CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines

Petitioner Merck Ex-1015, 0977

of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0227] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some

Petitioner Merck Ex-1015, 0978

embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0228] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is

Petitioner Merck Ex-1015, 0979

approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0229] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from

Petitioner Merck Ex-1015, 0980

S. pneumoniae serotype 15B in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0230] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of

Petitioner Merck Ex-1015, 0981

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0231] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 1:1

Petitioner Merck Ex-1015, 0982

(w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0232] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A. In some embodiments, the average ratio of

Petitioner Merck Ex-1015, 0983

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines

Petitioner Merck Ex-1015, 0984

of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0233] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some

Petitioner Merck Ex-1015, 0985

embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0234] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of

Petitioner Merck Ex-1015, 0986

immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0235] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from

Petitioner Merck Ex-1015, 0987

S. pneumoniae serotype 22F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0236] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of

Petitioner Merck Ex-1015, 0988

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0237] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F. In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1,7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 1:1

Petitioner Merck Ex-1015, 0989

(w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions and vaccines of the invention may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0238] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average

Petitioner Merck Ex-1015, 0990

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

Petitioner Merck Ex-1015, 0991

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 0992

serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average

Petitioner Merck Ex-1015, 0993

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 5.5:1 and about 6.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

Petitioner Merck Ex-1015, 0994

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0239] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 0995

serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average

Petitioner Merck Ex-1015, 0996

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 5.5:1 and about 6.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0240] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

Petitioner Merck Ex-1015, 0997

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 0998

serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0241] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

Petitioner Merck Ex-1015, 0999

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1000

serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average

Petitioner Merck Ex-1015, 1001

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 5.5:1, 5.6:1, 5.7:1, 5.8:1, 5.9:1, 6:1, 6.1:1, 6.2:1, 6.3:1, 6.4:1, or 6.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

Petitioner Merck Ex-1015, 1002

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0242] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1003

serotype 3 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average

Petitioner Merck Ex-1015, 1004

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 5.5:1, 5.6:1, 5.7:1, 5.8:1, 5.9:1, 6:1, 6.1:1, 6.2:1, 6.3:1, 6.4:1, or 6.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

Petitioner Merck Ex-1015, 1005

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0243] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1006

serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0244] In some embodiments, a vaccine or immunogenic composition comprises:

Petitioner Merck Ex-1015, 1007

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1008

serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average

Petitioner Merck Ex-1015, 1009

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

Petitioner Merck Ex-1015, 1010

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 5.9:1 and about 6.1:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1011

serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0245] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

Petitioner Merck Ex-1015, 1012

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1013

serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 5.9:1 and about 6.1:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0246] In some embodiments, a vaccine or immunogenic composition comprises:

Petitioner Merck Ex-1015, 1014

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae*

Petitioner Merck Ex-1015, 1015

serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0247] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average

Petitioner Merck Ex-1015, 1016

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is about 3:1;

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average

Petitioner Merck Ex-1015, 1017

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average

Petitioner Merck Ex-1015, 1018

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1;

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 6:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1;

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1; and

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average

Petitioner Merck Ex-1015, 1019

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1.

[0248] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is about 3:1;

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;

Petitioner Merck Ex-1015, 1020

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 6:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;

Petitioner Merck Ex-1015, 1021

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1; and

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1.

[0249] In some embodiments, a vaccine or immunogenic composition comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average

Petitioner Merck Ex-1015, 1022

ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1; and

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1.

Immunogenic and Vaccine Compositions

[0250] Another aspect of the disclosure provides compositions that include one or more immunogenic complexes described herein. For example, an immunogenic composition, *e.g.*, vaccine composition, can include one or more immunogenic complexes described herein. In some embodiments, such compositions can include a plurality of one type of immunogenic complex described herein. For example, a composition can include a population of one type of immunogenic complex, where all of the immunogenic complexes include the same antigenic polypeptide and the same antigenic polysaccharide. Additionally or alternatively, such compositions can include a plurality of more than one type of immunogenic complex described herein. For example, a composition can include populations of different types of immunogenic

Petitioner Merck Ex-1015, 1023

complexes. In some embodiments, a composition can include a population of a first type of immunogenic complex and a population of a second type of immunogenic complex, where the first type and the second type of the immunogenic complex have different antigenic polypeptides and/or different antigenic polysaccharides. In some embodiments, a composition can include a population of a first type of immunogenic complex and a population of a second type of immunogenic complex, where the first type and the second type of the immunogenic complex include the same antigenic polypeptide and different antigenic polysaccharides (*e.g.*, polysaccharides of different serotypes). In some embodiments, immunogenic complexes described herein are formulated into a pharmaceutical composition. In some embodiments a pharmaceutical composition may be a vaccine. In some embodiments a pharmaceutical composition comprises a pharmaceutically acceptable carrier. In some embodiments a pharmaceutical composition comprises an adjuvant.

[0251] In some embodiments, an immunogenic composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or more) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1024

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6D capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6E capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6G capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6H capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1025

(14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9L capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1026

(23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(25) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(26) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(27) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(28) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(29) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(30) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11D capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(31) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11E capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1027

(32) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(33) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(34) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(35) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(36) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 13 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(37) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(38) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(39) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(40) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1028

(41) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(42) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(43) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(44) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(45) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(46) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(47) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(48) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(49) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1029

(50) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(51) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(52) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(53) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(54) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(55) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(56) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 21 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(57) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(58) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1030

(59) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(60) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(61) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(62) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(63) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(64) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(65) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 25A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(66) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 25F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(67) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 27 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1031

(68) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 28A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(69) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 28F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(70) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 29 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(71) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 31 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(72) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 32A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(73) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 32F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(74) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(75) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(76) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1032

(77) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33D capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(78) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33E capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(79) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(80) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 34 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(81) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(82) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(83) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(84) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(85) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 36 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1033

(86) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 37 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(87) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 38 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(88) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 39 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(89) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 40 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(90) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 41A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(91) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 41F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(92) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 42 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(93) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 43 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(94) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 44 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1034

(95) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 45 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(96) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 46 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(97) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 47A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(98) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 47F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(99) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 48 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0252] In some embodiments, an immunogenic composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 21, 22, 23, or 24) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1035

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1036

(13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1037

(22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0253] In some embodiments, a vaccine composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1038

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

Petitioner Merck Ex-1015, 1039

(15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0254] In some embodiments, a vaccine composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, or 9) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1040

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

Vaccine compositions

[0255] In some embodiments, a vaccine composition is a polyvalent or multivalent vaccine. In some embodiments, the valency of a vaccine composition refers to the number of species of immunogenic complexes present in the vaccine composition. The valency of a vaccine described herein is not limiting with respect to the total antigens present in said pharmaceutical composition, immunogenic complex, or vaccine, or to the number of pathogen strains for which administration of said pharmaceutical composition, immunogenic complex, immunogenic composition, or vaccine composition may induce an immune-protective response. In a non-limiting example, a 24-valent vaccine composition may comprise more than 24 antigenic components (*e.g.*, peptide and/or polysaccharide components) and may induce an immunoprotective response against more than 24 pathogens, or pathogenic serotypes or strains.

[0256] In some embodiments, a vaccine composition comprises between 1-50 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-40 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-35 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-30 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-30 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-24 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-15 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-9 species of immunogenic complexes. In some embodiments, a vaccine composition comprises between 1-5 species of immunogenic complexes. In some embodiments, a vaccine is a polyvalent vaccine.

Petitioner Merck Ex-1015, 1041

[0257] In some embodiments, a vaccine composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 1 type of immunogenic complex. In some embodiments, a vaccine composition comprises 2 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 4 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 6 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 7 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 8 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 9 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 10 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 11 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 12 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 13 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 14 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 15 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 16 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 17 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 18 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 19 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 20 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 21 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 22 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 23 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 24 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 25 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 26 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 27 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 28 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 29 species of immunogenic complexes. In

Petitioner Merck Ex-1015, 1042

some embodiments, a vaccine composition comprises 30 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 35 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 40 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 45 species of immunogenic complexes. In some embodiments, a vaccine composition comprises 50 species of immunogenic complexes.

[0258] In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharides in the vaccine composition from each immunogenic complex is about the same, *e.g.*, present in a w/w ratio of about 1:1. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.20 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.25 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 1 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 1.5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 2 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 2.5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 3 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 3.5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 4 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 4.5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 5.5 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 6 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 7 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 8

Petitioner Merck Ex-1015, 1043

μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 9 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 10 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 11 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 12 μg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is more than 12 μg, *e.g.*, 13 μg, 14 μg, 15 μg, 16 μg, 17 μg, 18 μg, 19 μg, 20 μg, 21 μg, 22 μg, 23 μg, 24 μg, 25 μg, or more.

[0259] In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharides in the vaccine composition contributed by each immunogenic complex is different, *e.g.*, present in a w/w ratio that is not about 1:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:2. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:3. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:4. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:5. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:6. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in

Petitioner Merck Ex-1015, 1044

amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:7. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:8. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:9. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharide in the vaccine composition contributed by a first immunogenic complex and a second immunogenic complex is 1:10. In some embodiments, the vaccine composition comprises a mixture of immunogenic complexes, such that the weight of polysaccharide in a vaccine contributed by an immunogenic complex ranges from about 0.20 μg to about 6 μg. In some embodiments, the vaccine composition comprises a mixture of immunogenic complexes, such that the weight of polysaccharide in a vaccine contributed by an immunogenic complex ranges from about 0.20 μg to about 12 μg. In some embodiments, the vaccine composition comprises a mixture of immunogenic complexes, such that the weight of polysaccharides in the vaccine contributed by each immunogenic complex ranges from about 0.20 μg to about 20 μg. In some embodiments, the vaccine composition comprises a mixture of immunogenic complexes, such that the weight of polysaccharides in the vaccine contributed by each immunogenic complex ranges from about 0.20 μg to about 40 μg.

[0260] In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is about the same, *e.g.*, present in a w/w protein:PS ratio of about 1:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition)

Petitioner Merck Ex-1015, 1045

is present in a w/w protein:PS ratio of about 2:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 3:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 4:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 5:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 6:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 7:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 8:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 9:1. In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions)

Petitioner Merck Ex-1015, 1046

in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex (*e.g.*, in an immunogenic composition) is present in a w/w protein:PS ratio of about 10:1.

[0261] In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 0.20 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 0.40 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 1 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 2 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 3 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 4 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 5 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 6 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 7 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 8 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 9 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 10 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 11 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 12 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 14 μg. In some embodiments, the combined weight of polysaccharides and polypeptides

Petitioner Merck Ex-1015, 1047

in the vaccine contributed by each immunogenic composition is about 16 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 18 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 20 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 21 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 22 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 23 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 24 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 25 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 30 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 40 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 50 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 60 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 70 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 80 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 90 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 100 μg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 110 μg.

Petitioner Merck Ex-1015, 1048

[0262] In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the combined weight of polysaccharides and polypeptides in the vaccine composition contributed by each immunogenic complex is different, *e.g.*, present in a w/w protein:PS ratio that is not about 1:1, *e.g.*, a protein:PS ratio that is 2:1, 3:1, 4:1. 5:1. 6:1, 7:1, 8:1, 9:1, or 10:1. In some embodiments, the vaccine composition comprises a mixture of immunogenic complexes, such that the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex ranges from about 0.4 μg to about 110 μg.

