugated to CRM₁₉₇.

14. The multivalent pneumococcal conjugate composition of claim 2, wherein the capsular polysaccharides from serotypes 1, 3, 15B, and 22F are conjugated to the tetanus toxoid, and the capsular polysaccharides from serotypes 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15C, 18C, 19A, 19F, 23A, 23B, 23F, 24F, 33F, and 35B are conjugated to CRM₁₉₇.

15. The multivalent pneumococcal conjugate composition of claim 2, wherein the capsular polysaccharides from serotypes 3, 5, 15B, and 22F are conjugated to the tetanus toxoid, and the capsular polysaccharides from serotypes 1, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15C, 18C, 19A, 19F, 23A, 23B, 23F, 24F, 33F, and 35B are conjugated to CRM₁₉₇.

16. The multivalent pneumococcal conjugate composition of any one of the preceding claims, further comprising an adjuvant.

17. The multivalent pneumococcal conjugate composition of claim 16, wherein the adjuvant is an aluminum-based adjuvant.

18. The multivalent pneumococcal conjugate composition of claim 17, wherein the adjuvant is selected from the group consisting of aluminum phosphate, aluminum sulfate, and aluminum hydroxide.
19. The multivalent pneumococcal conjugate composition of claim 18, wherein the adjuvant is aluminum phosphate.
20. The use of the multivalent pneumococcal conjugate composition of any of the preceding claims for prophylaxis against *Streptococcus pneumoniae* infection or disease in a subject.
21. A vaccine comprising the multivalent pneumococcal conjugate composition of any one of claims 1-19 and a pharmaceutically acceptable excipient.
22. A method for prophylaxis of *Streptococcus pneumoniae* infection or disease in a subject, the method comprising administering a prophylactically effective amount of the multivalent pneumococcal conjugate composition of any one of claims 1-19 or the vaccine of claim 21 to the subject.
23. The method of claim 22, wherein the subject is a human who is at least 50 years old and the disease is pneumonia or invasive pneumococcal disease (IPD).
24. The method of claim 22, wherein the subject is a human who is at least 6 weeks old and the disease is pneumonia, invasive pneumococcal disease (IPD), or acute otitis media (AOM).
25. The method of claim 24, wherein the subject is 6 weeks to 5 years of age, 2 to 15 months of age, or 6 to 17 years of age.
26. The use of claim 20 or the method of claim 22, wherein the subject is a human.
27. The method of any one of claims 22-26, wherein the multivalent pneumococcal conjugate composition or the vaccine is administered by intramuscular injection.

28. The method of any one of claims 22-27, wherein the multivalent pneumococcal conjugate composition or the vaccine is administered as part of an immunization series.

29. An immunogenic composition comprising at least one polysaccharide-protein conjugate, wherein the polysaccharide in the at least one polysaccharide-protein conjugate is a capsular polysaccharide from *Streptococcus pneumoniae* serotype 15A, serotype 15C, serotype 23A, serotype 23B, serotype 24F, or serotype 35B.

30. A method of making a capsular polysaccharide from *Streptococcus pneumoniae* serotype 15A, serotype 15C, serotype 23A, serotype 23B, serotype 24F, or serotype 35B as described herein.

31. The method of claim 30, wherein the serotype is serotype 15A and the method comprises:

(i) subjecting a purified *Streptococcus pneumoniae* serotype 15A polysaccharide to an acid hydrolysis reaction and heat or a microfluidizer, and then reacting with an oxidizing agent to produce an activated *Streptococcus pneumoniae* serotype 15A polysaccharide;

(ii) optionally lyophilizing the activated *Streptococcus pneumoniae* serotype 15A polysaccharide and a carrier protein;

(iii) suspending the activated *Streptococcus pneumoniae* serotype 15A polysaccharide and the carrier protein in dimethyl sulfoxide (DMSO);

(iv) reacting the activated *Streptococcus pneumoniae* serotype 15A polysaccharide and the carrier protein with a reducing agent to produce *Streptococcus pneumoniae* serotype 15A polysaccharide-carrier protein conjugate; and

(v) capping unreacted aldehydes in the *Streptococcus pneumoniae* serotype 15A polysaccharide-carrier protein conjugate to prepare an immunogenic conjugate comprising the *Streptococcus pneumoniae* serotype 15A polysaccharide covalently linked to the carrier protein.

32. The method of claim 30, wherein the serotype is serotype 15C and the method comprises:

(i) reacting a purified *Streptococcus pneumoniae* serotype 15C polysaccharide with an oxidizing agent to produce an activated *Streptococcus pneumoniae* serotype 15C polysaccharide;

(ii) optionally lyophilizing the activated *Streptococcus pneumoniae* serotype 15C polysaccharide and a carrier protein;

(iii) suspending the activated *Streptococcus pneumoniae* serotype 15C polysaccharide and the carrier protein in dimethyl sulfoxide (DMSO) or phosphate buffer;

(iv) reacting the mixture of the activated serotype 15C polysaccharide and the carrier protein with a reducing agent to produce a serotype 15C polysaccharide-carrier protein conjugate; and

(v) capping unreacted aldehydes in the serotype 15C polysaccharide-carrier protein conjugate to prepare an immunogenic conjugate comprising the *Streptococcus pneumoniae* serotype 15C polysaccharide covalently linked to the carrier protein.