[0263] In some embodiments, a vaccine composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or more) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1049

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6D capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6E capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6G capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6H capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1050

(15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9L capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1051

(24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(25) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(26) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(27) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(28) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(29) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(30) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11D capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(31) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11E capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(32) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1052

(33) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(34) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(35) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(36) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 13 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(37) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(38) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(39) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(40) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(41) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1053

(42) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(43) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(44) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(45) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(46) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(47) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(48) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(49) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(50) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1054

(51) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(52) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(53) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(54) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(55) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(56) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 21 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(57) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(58) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(59) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1055

(60) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(61) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(62) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(63) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(64) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(65) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 25A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(66) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 25F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(67) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 27 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(68) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 28A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1056

(69) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 28F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(70) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 29 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(71) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 31 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(72) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 32A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(73) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 32F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(74) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(75) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(76) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(77) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33D capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1057

(78) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33E capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(79) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(80) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 34 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(81) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(82) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(83) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(84) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(85) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 36 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(86) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 37 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1058

(87) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 38 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(88) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 39 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(89) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 40 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(90) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 41A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(91) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 41F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(92) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 42 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(93) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 43 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(94) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 44 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(95) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 45 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1059

(96) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 46 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(97) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 47A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(98) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 47F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(99) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 48 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0264] In some embodiments, a vaccine composition comprises two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 21, 22, 23, or 24) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1060

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1061

(14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1062

(23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0265] In some embodiments, a vaccine composition comprises two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1063

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

Petitioner Merck Ex-1015, 1064

[0266] In some embodiments, a vaccine composition comprises two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or 9) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

Petitioner Merck Ex-1015, 1065

(9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0267] In some embodiments, a vaccine composition comprises:

(1) a first immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) a second immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) a third immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) a fourth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) a fifth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) a sixth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) a seventh immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an eighth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1066

(9) a ninth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) a tenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an eleventh immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) a twelfth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(13) a thirteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(14) a fourteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(15) a fifteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(16) a sixteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(17) a seventeenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1067

(18) an eighteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(19) a nineteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(20) a twentieth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(21) a twenty-first immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(22) a twenty-second immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(23) a twenty-third immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(24) a twenty-fourth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0268] In some embodiments, a vaccine composition comprises:

(1) a first immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) a second immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1068

(3) a third immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) a fourth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) a fifth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) a sixth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(7) a seventh immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an eighth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(9) a ninth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(10) a tenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(11) an eleventh immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(12) a twelfth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1069

(13) a thirteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(14) a fourteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(15) a fifteenth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0269] In some embodiments, a vaccine composition comprises:

(1) a first immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) a second immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) a third immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) a fourth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) a fifth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) a sixth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

Petitioner Merck Ex-1015, 1070

(7) a seventh immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(8) an eighth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(9) a ninth immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

Conjugated Immunogenic Complexes; Immunogenic and Vaccine Compositions Comprising Same

[0270] In some embodiments, one or more polypeptides (*e.g.*, antigenic polypeptides) of immunogenic complexes are conjugated to one or more polysaccharides. In some embodiments, one or more conjugated polysaccharides comprise a capsular polysaccharide of *S. pneumoniae*. In some embodiments, one or more polypeptides of conjugated immunogenic complex comprise an antigenic polypeptide of *S. pneumoniae*. In some embodiments, an antigenic polypeptide of a conjugated immunogenic complex is or comprises a fusion protein. In some such embodiments, a fusion protein of a conjugated immunogenic complex is or comprises CP1 fusion protein.

[0271] In some embodiments, a conjugated immunogenic complex comprises one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48. In some embodiments, a conjugated immunogenic complex comprises one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, an immunogenic complex comprises

Petitioner Merck Ex-1015, 1071

one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F. In some embodiments, an immunogenic complex comprises one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B.

[0272] In some embodiments, a conjugated immunogenic complex comprises:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0273] In some embodiments, a conjugated immunogenic complex comprises:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or are derived, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0274] In some embodiments, a conjugated immunogenic complex comprises:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0275] In some embodiments, a conjugated immunogenic complex comprises:

(a) one or more CP1 proteins; and

Petitioner Merck Ex-1015, 1072

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0276] In some embodiments, an immunogenic composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48,
wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0277] In some embodiments, an immunogenic composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0278] In some embodiments, an immunogenic composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

Petitioner Merck Ex-1015, 1073

[0279] In some embodiments, an immunogenic composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0280] In some embodiments, a vaccine composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 protein; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48,
wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0281] In some embodiments, a vaccine composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0282] In some embodiments, a vaccine composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

Petitioner Merck Ex-1015, 1074

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

[0283] In some embodiments, a vaccine composition comprises one or more conjugated immunogenic complexes comprising:

(a) one or more CP1 proteins; and

(b) one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B, wherein the one or more CP1 proteins are covalently attached to the one or more polysaccharides.

Uses of Immunogenic and Vaccine Compositions

[0284] In some embodiments, an immunogenic complex described herein that includes one or more antigenic polysaccharides is characterized in that one or more of the opsonization potential, or immune response to one or more antigenic polysaccharides is increased relative to a predetermined level, as measured by ELISA and or by a functional antibody assay. In some embodiments, one or more of the opsonization potential, immune response to the one or more antigenic polysaccharides is increased at least 1-fold, 2-fold, 3-fold, 4-fold, or 5-fold relative to a predetermined level, as measured by ELISA and or by a functional antibody assay. In some embodiments, the predetermined level is a pre-immune level. In some embodiments, the predetermined level is a pre-immune level. In some embodiments, one or more polypeptide antigens are carrier proteins for one or more antigenic polysaccharides.

[0285] In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces an immune response against one or more pathogens in the subject at a level greater than a composition comprising an antigenic polysaccharide alone. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces an immune response against one or more pathogens in the subject at a level greater than a composition comprising a polypeptide antigen alone. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response.

Petitioner Merck Ex-1015, 1075

[0286] In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces an immune response against *S. pneumoniae*. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces an immune response against one or more serotypes of *S. pneumoniae*. In some embodiments, such an immune response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic complex described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an immune response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic complex described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an immune response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic complex described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response against one or more serotypes of *S. pneumoniae*. In some embodiments, such a protective response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic complex described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such a protective response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic complex described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such a protective response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic complex described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and

Petitioner Merck Ex-1015, 1076

(ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0287] In some embodiments, the immune response is an antibody or B cell response. In some embodiments, the immune response is a T cell response. In some embodiments, the immune response is an innate immune response. In some embodiments, the immune response is a CD4+ T cell response, including T_H1, T_H2, or T_H17 response, or a CD8+ T cell response, or a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response. In some embodiments, the immune response is an antibody or B cell response, and a T cell response. In some embodiments, the immune response is an antibody or B cell response, a T cell response, and an innate immune response. In some embodiments, the immune response is a protective immune response.

[0288] In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces antibody production against one or more pathogens in the subject at a level greater than a composition comprising an antigenic polysaccharide alone. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces antibody production against one or more pathogens in the subject at level greater than a composition comprising a polypeptide antigen alone.

[0289] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against one or more pathogens in the subject at a level greater than a composition comprising an antigenic polysaccharide alone. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against one or more pathogens in the subject at a level greater than a composition comprising a polypeptide antigen alone. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response.

[0290] The *S. pneumoniae* immunogenic compositions and vaccines described herein may be used for prophylactic and/or therapeutic treatment of *S. pneumoniae*. Accordingly, this application provides a method for immunizing a subject suffering from or susceptible to *S. pneumoniae* infection, comprising administering an immunologically effective amount of any of

Petitioner Merck Ex-1015, 1077

the immunogenic compositions or vaccine formulations described herein. The subject receiving the vaccination may be a male or a female, and may be an infant, child, adolescent, or adult. In some embodiments, the subject being treated is a human. In other embodiments, the subject is a non-human animal. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response against one or more serotypes of *S. pneumoniae*.

[0291] In prophylactic embodiments, a vaccine composition (*e.g.*, ones as described and/or utilized herein) is administered to a subject to induce an immune response that can help protect against the establishment of *S. pneumoniae*, for example by protecting against colonization, the first and necessary step in disease. In some embodiments, such an immune response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a vaccine composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an immune response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a vaccine composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s) (non-vaccine types, NVTs). In some embodiments, such an immune response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a vaccine composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). Thus, in some aspects, the method inhibits infection by *S. pneumoniae* in a non-colonized or uninfected subject. In another aspect, the method may reduce the duration of colonization in a subject who is already colonized.

[0292] In therapeutic embodiments, the vaccine may be administered to a subject suffering from *S. pneumoniae* infection, in an amount sufficient to treat the subject. Treating the subject, in this case, refers to reducing *S. pneumoniae* symptoms and/or bacterial load and/or sequelae in an infected subject. In some embodiments, treating the subject refers to reducing the duration of symptoms or sequelae, or reducing the intensity of symptoms or sequelae. In some

Petitioner Merck Ex-1015, 1078

embodiments, the vaccine reduces transmissibility of *S. pneumoniae* from the vaccinated subject. In certain embodiments, the reductions described above are at least 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.

[0293] In therapeutic embodiments, the vaccine is administered to a subject post-infection. The vaccine may be administered shortly after infection, *e.g.* before symptoms or sequelae manifest, or may be administered during or after manifestation of symptoms or sequelae.

[0294] In some embodiments, the vaccine compositions of the invention confer protective immunity, allowing a vaccinated subject to exhibit delayed onset of symptoms or sequelae, or reduced severity of symptoms or sequelae, as the result of his or her exposure to the vaccine. In certain embodiments, the reduction in severity of symptoms or sequelae is at least 25%, 40%, 50%, 60%, 70%, 80%, or 90%. In particular embodiments, vaccinated subjects may display no symptoms or sequelae upon contact with *S. pneumoniae*, do not become colonized by *S. pneumoniae*, or both. Protective immunity is typically achieved by one or more of the following mechanisms: mucosal, humoral, or cellular immunity. Mucosal immunity is primarily the result of secretory IgA (sIGA) antibodies on mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts. The sIGA antibodies are generated after a series of events mediated by antigen-processing cells, B and T lymphocytes, that result in sIGA production by B lymphocytes on mucosa-lined tissues of the body. Humoral immunity is typically the result of IgG antibodies and IgM antibodies in serum. Cellular immunity can be achieved through cytotoxic T lymphocytes or through delayed-type hypersensitivity that involves macrophages and T lymphocytes, as well as other mechanisms involving T cells without a requirement for antibodies. In particular, cellular immunity may be mediated by T_H1 or T_H17 cells.

[0295] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against *S. pneumoniae*. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or more) serotypes of *S. pneumoniae*. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to

Petitioner Merck Ex-1015, 1079

a subject, induces an immune response against all serotypes of *S. pneumoniae* comprised in such immunogenic composition or vaccine. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or more) serotypes of *S. pneumoniae*. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response against all serotypes of *S. pneumoniae* comprised in such immunogenic composition or vaccine.

[0296] In some embodiments, the immune response is an antibody or B cell response. In some embodiments, the immune response is a T cell response. In some embodiments, the immune response is an innate immune response. In some embodiments, the immune response is a CD4+ T cell response, including T_H1, T_H2, or T_H17 response, or a CD8+ T cell response, or a CD4+ and CD8+ T cell response, or CD4-/CD8- T cell response. In some embodiments, the immune response is an antibody or B cell response, and a T cell response. In some embodiments, the immune response is an antibody or B cell response, a T cell response, and an innate immune response. In some embodiments, the immune response is a protective immune response.

[0297] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an antibody or B cell response against one or more pathogens in the subject at a level greater than a composition comprising an antigenic polysaccharide alone. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an antibody or B cell response against one or more pathogens in the subject at level greater than a composition comprising a polypeptide antigen alone. In some embodiments, the immune response is a protective immune response.

[0298] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces a T cell response against one or more pathogens in the subject at a level greater than a composition comprising an antigenic polysaccharide alone. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces a T cell response against one or more pathogens in the subject at level greater than a composition comprising a polypeptide antigen alone. In some embodiments, the immune response is a protective immune response.

Petitioner Merck Ex-1015, 1080

[0299] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents Invasive Pneumococcal Disease (IPD) due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents bacteremia due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents sepsis due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents organ damage due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents meningitis due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents pneumonia due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents otitis media due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents sinusitis due to infection by *S. pneumoniae*.

[0300] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of Invasive Pneumococcal Disease (IPD) due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of bacteremia due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of sepsis due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of organ

Petitioner Merck Ex-1015, 1081

damage due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of meningitis due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of pneumonia due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of otitis media due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of sinusitis due to infection by *S. pneumoniae*.

[0301] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of Invasive Pneumococcal Disease (IPD) due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of bacteremia due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of sepsis due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of organ damage due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of meningitis due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of pneumonia due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of otitis media due to infection by *S. pneumoniae*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of sinusitis due to infection by *S. pneumoniae*.

Petitioner Merck Ex-1015, 1082

[0302] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits transmission of *S. pneumoniae* from the subject to another subject. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits colonization by *S. pneumoniae* in the subject. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits colonization by *S. pneumoniae* in the nasopharynx of the subject.

[0303] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against one or more pathogens in the subject at a level greater than a control composition. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces a protective immune response against one or more pathogens in the subject at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0304] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody of one or more *S. pneumoniae* serotypes. In some embodiments the

Petitioner Merck Ex-1015, 1083

subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0305] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody of one or more *S. pneumoniae* serotypes. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0306] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A , 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that

Petitioner Merck Ex-1015, 1084

elicited by administration of PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody of one or more *S. pneumoniae* serotypes. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0307] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody of one or more *S. pneumoniae* serotypes. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0308] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F

Petitioner Merck Ex-1015, 1085

and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity non-inferior to (*e.g.*, immunogenicity at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody of one or more *S. pneumoniae* serotypes. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0309] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F

Petitioner Merck Ex-1015, 1086

and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity superior to that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody of one or more *S. pneumoniae* serotypes. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0310] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include

Petitioner Merck Ex-1015, 1087

polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against (i) one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0311] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a

Petitioner Merck Ex-1015, 1088

functional antibody titer at least as effective as) that elicited by administration of PCV13 against (i) one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0312] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against (i) one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14,

Petitioner Merck Ex-1015, 1089

15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0313] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 6A and 20B. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against each of *S. pneumoniae* serotypes 6A and 20B. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PPSV23 against (i) one or more of *S. pneumoniae* serotypes 6A and 20B, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is

Petitioner Merck Ex-1015, 1090

a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0314] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against (i) one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

Petitioner Merck Ex-1015, 1091

[0315] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 against (i) one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0316] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23

Petitioner Merck Ex-1015, 1092

against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against (i) one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0317] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 6A and 20B. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against each of *S. pneumoniae* serotypes 6A and 20B. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine

Petitioner Merck Ex-1015, 1093

types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PPSV23 against (i) one or more of *S. pneumoniae* serotypes 6A and 20B, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0318] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A,

Petitioner Merck Ex-1015, 1094

12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against (i) one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against (i) one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

Petitioner Merck Ex-1015, 1095

[0319] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer non-inferior to (*e.g.*, a functional antibody titer at least as effective as) that elicited by administration of PCV13 in combination with PPSV23 against (i) one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

Petitioner Merck Ex-1015, 1096

[0320] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against (i) one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against (i) one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B,

Petitioner Merck Ex-1015, 1097

17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0321] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against (i) one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not

Petitioner Merck Ex-1015, 1098

include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0322] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against (i) one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against one or more *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C,

Petitioner Merck Ex-1015, 1099

19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments the amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the total amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the pre-determined threshold is a blood serum or plasma concentration between about 0.20 μg/mL to about 0.35 μg/mL. In some embodiments the pre-determined threshold is at least about 0.35 μg/mL. In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0323] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against (i) one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the immunogenicity

Petitioner Merck Ex-1015, 1100

is determined by measuring the amount of anti-capsular polysaccharide antibody against one or more *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In some embodiments the amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the total amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the pre-determined threshold is a blood serum or plasma concentration between about 0.20 μg/mL to about 0.35 μg/mL. In some embodiments the pre-determined threshold is at least about 0.35 μg/mL. In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0324] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against (i) one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein

Petitioner Merck Ex-1015, 1101

such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against one or more *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F. In some embodiments the amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the total amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the pre-determined threshold is a blood serum or plasma concentration between about 0.20 μg/mL to about 0.35 μg/mL. In some embodiments the pre-determined threshold is at least about 0.35 μg/mL. In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0325] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, (i) elicits a functional antibody titer superior to that elicited by administration of PCV13 against one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F; and (ii) elicits immunogenicity against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, (i) elicits a functional antibody titer superior to that elicited by administration of PCV13 against each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F; and (ii) elicits immunogenicity against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F,

Petitioner Merck Ex-1015, 1102

18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits (i) a functional antibody titer superior to that elicited by administration of PCV13 and (ii) immunogenicity (*e.g.*, an innate immune response, an antibody or B cell response, and/or a T cell response), against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits (i) a functional antibody titer superior to that elicited by administration of PCV13 and (ii) immunogenicity (*e.g.*, an innate immune response, an antibody or B cell response, and/or a T cell response), against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and also against one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the total amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the pre-determined threshold is a blood serum or plasma concentration between about 0.20 μg/mL to about 0.35 μg/mL. In some embodiments the pre-determined threshold is at least about 0.35 μg/mL. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

Petitioner Merck Ex-1015, 1103

[0326] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, (i) elicits a functional antibody titer superior to that elicited by administration of PPSV23 against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F; and (ii) elicits immunogenicity against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, (i) elicits a functional antibody titer superior to that elicited by administration of PPSV23 against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F; and (ii) elicits immunogenicity against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits (i) a functional antibody titer superior to that elicited by administration of PPSV23 and (ii) immunogenicity (*e.g.*, an innate immune response, an antibody or B cell response, and/or a T cell response), against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits (i) a functional antibody titer superior to that elicited by administration of PPSV23 and (ii) immunogenicity (*e.g.*, an innate immune response, an antibody or B cell response, and/or a T cell response), against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and also against one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the amount of

Petitioner Merck Ex-1015, 1104

anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the total amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the pre-determined threshold is a blood serum or plasma concentration between about 0.20 μg/mL to about 0.35 μg/mL. In some embodiments the pre-determined threshold is at least about 0.35 μg/mL. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0327] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, (i) elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F; and (ii) elicits immunogenicity against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, (i) elicits a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 against each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F; and (ii) elicits immunogenicity against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits (i) a functional antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 and (ii) immunogenicity (*e.g.*, an innate immune response, an antibody or B cell response, and/or a T cell response), against one or more *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, elicits (i) a functional

Petitioner Merck Ex-1015, 1105

antibody titer superior to that elicited by administration of PCV13 in combination with PPSV23 and (ii) immunogenicity (*e.g.*, an innate immune response, an antibody or B cell response, and/or a T cell response), against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and also against one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments, the immunogenicity is determined by measuring the amount of anti-capsular polysaccharide antibody against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, the functional antibody titer is measured by an opsonophagocytic assay (*e.g.*, ones as described herein). In some embodiments the amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the total amount of anti-capsular polysaccharide antibody measured is an amount that meets a pre-determined threshold. In some embodiments the pre-determined threshold is a blood serum or plasma concentration between about 0.20 μg/mL to about 0.35 μg/mL. In some embodiments the pre-determined threshold is at least about 0.35 μg/mL. In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0328] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits an immunoprotective response against one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits an immunoprotective response against each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14,

Petitioner Merck Ex-1015, 1106

15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits an immunoprotective response against (i) at least one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs).

[0329] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 by (i) at least one or more of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0330] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine

Petitioner Merck Ex-1015, 1107

described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 by (i) at least one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0331] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F to a greater degree than administration of PCV13. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 by (i) at least one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A,

Petitioner Merck Ex-1015, 1108

11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0332] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PPSV23 by (i) at least one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some

Petitioner Merck Ex-1015, 1109

embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0333] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 6A and 20B to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 6A and 20B to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PPSV23 by (i) at least one or more of *S. pneumoniae* serotypes 6A and 20B, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0334] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F,

Petitioner Merck Ex-1015, 1110

20B, 22F, 23F, and 33F to a greater degree than administration of PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PPSV23 by (i) at least one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older.

[0335] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 in combination with PPSV23 by (i) at least one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20A, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae*

Petitioner Merck Ex-1015, 1111

serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0336] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 in combination with PPSV23 by (i) at least one or more of *S. pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of

Petitioner Merck Ex-1015, 1112

age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.

[0337] In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization by each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F to a greater degree than administration of PCV13 in combination with PPSV23. In some embodiments an immunogenic composition or vaccine described herein, upon administration to a subject, reduces *S. pneumoniae* colonization to a greater degree than administration of PCV13 in combination with PPSV23 by (i) at least one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F, and (ii) one or more additional *S. pneumoniae* serotypes, wherein such an immunogenic composition or vaccine does not include polysaccharide(s) present in at least one or more of such additional *S. pneumoniae* serotypes (non-vaccine types, NVTs). In some embodiments the subject is a human. In some embodiments the human is between about 2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, the PCV13 is administered before the PPSV23. In some embodiments, the PCV13 is administered after the PPSV23. In some embodiments, the PCV13 is administered at approximately the same time as the PPSV23.In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response that can help protect

Petitioner Merck Ex-1015, 1113

against the establishment of *S. pneumoniae*, at a level greater than a control composition. In some embodiments, an immunogenic composition or vaccine described herein protects against colonization at a level greater than a control composition. In some embodiments, an immunogenic composition or vaccine described herein inhibits infection by *S. pneumoniae* in a non-colonized or uninfected subject at a level greater than a control composition. In some embodiments, an immunogenic composition or vaccine described herein reduces the duration of colonization by *S. pneumoniae* in a subject who is already colonized at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0338] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against *S. pneumoniae* at a level greater than a control composition. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against one or more serotypes of *S. pneumoniae* at a level greater than a control composition. In some embodiments, the immune response is an antibody or B cell response. In some embodiments, the immune response is a T cell response. In some embodiments, the immune response is an innate immune response. In some embodiments, the immune response is a CD4+ T cell response, including T_H1, T_H2, or T_H17 response, or a CD8+ T cell response, or a CD4+ and CD8+ T cell response, or CD4-/CD8- T cell response. In some embodiments, the immune response is an antibody or B cell response, and a T cell response. In some embodiments, the immune response is an antibody or B cell response, a T cell response, and an innate immune response. In some embodiments, the immune response is a protective immune response. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about

Petitioner Merck Ex-1015, 1114

24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0339] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an antibody or B cell response against one or more pathogens in the subject at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0340] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an antibody or B cell response against one or more pathogens in the subject at level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0341] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces a T cell response against one or more pathogens in the subject at a level greater than a control composition. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces a T cell response against one or more pathogens in the subject at level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%,

Petitioner Merck Ex-1015, 1115

about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0342] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents Invasive Pneumococcal Disease (IPD) due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents bacteremia due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents sepsis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents organ damage due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents meningitis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents pneumonia due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents otitis media due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents sinusitis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%,

Petitioner Merck Ex-1015, 1116

about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0343] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of Invasive Pneumococcal Disease (IPD) due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of bacteremia due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of sepsis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of organ damage due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of meningitis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of pneumonia due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits or reduces the rate of occurrence of otitis media due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine

Petitioner Merck Ex-1015, 1117

described herein inhibits or reduces the rate of occurrence of sinusitis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0344] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of Invasive Pneumococcal Disease (IPD) due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of bacteremia due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of sepsis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of organ damage due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, the control composition may be PCV13 or PPSV23. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of meningitis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of pneumonia due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of otitis media due to

Petitioner Merck Ex-1015, 1118

infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of sinusitis due to infection by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0345] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits transmission of *S. pneumoniae* from the subject to another subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits colonization by *S. pneumoniae* in the subject at a level greater than a control composition. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits colonization by *S. pneumoniae* in the nasopharynx of the subject at a level greater than a control composition. In some embodiments, the level greater is about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% of the control composition. In some embodiments, the control composition may be PCV13 or PPSV23.