33. The method of claim 30, wherein the serotype is serotype 23A and the method comprises:

(i) reacting a purified *Streptococcus pneumoniae* serotype 23A with an oxidizing agent to produce an activated *Streptococcus pneumoniae* serotype 23A polysaccharide;

(ii) optionally lyophilizing the activated *Streptococcus pneumoniae* serotype 23A polysaccharide and a carrier protein;

(iii) suspending the activated *Streptococcus pneumoniae* serotype 23A polysaccharide and the carrier protein in dimethyl sulfoxide (DMSO) or phosphate buffer;

(iv) reacting the mixture of the activated *Streptococcus pneumoniae* serotype 23A polysaccharide and the carrier protein with a reducing agent to produce a *Streptococcus pneumoniae* serotype 23A polysaccharide-carrier protein conjugate; and

(v) capping unreacted aldehydes in the *Streptococcus pneumoniae* serotype 23A polysaccharide-carrier protein conjugate to prepare an immunogenic conjugate comprising the *Streptococcus pneumoniae* serotype 23A polysaccharide covalently linked to the carrier protein.

34. The method of claim 30, wherein the serotype is serotype 23B and the method comprises:

(i) reacting a purified *Streptococcus pneumoniae* serotype 23B with an oxidizing agent to produce an activated *Streptococcus pneumoniae* serotype 23B polysaccharide;

(ii) optionally lyophilizing the activated *Streptococcus pneumoniae* serotype 23B polysaccharide and a carrier protein;

(iii) suspending the activated *Streptococcus pneumoniae* serotype 23B polysaccharide and the carrier protein in dimethyl sulfoxide (DMSO);

(iv) reacting the mixture of the activated *Streptococcus pneumoniae* serotype 23B polysaccharide and the carrier protein with a reducing agent to produce a *Streptococcus pneumoniae* serotype 23B polysaccharide-carrier protein conjugate; and

(v) capping unreacted aldehydes in the *Streptococcus pneumoniae* serotype 23B polysaccharide-carrier protein conjugate to prepare an immunogenic conjugate comprising the *Streptococcus pneumoniae* serotype 23B polysaccharide covalently linked to the carrier protein.

35. The method of claim 30, wherein the serotype is serotype 24F and the method comprises:

(i) subjecting a purified *Streptococcus pneumoniae* serotype 24F polysaccharide to an acid hydrolysis reaction or a microfluidizer, and then reacting with an oxidizing agent to produce an activated *Streptococcus pneumoniae* serotype 24F polysaccharide;

(ii) optionally lyophilizing the activated *Streptococcus pneumoniae* serotype 24F polysaccharide and a carrier protein;

(iii) suspending the activated *Streptococcus pneumoniae* serotype 24F polysaccharide and the carrier protein in dimethyl sulfoxide (DMSO) or phosphate buffer;

(iv) reacting the activated *Streptococcus pneumoniae* serotype 24F polysaccharide and the carrier protein with a reducing agent to produce *Streptococcus pneumoniae* serotype 24F polysaccharide-carrier protein conjugate; and

(v) capping unreacted aldehydes in the *Streptococcus pneumoniae* serotype 24F polysaccharide-carrier protein conjugate to prepare an immunogenic conjugate comprising the *Streptococcus pneumoniae* serotype 24F polysaccharide covalently linked to the carrier protein.

36. The method of claim 30, wherein the serotype is serotype 35B and the method comprises:

(i) reacting a purified *Streptococcus pneumoniae* serotype 35B with an oxidizing agent to produce an activated *Streptococcus pneumoniae* serotype 35B polysaccharide;

(ii) optionally lyophilizing the activated *Streptococcus pneumoniae* serotype 35B polysaccharide and a carrier protein;

(iii) suspending the activated *Streptococcus pneumoniae* serotype 35B polysaccharide and the carrier protein in dimethyl sulfoxide (DMSO) or phosphate buffer;

(iv) reacting the activated *Streptococcus pneumoniae* serotype 35B polysaccharide and the carrier protein with a reducing agent to produce *Streptococcus pneumoniae* serotype 35B polysaccharide-carrier protein conjugate; and

(v) capping unreacted aldehydes in the *Streptococcus pneumoniae* serotype 35B polysaccharide-carrier protein conjugate to prepare an immunogenic conjugate comprising the *Streptococcus pneumoniae* serotype 35B polysaccharide covalently linked to the carrier protein.