[0346] In some embodiments, an immunogenic composition or vaccine described herein is administered to a subject between about 6 weeks and about 5 years (*e.g.*, prior to the 6th birthday) for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

Petitioner Merck Ex-1015, 1119

[0347] In some embodiments, a MAPS24 vaccine is administered to a subject between about 6 weeks and about 5 years (*e.g.*, prior to the 6th birthday) for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0348] In some embodiments an immunogenic composition or vaccine described herein is administered to a subject between about 6 years and about 17 years (*e.g.*, prior to the 18th birthday) for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0349] In some embodiments, a MAPS24 vaccine is administered to a subject between about 6 years and about 17 years (*e.g.*, prior to the 18th birthday) for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0350] In some embodiments an immunogenic composition or vaccine described herein is administered to a subject 18 years or older for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0351] In some embodiments, a MAPS24 vaccine is administered to a subject 18 years or older for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0352] In some embodiments an immunogenic composition or vaccine described herein is administered to a subject 18 years or older for active immunization for the prevention of pneumonia caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0353] In some embodiments, a MAPS24 vaccine is administered to a subject 18 years or older for active immunization for the prevention of pneumonia caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

Petitioner Merck Ex-1015, 1120

[0354] In some embodiments an immunogenic composition or vaccine described herein is administered to a subject 18 years or older for active immunization for the prevention of invasive disease and pneumonia caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

[0355] In some embodiments, a MAPS24 vaccine is administered to a subject 18 years or older for active immunization for the prevention of invasive disease and pneumonia caused by *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F.

Antibody Compositions

[0356] Some embodiments provide for an antibody composition comprising antibodies raised in a mammal immunized with an immunogenic complex of the invention. In some embodiments, an antibody comprises at least one antibody selected from the group consisting of mAbs and anti-idiotype antibodies. In some embodiments, an antibody composition comprises an isolated gamma globulin fraction. In some embodiments, an antibody composition comprises polyclonal antibodies. In some embodiments, the antibody composition is administered to a subject. In some embodiments, the antibody composition administered to a subject confers passive immunization.

Vaccine Formulations

[0357] Optimal amounts of components for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects can receive one or several booster immunizations adequately spaced in time.

[0358] The immunogenic complexes described herein, and/or preparations thereof may be formulated in a unit dosage form for ease of administration and uniformity of dosage. The specific therapeutically effective dose level for any particular subject or organism may depend upon a variety of factors including the severity or degree of risk of infection; the activity of the specific vaccine or vaccine composition employed; other characteristics of the specific vaccine or vaccine composition employed; the age, body weight, general health, sex of the subject, diet of the subject, pharmacokinetic condition of the subject, the time of administration (*e.g.*, with

Petitioner Merck Ex-1015, 1121

regard to other activities of the subject such as eating, sleeping, receiving other medicines including other vaccine doses, etc.), route of administration, rate of excretion of the specific vaccine or vaccine composition employed; vaccines used in combination or coincidental with the vaccine composition employed; and like factors well known in the medical arts.

[0359] Immunogenic complexes for use in accordance with the present disclosure may be formulated into compositions (*e.g.*, pharmaceutical compositions) according to known techniques. Vaccine preparation is generally described in Vaccine Design (Powell and Newman, 1995). For example, an immunologically amount of a vaccine product can be formulated together with one or more organic or inorganic, liquid or solid, pharmaceutically suitable carrier materials. Preparation of pneumococcal polysaccharide and conjugate vaccines is described, for example, in USSN 11/395,593, filed March 31, 2006, the contents of which are incorporated herein by reference.

[0360] In general, pharmaceutically acceptable carrier(s) include solvents, dispersion media, and the like, which are compatible with pharmaceutical administration. For example, materials that can serve as pharmaceutically acceptable carriers include, but are not limited to sugars such as lactose, glucose, dextrose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; polyols such as glycerol, propylene glycol, and liquid polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as preservatives, and antioxidants can also be present in the composition, according to the judgment of the formulator (Martin, 1975).

[0361] Vaccines may be formulated by combining one or more of the immunogenic complexes disclosed herein with carriers and/or other optional components by any available means including, for example, conventional mixing, granulating, dissolving, lyophilizing, or similar processes.

Petitioner Merck Ex-1015, 1122

[0362] Vaccine compositions useful in the provided methods may be lyophilized up until they are about to be used, at which point they are extemporaneously reconstituted with diluent. In some embodiments, vaccine components or compositions are lyophilized in the presence of one or more other components (*e.g.*, adjuvants), and are extemporaneously reconstituted with saline solution. Alternatively, individual components, or sets of components may be separately lyophilized and/or stored (*e.g.*, in a vaccination kit), the components being reconstituted and either mixed prior to use or administered separately to the subject.

[0363] Lyophilization can produce a more stable composition (for instance by preventing or reducing breakdown of polysaccharide antigens). Lyophilizing of vaccines or vaccine components is well known in the art. Typically, a liquid vaccine or vaccine component is freeze dried, often in the presence of an anti-caking agent (such as, for example, sugars such as sucrose or lactose). In some embodiments, the anti-caking agent is present, for example, at an initial concentration of 10-200 mg/ml. Lyophilization typically occurs over a series of steps, for instance a cycle starting at -69° C, gradually adjusting to -24°C over 3 h, then retaining this temperature for 18 h, then gradually adjusting to -16°C over 1 h, then retaining this temperature for 6 h, then gradually adjusting to +34°C over 3 h, and finally retaining this temperature over 9 h.

[0364] In some embodiments, a vaccine is a liquid. In some embodiments the liquid is a reconstituted lyophylate. In some embodiments a vaccine has a pH of about 5, about 6, about 7, or about 8. In some embodiments a vaccine has a pH between about 5 and about 7.5. In some embodiments a vaccine has a pH between 5 and 7.5. In some embodiments a vaccine has a pH between about 5.3 and about 6.3. In some embodiments a vaccine has a pH between 5.3 and 6.3. In some embodiments a vaccine has a pH of about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5.

[0365] Vaccines or vaccine components for use in accordance with the present invention may be incorporated into liposomes, cochleates, biodegradable polymers such as poly-lactide, poly-glycolide and poly-lactide-co-glycolides, or immune-stimulating complexes (ISCOMs).

Petitioner Merck Ex-1015, 1123

[0366] In certain situations, it may be desirable to prolong the effect of a vaccine or for use in accordance with the present invention, for example by slowing the absorption of one or more vaccine components. Such delay of absorption may be accomplished, for example, by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the product then depends upon its rate of dissolution, which in turn, may depend upon size and form. Alternatively, or additionally, delayed absorption may be accomplished by dissolving or suspending one or more vaccine components in an oil vehicle. Injectable depot forms can also be employed to delay absorption. Such depot forms can be prepared by forming microcapsule matrices of one or more vaccine components a biodegradable polymers network. Depending upon the ratio of polymer to vaccine component, and the nature of the particular polymer(s) employed, the rate of release can be controlled.

[0367] Examples of biodegradable polymers that can be employed in accordance with the present invention include, for example, poly(orthoesters) and poly(anhydrides). One particular exemplary polymer is polylactide-polyglycolide.

[0368] Depot injectable formulations may also be prepared by entrapping the product in liposomes or microemulsions, which are compatible with body tissues.

[0369] Polymeric delivery systems can also be employed in non-depot formulations including, for example, oral formulations. For example, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid, etc., can be used in oral formulations. Polysaccharide antigens or conjugates may be formulated with such polymers, for example to prepare particles, microparticles, extrudates, solid dispersions, admixtures, or other combinations in order to facilitate preparation of useful formulations (*e.g.*, oral).

[0370] Vaccines for use in accordance with the present invention include immunogenic compositions, and may additionally include one or more additional active agents (*i.e.*, agents that exert a biological effect – not inert ingredients). For example, it is common in vaccine preparation to include one or more adjuvants. It will be appreciated that such additional agents may be formulated together with one or more other vaccine components, or may be maintained separately and combined at or near the time of administration. In some embodiments, such

Petitioner Merck Ex-1015, 1124

additional components may be administered separately from some or all of the other vaccine components, within an appropriate time window for the relevant effect to be achieved.

Adjuvants

[0371] The vaccine formulations and immunogenic compositions described herein may include an adjuvant. Adjuvants, generally, are agents that enhance the immune response to an antigen. Adjuvants can be broadly separated into two classes, based on their principal mechanisms of action: vaccine delivery systems and immunostimulatory adjuvants (see, *e.g.*, Singh et al, 2003). In most vaccine formulations, the adjuvant provides a signal to the immune system so that it generates a response to the antigen, and the antigen is required for driving the specificity of the response to the pathogen. Vaccine delivery systems are often particulate formulations, *e.g.*, emulsions, microparticles, immune-stimulating complexes (ISCOMs), nanoparticles, which may be, for example, particles and/or matrices, and liposomes. In contrast, immunostimulatory adjuvants are sometimes from or derived from pathogens and can represent pathogen associated molecular patterns (PAMP), *e.g.*, lipopolysaccharides (LPS), monophosphoryl lipid A (MPL), or CpG-containing DNA, which activate cells of the innate immune system.

[0372] Alternatively, adjuvants may be classified as organic and inorganic. Inorganic adjuvants include alum salts such as aluminum phosphate, amorphous aluminum hydroxyphosphate sulfate, and aluminum hydroxide, which are commonly used in human vaccines. Organic adjuvants comprise organic molecules including macromolecules. Non-limiting examples of organic adjuvants include cholera toxin/toxoids, other enterotoxins/toxoids or labile toxins/toxoids of Gram-negative bacteria, interleukins (*e.g.*, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, etc.), interferons (*e.g.*, gamma interferon), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), and tumor necrosis factor (TNF).

[0373] Adjuvants may also be classified by the response they induce. In some embodiments, the adjuvant induces the generation, proliferation, or activation of T_H1 cells or T_H2 cells. In other embodiments, the adjuvant induces the generation, proliferation, or activation of B cells. In yet other embodiments, the adjuvant induces the activation of antigen-presenting

Petitioner Merck Ex-1015, 1125

cells. These categories are not mutually exclusive; in some cases, an adjuvant activates more than one type of cell.

[0374] In certain embodiments, the adjuvant induces the generation, proliferation, or activation of T_H17 cells. The adjuvant may promote the CD4+ or CD8+ T cells to secrete IL-17. In some embodiments, an adjuvant that induces the generation, proliferation, or activation of T_H17 cells is one that produces at least a 2-fold, and in some cases a 10-fold, experimental sample to control ratio in the following assay. In the assay, an experimenter compares the IL-17 levels secreted by two populations of cells: (1) cells from animals immunized with the adjuvant and a polypeptide known to induce T_H17 generation, proliferation, or activation, and (2) cells from animals treated with the adjuvant and an irrelevant (control) polypeptide. An adjuvant that induces the generation, proliferation, or activation of T_H17 cells may cause the cells of population (1) to produce more than 2-fold, or more than 10-fold more IL-17 than the cells of population (2). IL-17 may be measured, for example, by ELISA or ELISPOT. Certain toxins, such as cholera toxin and labile toxin (produced by enterotoxigenic *E. coli*, or ETEC), activate a T_H17 response. Thus, in some embodiments, the adjuvant is a toxin or toxoid. Cholera toxin was successfully used in the mouse model to induce protective immunity in conjunction with certain polypeptides from Table 1 (see Examples 5-8). One form of labile toxin is produced by Intercell. Mutant derivates of labile toxin (toxoids) that are active as adjuvants but significantly less toxic can be used as well. Exemplary detoxified mutant derivatives of labile toxin include mutants lacking ADP-ribosyltransferase activity. Particular detoxified mutant derivatives of labile toxin include LTK7 (Douce et al, 1995) and LTK63 (Williams et al, 2004), LT-G192 (Douce et al, 1999), and LTR72 (Giuliani et al, 1998).

[0375] In some embodiments, the adjuvant comprises a VLP (virus-like particle). One such adjuvant platform, Alphavirus replicons, induces the activation of T_H17 cells using alphavirus and is produced by Alphavax. In certain embodiments of the Alphavirus replicon system, alphavirus may be engineered to express an antigen of interest, a cytokine of interest (for example, IL-17 or a cytokine that stimulates IL-17 production), or both, and may be produced in a helper cell line. More detailed information may be found in U.S. Patent Nos. 5,643,576 and 6,783,939. In some embodiments, a vaccine formulation is administered to a subject in combination with a nucleic acid encoding a cytokine.

Petitioner Merck Ex-1015, 1126

[0376] Certain classes of adjuvants activate toll-like receptors (TLRs) in order to activate a T_H17 response. TLRs are well known proteins that may be found on leukocyte membranes, and recognize foreign antigens (including microbial antigens). Administering a known TLR ligand together with an antigen of interest (for instance, as a fusion protein) can promote the development of an immune response specific to the antigen of interest. One exemplary adjuvant that activates TLRs comprises Monophosphoryl Lipid A (MPL). Traditionally, MPL has been produced as a detoxified lipopolysaccharide (LPS) endotoxin obtained from Gram-negative bacteria, such as *S. minnesota*. In particular, sequential acid and base hydrolysis of LPS produces an immunoactive lipid A fraction (which is MPL), and lacks the saccharide groups and all but one of the phosphates present in LPS. A number of synthetic TLR agonists (in particular, TLR-4 agonists) are disclosed in Evans et al, 2003. Like MPL adjuvants, these synthetic compounds activate the innate immune system via TLR. Another type of TLR agonist is a synthetic phospholipid dimer, for example E6020 (Ishizaka et al, 2007). Various TLR agonists (including TLR-4 agonists) have been produced and/or sold by, for example, the Infectious Disease Research Institute (IRDI), Corixa, Esai, Avanti Polar Lipids, Inc., and Sigma Aldrich. Another exemplary adjuvant that activates TLRs comprises a mixture of MPL, Trehalose Dicoynomycolate (TDM), and dioctadecyldimethylammonium bromide (DDA). Another TLR-activating adjuvant is R848 (resiquimod).

[0377] In some embodiments, the adjuvant is or comprises a saponin. Typically, the saponin is a triterpene glycoside, such as those isolated from the bark of the *Quillaja saponaria* tree. A saponin extract from a biological source can be further fractionated (*e.g.*, by chromatography) to isolate the portions of the extract with the best adjuvant activity and with acceptable toxicity. Typical fractions of extract from *Quillaja saponaria* tree used as adjuvants are known as fractions A and C.

[0378] In certain embodiments, combinations of adjuvants are used. Three exemplary combinations of adjuvants are MPL and alum, E6020 and alum, and MPL and an ISCOM.

[0379] Adjuvants may be covalently or non-covalently bound to antigens. In some embodiments, the adjuvant may comprise a protein which induces inflammatory responses through activation of antigen-presenting cells (APCs). In some embodiments, one or more of these proteins can be recombinantly fused with an antigen of choice, such that the resultant

Petitioner Merck Ex-1015, 1127

fusion molecule promotes dendritic cell maturation, activates dendritic cells to produce cytokines and chemokines, and ultimately, enhances presentation of the antigen to T cells and initiation of T cell responses (*e.g.*, see Wu et al, 2005).

[0380] In some embodiments, immunogenic complexes described herein are formulated and/or administered in combination with an adjuvant. In some embodiments, the adjuvant is selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphate aluminum hydroxide. In some embodiments, the adjuvant comprises aluminum phosphate. In some embodiments, the adjuvant is aluminum phosphate.

[0381] Typically, the same adjuvant or mixture of adjuvants is present in each dose of a vaccine. Optionally, however, an adjuvant may be administered with the first dose of vaccine and not with subsequent doses (*i.e.*, booster shots). Alternatively, a strong adjuvant may be administered with the first dose of vaccine and a weaker adjuvant or lower dose of the strong adjuvant may be administered with subsequent doses. The adjuvant can be administered before the administration of the antigen, concurrent with the administration of the antigen or after the administration of the antigen to a subject (sometimes within 1, 2, 6, or 12 hours, and sometimes within 1, 2, or 5 days). Certain adjuvants are appropriate for human subjects, non-human animals, or both.

[0382] Vaccines for use in accordance with the present invention may include, or be administered concurrently with, other antimicrobial therapy. For example, such vaccines may include or be administered with one or more agents that kills or retards growth of a pathogen. Such agents include, for example, penicillin, vancomycin, erythromycin, azithromycin, and clarithromycin, cefotaxime, ceftriaxone, levoflaxin, gatifloxacin.

[0383] Alternatively or additionally, vaccines for use in accordance with the present invention may include, or be administered with, one or more other vaccines or therapies. For example, one or more non-pneumococcal antigens may be included in or administered with the vaccines.

Additional Components and Excipients

[0384] In addition to the antigens and the adjuvants described above, a vaccine formulation or immunogenic composition may include one or more additional components.

Petitioner Merck Ex-1015, 1128

[0385] In certain embodiments, the vaccine formulation or immunogenic composition may include one or more stabilizers such as sugars (such as sucrose, glucose, or fructose), phosphate (such as sodium phosphate dibasic, potassium phosphate monobasic, dibasic potassium phosphate, or monosodium phosphate), glutamate (such as monosodium L-glutamate), gelatin (such as processed gelatin, hydrolyzed gelatin, or porcine gelatin), amino acids (such as arginine, asparagine, histidine, L-histidine, alanine, valine, leucine, isoleucine, serine, threonine, lysine, phenylalanine, tyrosine, and the alkyl esters thereof), inosine, or sodium borate.

[0386] In certain embodiments, the vaccine formulation or immunogenic composition includes one or more buffers such as a mixture of sodium bicarbonate and ascorbic acid. In some embodiments, the vaccine formulation may be administered in saline, such as phosphate buffered saline (PBS), or distilled water.

[0387] In certain embodiments, the vaccine formulation or immunogenic composition includes one or more surfactants, for example, but not limited to, polysorbate 80 (TWEEN 80), polysorbate 20 (TWEEN 20), Polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether (TRITON X-100), and 4-(1,1,3,3-Tetramethylbutyl)phenol polymer with formaldehyde and oxirane (TYLOXAPOL). A surfactant can be ionic or non-ionic.

[0388] In certain embodiments, the vaccine formulation or immunogenic composition includes one or more salts such as sodium chloride, ammonium chloride, calcium chloride, or potassium chloride.

[0389] In certain embodiments, a preservative is included in the vaccine or immunogenic composition. In other embodiments, no preservative is used. A preservative is most often used in multi-dose vaccine vials, and is less often needed in single-dose vaccine vials. In certain embodiments, the preservative is 2-phenoxyethanol, methyl and propyl parabens, benzyl alcohol, and/or sorbic acid.

Methods of Administration

[0390] In some embodiments, immunogenic complexes are administered to a subject at risk of developing pneumococcal disease, *e.g.* an infant, a toddler, a juvenile, or an older adult. In some embodiments the subject is a human. In some embodiments the human is between about

Petitioner Merck Ex-1015, 1129

2 weeks of age and about 6 weeks of age. In some embodiments the human is between about 6 weeks of age and about 6 years of age. In some embodiments the human is between about 6 years of age and about 18 years of age. In some embodiments the human is between about 18 years of age and about 50 years of age. In some embodiments the human is about 50 years of age or older. In some embodiments, immunogenic complexes are administered to a subject at elevated risk of developing pneumococcal disease, *e.g.*, immunocompromised subjects, subjects having sickle cell disease or other hemoglobinopathies, congenital or acquired asplenia, splenic dysfunction, chronic renal failure or nephrotic syndrome, diseases associated with treatment with immunosuppressive drugs or radiation therapy (including malignant neoplasm, leukemia, lymphomas, Hodgkin's disease, or solid organ transplantation), congenital or acquired immunodeficiency, HIV infection, cerebrospinal fluid leaks, cochlear implant(s), chronic heart disease, chronic lung disease, diabetes mellitus, alcoholism, chronic liver disease, cigarette smoking, asthma, generalized malignancy, multiple myeloma, or solid organ transplantation. It will be appreciated that a subject can be considered at risk for developing a disease without having been diagnosed with any symptoms of the disease. For example, if the subject is known to have been, or to be intended to be, in situations with relatively high risk of infection, that subject will be considered at risk for developing the disease.

[0391] Any effective route of administration may be utilized such as, for example, oral, nasal, enteral, parenteral, intramuscular or intravenous, subcutaneous, transdermal, intradermal, rectal, vaginal, topical, ocular, pulmonary, or by contact application. In some embodiments, vaccine compositions may be injected (*e.g.*, via intramuscular, intraperitoneal, intradermal and/or subcutaneous routes); or delivered via the mucosa (*e.g.*, to the oral/alimentary, respiratory, and/or genitourinary tracts). Intranasal administration of vaccines may be particularly useful in some contexts, for example for treatment of pneumonia or otitis media (as nasopharyngeal carriage of pneumococci can be more effectively prevented, thus attenuating infection at its earliest stage). In some embodiments of the invention, it may be desirable to administer different doses of a vaccine by different routes; in some embodiments, it may be desirable to administer different components of one dose via different routes. In some embodiments, an immunogenic composition or vaccine disclosed herein is administered intramuscularly. In some embodiments, an immunogenic composition or vaccine disclosed herein is administered subcutaneously.

Petitioner Merck Ex-1015, 1130

[0392] In some embodiments of the present invention, pharmaceutical compositions (*e.g.*, vaccines) are administered intradermally. Conventional technique of intradermal injection, the "Mantoux procedure", comprises steps of cleaning the skin, and then stretching with one hand, and with the bevel of a narrow gauge needle (26-31 gauge) facing upwards the needle is inserted at an angle of between 10-15°. Once the bevel of the needle is inserted, the barrel of the needle is lowered and further advanced while providing a slight pressure to elevate it under the skin. The liquid is then injected very slowly thereby forming a bleb or bump on the skin surface, followed by slow withdrawal of the needle.

[0393] Devices that are specifically designed to administer liquid agents into or across the skin have been described, for example the devices described in WO 99/34850 and EP 1092444, also the jet injection devices described for example in WO 01/13977; US Patent No. 5,480,381, US Patent No. 5,599,302, US Patent No. 5,334,144, US Patent No. 5,993,412, US Patent No. 5,649,912, US Patent No. 5,569,189, US Patent No. 5,704,911, US Patent No. 5,383,851, US Patent No. 5,893,397, US Patent No. 5,466,220, US Patent No. 5,339,163, US Patent No. 5,312,335, US Patent No. 5,503,627, US Patent No. 5,064,413, US Patent No. 5,520,639, US Patent No. 4,596,556, US Patent No. 4,790,824, US Patent No. 4,941,880, US Patent No. 4,940,460, WO 97/37705 and WO 97/13537. Other methods of intradermal administration of the vaccine preparations may include conventional syringes and needles, or devices designed for ballistic delivery of solid vaccines (WO 99/27961), or transdermal patches (WO 97/48440; WO 98/28037); or applied to the surface of the skin (transdermal or transcutaneous delivery WO 98/20734; WO 98/28037).

[0394] As described above, pharmaceutical compositions (*e.g.*, vaccines) may be administered as a single dose or as multiple doses. It will be appreciated that an administration is a single "dose" so long as all relevant components are administered to a subject within a window of time; it is not necessary that every component be present in a single composition. For example, administration of two different immunogenic compositions, within a period of less than 24 h, is considered a single dose. To give but one example, immunogenic compositions having different antigenic components may be administered in separate compositions, but as part of a single dose. As noted above, such separate compositions may be administered via different routes or via the same route. Alternatively or additionally, in embodiments wherein a vaccine

Petitioner Merck Ex-1015, 1131

comprises a combination of immunogenic compositions and additional types of active agents, immunogenic compositions may be administered via one route, and a second active agent may be administered by the same route or by a different route.

[0395] Pharmaceutical compositions (*e.g.*, vaccines) are administered in such amounts and for such time as is necessary to achieve a desired result. In certain embodiments of the present invention, a vaccine composition comprises an immunologically effective amount of at least immunogenic composition. The exact amount required to achieve an immunologically effective amount may vary, depending on the immunogenic composition, and from subject to subject, depending on the species, age, and general condition of the subject, the stage of the disease, the particular pharmaceutical mixture, its mode of administration, and the like.

[0396] The amount of polypeptide antigen(s), polysaccharide antigen(s) or conjugate(s) in each pharmaceutical composition (*e.g.*, vaccine) dose is selected to allow the vaccine, when administered as described herein, to induce an appropriate immune-protective response without significant, adverse side effects.

[0397] In some embodiments, a pharmaceutical composition comprising an immunogenic complex disclosed herein induces a T_H1 and/or T_H17 cell response upon administration to a subject. In some embodiments, a pharmaceutical composition comprising an immunogenic complex disclosed herein induces an opsonic/bactericidal response against *S. pneumoniae* upon administration to a subject. In some embodiments, a pharmaceutical composition comprising an immunogenic complex disclosed herein reduces rate of transmission and/or colonization of the mucosal surfaces by *S. pneumoniae* upon administration to a subject.

[0398] In some embodiments, a pharmaceutical composition comprising an immunogenic complex disclosed herein reduces rate of transmission and/or colonization of the nasopharynx or the lungs by *S. pneumoniae* upon transmission.

[0399] Some embodiments provide for a method of immunizing a subject against *S. pneumoniae* infection comprising administering to the subject an immunologically effective amount of an immunogenic complex described herein. Some embodiments provide for a method of immunizing a subject against *S. pneumoniae* infection comprising administering to the subject an immunologically effective amount of an immunogenic composition described herein. Some

Petitioner Merck Ex-1015, 1132

embodiments provide for a method of immunizing a subject against *S. pneumoniae* infection comprising administering to the subject an immunologically effective amount of a vaccine composition described herein. Some embodiments provide for a method of immunizing a subject against *S. pneumoniae* infection comprising administering to the subject an immunologically effective amount of a pharmaceutical composition described herein.

Combination Prophylaxis or Combination Therapy

[0400] In some embodiments, an immunogenic complex, immunogenic composition, vaccine, or pharmaceutical composition disclosed herein may be administered in combination with another agent. In some embodiments, the agent is or comprises PCV13. In some embodiments, the agent is or comprises PPSV23. In some embodiments, the agent is or comprises an antibiotic.

Dosing

[0401] In some embodiments, administration of a vaccine (*e.g.*, a vaccine composition) described herein may involve the delivery of a single dose. In some embodiments, administration may involve an initial dose followed by one or several additional immunization doses, adequately spaced. An immunization schedule is a program for the administration of one or more specified doses of one or more specified pneumococcal vaccines, by one or more specified routes of administration, at one or more specified ages of a subject.

[0402] The present disclosure provides immunization methods that involve administering at least one dose of a vaccine to an infant subject. In some embodiments, the infant subject is 18 months old or younger. In some embodiments, the infant subject is 12 months old or younger. In some embodiments, the infant subject has previously received one or more doses of a conjugated pneumococcal polysaccharide vaccine; in other embodiments, the infant subject is naïve to pneumococcal vaccines. In some embodiments, the infant subject has previously been infected with, or exposed to infection by *S. pneumoniae*.

[0403] The present disclosure provides immunization methods that involve administering at least one dose of a vaccine to a toddler subject. In some embodiments, the toddler subject is 5 years old or younger. In some embodiments, the toddler subject is 4 years old or younger. In some embodiments, the toddler subject has previously received one or more doses of a

Petitioner Merck Ex-1015, 1133

conjugated pneumococcal polysaccharide vaccine; in other embodiments, the toddler subject is naïve to pneumococcal vaccines. In some embodiments, the toddler subject has previously been infected with, or exposed to infection by *S. pneumoniae*.

[0404] The present disclosure provides immunization methods that involve administering at least one dose of a vaccine to a juvenile subject. In some embodiments, the juvenile subject is 18 years old or younger. In some embodiments, the juvenile subject is 15 years old or younger. In some embodiments, the juvenile subject has previously received one or more doses of a conjugated pneumococcal polysaccharide vaccine; in other embodiments, the juvenile subject is naïve to pneumococcal vaccines. In some embodiments, the juvenile subject has previously been infected with, or exposed to infection by *S. pneumoniae*.

[0405] The present disclosure provides immunization methods that involve administering at least one dose of a vaccine to an adult subject. In some embodiments, the adult subject is older than about 50 years of age. In some embodiments, the adult subject is older than about 65 years of age. In some embodiments, the adult subject has previously received one or more doses of a conjugated pneumococcal polysaccharide vaccine; in other embodiments, the adult subject is naïve to pneumococcal vaccines. In some embodiments, the adult subject has previously been infected with, or exposed to infection by *S. pneumoniae*.

[0406] Immunization schedules of the present disclosure are provided to induce an immune response (*e.g.*, an immunoprotective response) in a subject sufficient to reduce at least one measure selected from the group consisting of incidence, prevalence, frequency, and/or severity of at least one infection, disease, or disorder, and/or at least one surrogate marker of the infection, disease, or disorder, in a population and/or subpopulation of the subject(s). A supplemental immunization schedule is one which has this effect relative to the standard schedule which it supplements. A supplemental schedule may call for additional administrations and/or supra-immunogenic doses of the immunogenic compositions disclosed herein, found in the standard schedule, or for the administration of vaccines not part of the standard schedule. A full immunization schedule of the present invention may comprise both a standard schedule and a supplemental schedule. Exemplary sample vaccination schedules are provided for illustrative purposes. Detailed descriptions of methods to assess immunogenic response discussed herein

Petitioner Merck Ex-1015, 1134

allow one to develop alterations to the sample immunization schedules without undue experimentation.

[0407] In some embodiments of the present disclosure, a first administration of a pneumococcal vaccine occurs when a subject is more than about 2 weeks old, more than about 5 weeks old, more than about 1 year old, more than about 2 years old, more than about 15 years old, or more than about 18 years old.

[0408] In some embodiments, a first administration of a pneumococcal vaccine occurs when a subject is about two months old. In some embodiments, a second administration of a pneumococcal vaccine occurs when a subject is about four months old. In some embodiments, a third administration of a pneumococcal vaccine occurs when a subject is about six months old. In some embodiments, a fourth administration of a pneumococcal vaccine occurs when a subject is between about twelve months old and about fifteen months old.

[0409] In some embodiments of the present disclosure, a first administration of a pneumococcal vaccine occurs when a subject is more than about 50 years old, more than about 55 years old, more than about 60 years old, more than about 65 years old, or more than about 70 years old.

[0410] In some embodiments of the disclosure, a single administration of vaccine is employed. It is possible that the purposes of the present invention can be served with a single administration, especially when one or more utilized vaccine polypeptide(s), polysaccharide(s) and/or immunogenic complex(es) or combinations thereof is/are strong, and in such a situation a single dose schedule is sufficient to induce a lasting immune-protective response.

[0411] In certain embodiments, it is desirable to administer two or more doses of vaccine, for greater immune-protective efficacy and coverage. Thus, in some embodiments, a number of doses is at least two, at least three or more doses. There is no set maximum number of doses, however it is good clinical practice not to immunize more often than necessary to achieve the desired effect.

[0412] Without being bound by theory, a first dose of vaccine administered according to the disclosure may be considered a "priming" dose. In certain embodiments, more than one dose

Petitioner Merck Ex-1015, 1135

is included in an immunization schedule. In such a scenario, a subsequent dose may be considered a "boosting" dose.

[0413] A priming dose may be administered to a naïve subject (a subject who has never previously received a conjugated polysaccharide vaccine). In some embodiments, a priming dose may be administered to a subject who has previously received conjugated polysaccharide vaccine at least five or more years previous to administration of an initial vaccine dose according to the invention. In other embodiments, a priming dose may be administered to a subject who has previously received a conjugated polysaccharide vaccine at least twenty or more years previous to administration of a priming vaccine according to the invention.

[0414] When an immunization schedule calls for two or more separate doses, the interval between doses is considered. The interval between two successive doses may be the same throughout an immunization schedule, or it may change as the subject ages. In immunization schedules of the present invention, once a first vaccine dose has been administered, there is a first interval before administration of a subsequent dose. A first interval is generally at least about 2 weeks, 1 month, 6 weeks, 2 months, 3 months, 6 months, 9 months, 12 months, or longer. Where more than one subsequent dose(s) are administered, second (or higher) intervals may be provided between such subsequent doses. In some embodiments, all intervals between subsequent doses are of the same length; in other embodiments, second intervals may vary in length. In some embodiments, the interval between subsequent doses may be at least about 12 months, at least about 15 months, at least about 18 months, at least about 21 months or at least about 2 years. In certain embodiments, the interval between doses may be up to 3 years, up to about 4 years, or up to about 5 years or 10 years or more. In certain embodiments, intervals between subsequent doses may decrease as the subject ages.

[0415] It will be appreciated by those skilled in the art that a variety of possible combinations and sub-combinations of the various conditions of timing of the first administration, shortest interval, largest interval and total number of administrations (in absolute terms, or within a stated period) exist, and all of these combinations and sub-combinations should be considered to be within the inventor's contemplation though not explicitly enumerated here.

Petitioner Merck Ex-1015, 1136

Assays for Determining Immune Response

[0416] In some embodiments, a method of assessing the immunogenicity of an immunogenic composition described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by opsonophagocytic killing (OPK), serum bactericidal killing (SBA), agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of in *vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction of mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition. In some embodiments, the immune response is compared to a control composition. In some embodiments, a control composition may comprise an antigenic polysaccharide present in the immunogenic composition and not comprise an antigenic polypeptide present in the immunogenic composition. In some embodiments, a control composition may comprise an antigenic polypeptide present in the immunogenic composition and not comprise an antigenic polysaccharide present in the immunogenic composition. In some embodiments, a control composition may comprise an adjuvant present in the immunogenic composition, and not comprise an antigenic polysaccharide and/or an immunogenic polypeptide present in the immunogenic composition.

[0417] In some embodiments, a method of assessing the potency of an immunogenic composition described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by OPK, serum bactericidal killing (SBA), internalization, activity neutralization, agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization).

Petitioner Merck Ex-1015, 1137

Parameters of *in vivo* assays include bacterial clearance or reduction from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction of mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition. In some embodiments, the immune response is compared to a control composition. In some embodiments, a control composition may comprise an antigenic polysaccharide present in the immunogenic composition and not comprise an antigenic polypeptide present in the immunogenic composition. In some embodiments, a control composition may comprise an antigenic polypeptide present in the immunogenic composition and not comprise an antigenic polysaccharide present in the immunogenic composition. In some embodiments, a control composition may comprise an adjuvant present in the immunogenic composition, and not comprise an antigenic polysaccharide and/or an immunogenic polypeptide present in the immunogenic composition.

[0418] In some embodiments, a method of assessing the immunogenicity of a vaccine composition described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by OPK, serum bactericidal killing (SBA), agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of *in vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction of mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition. In some embodiments, the immune response is compared to a control composition. In some embodiments, a control composition may comprise an antigenic polysaccharide present in the vaccine composition and not comprise an antigenic polypeptide present in the vaccine composition. In some embodiments, a control composition may comprise an antigenic polypeptide present in the vaccine composition and not comprise an antigenic polysaccharide

Petitioner Merck Ex-1015, 1138

present in the vaccine composition. In some embodiments, a control composition may comprise an adjuvant present in the vaccine composition, and not comprise an antigenic polysaccharide and/or an immunogenic polypeptide present in the vaccine composition.

[0419] In some embodiments, a method of assessing the potency of a vaccine composition described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by OPK, serum bactericidal killing (SBA), agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of *in vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction of mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition. In some embodiments, the immune response is compared to a control composition. In some embodiments, a control composition may comprise an antigenic polysaccharide present in the vaccine composition and not comprise an antigenic polypeptide present in the vaccine composition. In some embodiments, a control composition may comprise an antigenic polypeptide present in the vaccine composition and not comprise an antigenic polysaccharide present in the vaccine composition. In some embodiments, a control composition may comprise an adjuvant present in the vaccine composition, and not comprise an antigenic polysaccharide and/or an immunogenic polypeptide present in the vaccine composition.

[0420] In some embodiments, a method of assessing the immunogenicity of a pharmaceutical composition described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by OPK, serum bactericidal killing (SBA), agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia,

Petitioner Merck Ex-1015, 1139

meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of *in vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction of mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition. In some embodiments, the immune response is compared to a control composition. In some embodiments, a control composition may comprise an antigenic polysaccharide present in the pharmaceutical composition and not comprise an antigenic polypeptide present in the pharmaceutical composition. In some embodiments, a control composition may comprise an antigenic polypeptide present in the pharmaceutical composition and not comprise an antigenic polysaccharide present in the pharmaceutical composition. In some embodiments, a control composition may comprise an adjuvant present in the pharmaceutical composition, and not comprise an antigenic polysaccharide and/or an immunogenic polypeptide present in the pharmaceutical composition.

[0421] In some embodiments, a method of assessing the potency of a pharmaceutical composition described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by OPK, serum bactericidal killing (SBA), agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of *in vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction of mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition. In some embodiments, the immune response is compared to a control composition. In some embodiments, a control composition may comprise an antigenic polysaccharide present in the pharmaceutical composition and not comprise an antigenic polypeptide present in the pharmaceutical composition. In some embodiments, a control composition may comprise an

Petitioner Merck Ex-1015, 1140

antigenic polypeptide present in the pharmaceutical composition and not comprise an antigenic polysaccharide present in the pharmaceutical composition. In some embodiments, a control composition may comprise an adjuvant present in the pharmaceutical composition, and not comprise an antigenic polysaccharide and/or an immunogenic polypeptide present in the pharmaceutical composition.

[0422] In some embodiments, a method of assessing the immunogenicity and/or potency of an immunogenic complex comprises evaluating an immune response to immunogenic or vaccine compositions comprising one or more immunogenic complexes. In some embodiments, the method of assessing the immunogenicity and/or potency of an immunogenic complex described herein comprises evaluating, measuring, and/or comparing an immune response using one or more *in vitro* bioassays, including B cell and T cell responses such as antibody levels by ELISA, multiplex ELISA, MSD, Luminex, flow cytometry, T_H1/T_H17 cell response, cytokine level measurement and functional antibody levels as measured by OPK, serum bactericidal killing (SBA), agglutination, motility, cytotoxicity, or adherence; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of *in vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction in mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition.

[0423] Generally speaking, it may be desirable to assess humoral responses, cellular responses, and/or interactions between the two. Where humoral responses are being assessed, antibody titers and/or types (*e.g.*, total IgG, IgG1, IgG2, IgM, IgA, etc.) to specific pathogen polysaccharides or polypeptides (either serotype-specific or conserved across two or more serotypes) may be determined, for example before and/or after administration of an initial or a boosting dose of vaccine (and/or as compared with antibody levels in the absence of antigenic stimulation). Cellular responses may be assessed by monitoring reactions such as delayed type hypersensitivity responses, etc. to the carrier protein. Cellular responses can also be measured directly by evaluating the response of peripheral blood mononuclear cells (PBMCs) monocytes to stimulation with the antigens of interest. Precursor and memory B cell populations may be

Petitioner Merck Ex-1015, 1141

assessed in enzyme linked immunospot (ELISpot) assays directed against specific pathogen polysaccharides or polypeptides.

[0424] Any of a variety of assays may be employed to detect levels and/or activity of antibodies in subject sera. Suitable assays include, for example, ligand binding assays, such as radioimmunoassay (RIAs), ELISAs, and multiplex assays (Luminex, Bioplex, MSD); functional assays, such as opsonophagocytic assays or internalization assays; and *in vivo* assays in animal models of pneumococcal disease (*e.g.* pneumonia, bacteremia, meningitis, sepsis, otitis media, nasopharyngeal colonization). Parameters of *in vivo* assays include bacterial clearance from mucosal surfaces or bloodstream, reduction or prevention of bacteremia, meningitis, sepsis, or otitis media, reduction or prevention of colonization of the nasopharynx, reduction in mortality, and passive and active protection following challenge with the pneumococcal pathogens that are the targets of the immunogenic composition.

[0425] The RIA method detects specific antibodies through incubation of sera with radio-labeled polysaccharides or polypeptides in suspension (*e.g.*, Schiffiman et al, 1980). The antigen-antibody complexes are then precipitated with ammonium sulfate and the radiolabeled pellets assayed for counts per minute (cpm).

[0426] In the ELISA detection method, specific antibodies from the sera of vaccinated subjects are quantitated by incubation with polysaccharides or polypeptides (either serotype-specific or conserved across two or more serotypes) which have been adsorbed to a solid support (*e.g.*, Koskela and Leinonen (1981); Kojima et al, 1990; Concepcion and Frasch, 2001). The bound antibody is detected using enzyme-conjugated secondary detection antibodies. The ELISA also allows isotyping and subclassing of the immune response (*i.e.*, IgM vs. IgG or IgG1 vs. IgG2) by using isotype- or subclass-specific secondary antibodies and can be adapted to evaluate the avidity of the antibodies (Anttila et al, 1998; Romero-Steiner et al, 2005). Multiplex assays (*e.g.*, Luminex) facilitate simultaneous detection of antibodies to multiple antigens. Capsular polysaccharide(s) or polypeptides are conjugated to spectrally distinct microspheres that are mixed and incubated with serum. The antibodies bound to the polysaccharides or polypeptides on the coated microspheres are detected using a secondary antibody (*e.g.*, R-Phycoerythrin-conjugated goat anti-human IgG).

Petitioner Merck Ex-1015, 1142

[0427] An approach for assessing functional antibody in serum is an opsonophagocytic assay (OPA) or a concentrated opsonophagocytic assay (COPA), which quantitates only the antibodies that can opsonize the bacteria, leading to ingestion and killing of the bacteria. The standard assay utilizes a human phagocytic effector cell, a source of complement, bacteria, and diluted sera. The assay readout is the serum endpoint titer at which there is $\geq$50% killing compared to bacteria incubated with complement and human cells alone (Romero-Steiner et al, 1997). This killing OPA can also be multiplexed by utilizing target strains of pathogen that carry different antibiotic resistance markers (Kim et al, 2003). Another type of multiplex opsonic assay is a nonkilling assay in which the uptake by phagocytic effector cells of fluorescent stained encapsulated pathogen or fluorescent microspheres conjugated with antigenic polysaccharides or polypeptides from a target pathogen in the presence of diluted sera plus a complement source is evaluated by flow cytometry (Martinez et al, 1999). Opsonic activity of serum antibody plus complement can also be evaluated by measuring the oxidative response of phagocytic human effector cells to ingested pathogen (Munro et al. 1985; Ojo-Amaize et al. 1995).

[0428] Certain *in vivo* model systems can be used to evaluate the protection afforded by serum antibodies induced by vaccines of the present invention. In such passive protection systems, mice or rats are challenged with the pathogen plus diluted sera, and the endpoint titer of the sera which provides protection against pneumonia, bacteremia, colonization of organs or tissues, or mortality is determined (Stack et al. 1998; Saeland et al. 2000).

[0429] In some embodiments, efficacy of vaccination may be determined by assaying one or more cytokine levels by stimulating T cells from a subject after vaccination. The one or more cytokine levels may be compared to the one or more cytokine levels in the same subject before vaccination. Increased levels of the one or more cytokine, such as a 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more increase over pre-immunization cytokine levels, would indicate an increased response to the vaccine. In some embodiments, the one or more cytokines are selected from GM-CSP; IL-1α; IL-1β; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-10; IL-12; IL-17A, IL-17F or other members of the IL-17 family; IL-22; IL-23; IFN-α; IFN-β; IFN-γ; MIP-1α; MIP-1β; TGF-β; TNFα, or TNF-β. In a non-limiting example, efficacy of vaccination may be determined by assaying IL-17 levels (particularly IL-17A) by stimulating T cells from a subject after vaccination. The IL-17 levels may be compared to IL-17 levels in

Petitioner Merck Ex-1015, 1143

the same subject before vaccination. Increased IL-17 (*e.g.*, IL-17A) levels, such as a 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more increase, would indicate an increased response to the vaccine.

[0430] In some embodiments, one may assay neutrophils in the presence of T cells or antibodies from the patient for pneumococcal killing. Increased pneumococcal killing, such as a 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more increase, would indicate an increased response to the vaccine. For example, one may measure T_H17 cell activation, where increased T_H17 cell activation, such as a 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more increase, correlates with an increased response to the vaccine. In another non-limiting example, one may measure T_H1 cell activation, where increased T_H1 cell activation, such as a 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more increase, correlates with an increased response to the vaccine. One may also measure levels of an antibody specific to the vaccine, where increased levels of the specific antibody, such as a 1.5 fold, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold or more increase, are correlated with increased vaccine efficacy. In certain embodiments, two or more of these assays are used. For example, one may measure IL-17 levels and the levels of vaccine-specific antibody. Alternatively, one may follow epidemiological markers such as incidence of, severity of, or duration of pneumococcal infection in vaccinated individuals compared to unvaccinated individuals.

[0431] Vaccine efficacy may also be assayed in various model systems such as the mouse challenge model. For instance, BALB/c or C57BL/6 strains of mice may be used. After administering the test vaccine to a subject (as a single dose or multiple doses), the experimenter administers a challenge dose of *S. pneumoniae*. In some cases, a challenge dose administered intranasally is sufficient to cause *S. pneumoniae* colonization (especially nasal colonization) in an unvaccinated animal, and in some cases a challenge dose administered via aspiration is sufficient to cause sepsis and a high rate of lethality in unvaccinated animals. In some cases, a challenge dose administered via intraperitoneal injection is sufficient to cause sepsis and a high rate of lethality in unvaccinated animals. In some cases, a challenge dose administered via intravenous injection is sufficient to cause sepsis and a high rate of lethality in unvaccinated

Petitioner Merck Ex-1015, 1144

animals. One can then measure the reduction in colonization or the reduction in lethality in vaccinated animals.

[0432] Certain *in vivo* model systems can be used to evaluate the protection afforded by serum antibodies induced by vaccines of the present invention. In such passive protection systems, mice or rats are challenged with the pathogen plus diluted sera, and the endpoint titer of the sera which provides protection against bacteremia, colonization of organs or tissues, or mortality is determined (Stack et al. 1998; Saeland et al. 2000).

Kits

[0433] The present disclosure also provides for kits for producing an immunogenic complex as disclosed herein which is useful for an investigator to tailor an immunogenic complex with their preferred antigens, *e.g.*, for research purposes to assess the effect of an antigen, or a combination of antigens on immune response. Such kits can be prepared from readily available materials and reagents. For example, such kits can comprise any one or more of the following materials: a container comprising a polysaccharide cross-linked with a plurality of first affinity molecules; a container comprising a complementary affinity molecule which associates with the first affinity molecule, wherein the complementary affinity molecule associates with an antigen or carrier protein; a container comprising an antigen; a container comprising a carrier protein; a container comprising an antigen associated with a complementary affinity molecule; a container comprising a carrier protein associated with a complementary affinity molecule.

[0434] In another embodiment, the kit comprises a container comprising a polysaccharide; a container comprising a plurality of first affinity molecules; and a container comprising a cross-linking reagent for cross-linking the first affinity molecules to the polysaccharide, for example, but not limited to, CDAP (l-cyano-4- dimethylaminopyridinium tetrafluoroborate), and EDC (l-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride).

[0435] In another embodiment, the kit comprises a container comprising an antigen or carrier protein, and a container comprising a complementary affinity molecule which associates with a first affinity molecule. In some embodiments, the kit further comprises a means to attach

Petitioner Merck Ex-1015, 1145

the complementary affinity molecule to the antigen or carrier protein, where the means can be by a cross-linking reagent or by some intermediary fusion protein.

[0436] In some embodiments, the kit can comprise at least one co-stimulation factor which can be added to the polymer. In some embodiments, the kit comprises a cross-linking reagent, for example, but not limited to, CDAP (l-cyano-4- dimethylaminopyridinium tetrafluoroborate); EDC (l-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride); sodium cyanoborohydride; cyanogen bromide; and ammonium bicarbonate/iodoacetic acid, for linking the co-factor to the polymer.

[0437] A variety of kits and components can be prepared for use in the methods described herein, depending upon the intended use of the kit, the particular target antigen and the needs of the user.

Exemplification

Example 1: S. pneumoniae Capsular Polysaccharides

Preparation and Purification

[0438] Methods of culturing pneumococci are well known in the art (*e.g.* Chase, 1967, Methods of Immunology and Immunochemistry 1:52). Methods of preparing pneumococcal capsular polysaccharides are also well known in the art (*e.g.*, European Patent No. EP0497524). Isolates of pneumococcal serotypes are available from the ATCC or the National Collection of Type Cultures operated by Public Health England.

[0439] *S. pneumoniae* is identified as non-motile, Gram-positive, lancet-shaped diplococci that are alpha-hemolytic on blood agar. Most but not all strains are encapsulated. Serotypes are differentiated on the basis of Neufeld Test (Quelling reaction) using specific antisera (*e.g.*, U.S. Pat. No. 5,847,112), latex agglutination, or multilocus sequence typing.

[0440] A frozen vial representing each of the *S. pneumoniae* serotypes present in the MAPS vaccine was thawed and used to generate a seed culture in appropriate pre-sterilized growth media. The seed culture was grown with temperature and pH control. The seed culture was transferred to a production fermenter that contained pre-sterilized growth media. The

Petitioner Merck Ex-1015, 1146

production culture was grown with temperature, pH and agitation rate control. The growth process was terminated with addition of an inactivating agent with a controlled temperature hold.

[0441] The purification process was initiated by removal of cell debris using a combination of centrifugation and filtration. The material was filtered followed by solvent-based fractionations to remove impurities and recover PS.

Specifications

[0442] All PS specifications in Table 1 were obtained from European Pharmacopoeia 9.0 Table 0966.-1, except for molecular size and serotype 6A, which are based on the manufacturer's certificate of analysis.

Table 1. Exemplary Polysaccharide (PS) Specifications

Molecular type†	Protein	Nucleic acids	Total nitrogen	Phosphorus	Molecular size (K_D)		Uronic acids	Hexos-amines	Methyl-pentoses	O-acetyl Groups
					‡	§				
1	≤ 2	≤ 2	3.5-6	0-1.5	≤ 0.15		≥ 45			≥ 1.8
2	≤ 2	≤ 2	0-1	0-1.0	≤ 0.15		≥ 15		≥ 38	
3	≤ 5	≤ 2	0-1	0-1.0	≤ 0.15		≥ 40			
4	≤ 3	≤ 2	4-6	0-1.5	≤ 0.15			≥ 40		
5	≤ 7.5	≤ 2	2.5-6.0	≤ 2		≤ 0.60	≥ 12	≥ 20		
6A	≤ 1.5	≤ 1.5	0-2	2.5-5.0		≤ 0.50			≥ 15	
6B	≤ 2	≤ 2	0-2	2.5-5.0		≤ 0.50			≥ 15	
7F	≤ 5	≤ 2	1.5-4.0	0-1.0	≤ 0.20				≥ 13	
8	≤ 2	≤ 2	0-1	0-1.0	≤ 0.15		≥ 25			
9N	≤ 2	≤ 1	2.2-4	0-1.0	≤ 0.20		≥ 20	≥ 28		
9V	≤ 2	≤ 2	0.5-3	0-1.0		≤ 0.45	≥ 15	≥ 13		
10A	≤ 7	≤ 2	0.5-3.5	1.5-3.5		≤ 0.65		≥ 12		
11A	≤ 3	≤ 2	0-2.5	2.0-5.0		≤ 0.40				≥ 9
12F	≤ 3	≤ 2	3-5	0-1.0	≤ 0.25			≥ 25		
14	≤ 5	≤ 2	1.5-4	0-1.0	≤ 0.30			≥ 20		
15B	≤ 3	≤ 2	1-3	2.0-4.5		≤ 0.55		≥ 15		
17F	≤ 2	≤ 2	0-1.5	0-3.5		≤ 0.45			≥ 20	
18C	≤ 3	≤ 2	0-1	2.4-4.9	≤ 0.15				≥ 14	

Petitioner Merck Ex-1015, 1147

Molecular type†	Protein	Nucleic acids	Total nitrogen	Phosphorus	Molecular size (K_D)		Uronic acids	Hexos-amines	Methyl-pentoses	O-acetyl Groups
					‡	§				
19A	≤ 2	≤ 2	0.6-3.5	3.0-7.0	≤ 0.45			≥ 12	≥ 20	
19F	≤ 3	≤ 2	1.4-3.5	3.0-5.5	≤ 0.20			≥ 12.5	≥ 20	
20	≤ 2	≤ 2	0.5-2.5	1.5-4.0		≤ 0.60		≥ 12		
22F	≤ 2	≤ 2	0-2	0-1.0		≤ 0.55	≥ 15		≥ 25	
23F	≤ 2	≤ 2	0-1	3.0-4.5	≤ 0.15				≥ 37	
33F	≤ 2.5	≤ 2	0-2	0-1.0		≤ 0.50				

Values shown are percentage contents of components of monovalent bulk PS.
† The different types are indicated using the Danish nomenclature.
‡ Cross-linked agarose for chromatography R.
§ Cross-linked agarose for chromatography R1.

Example 2: Preparation of Fusion Protein CP1

Vector Construction

[0443] Fusion protein CP1 comprises the biotin-binding portion of rhizavidin spanning amino acids 45 to 179 of the full-length protein encoded from the genome. The predicted signal sequences (amino acids 1-44) of rhizavidin were not incorporated. To optimize the expression level of CP1 in *E. coli*, the gene sequence that encodes rhizavidin polypeptide (amino acids 45-179; SEQ ID NO:2) was redesigned using *E. coli*-preferred expression codons, synthesized and cloned into plasmid pET24a(+). This synthetic rhizavidin gene was designated Rhavi.

[0444] To construct CP1, a DNA sequence encoding a flexible linker region consisting of seven amino acids (GGGGSSS; SEQ ID NO:3) was directly inserted into the 3' end of the synthetic Rhavi gene to provide separation from Rhavi and promote proper folding of the subsequent fusion protein. The genes encoding desired portions of SP1500 and SP0785 protein (not including predicted signal sequences; nucleic acid sequences SEQ ID NO:7 and SEQ ID NO:8, respectively) were synthesized and inserted into the Rhavi expression vector just beyond the linker region. Three residual amino acids (AAA) from a Not I restriction site of pET24a(+) separate SP1500 and SP0785, and may be referred to as the second linker of CP1.

[0445] A schematic of fusion protein CP1 is shown in Figure 2. The construction of the plasmid pET24a(+)-CP1 is shown in Figure 4 and the feature map in Table 2. The DNA sequence encoding fusion protein CP1 (Rhavi-GGGGSSS-SP1500-AAA-SP0785) was cloned

Petitioner Merck Ex-1015, 1148

into the pET-24a(+) vector using NdeI and XhoI restriction sites. Stop codons were inserted at the 3'-end of the CP1 DNA construct. After cloning was complete, DNA sequencing was performed to confirm the presence, orientation and sequence of the CP1 DNA construct inserted in the CP1/pET-24a(+) vector.

Table 2. Feature Map

Name	Start [bp]	End [bp]	Direction
Insert	2613	4922	Forward
ROP	247	59	Reverse
lacI	2138	1059	Reverse
P_T7_Inducible	2528	2569	Forward
LacO1	2549	2569	Forward
His	4923	4943	Forward
Term_T7	4951	5079	Forward
Ori_f1	5091	5546	Forward
Kanamycin-r	6400	5639	Reverse
Ori_ColE1	6619	7069	Forward

bp: base pair; His: Histidine tag; Laci: Lactose operon inhibitor r; LacO1: Lactose operator; Ori_ColE1: Colicin E1 origin of replication; Ori_f1: Origin of replication from F1 phage; Term_T7: T7 terminator; ROP: repressor of primer.

Establishment of Master Cell Bank (MCB)

[0446] To generate the Research Cell Bank (RCB), the CP1/pET-24a(+) vector was transformed into an *E. coli* expression strain.

[0447] Transformed *E. coli* cells were plated in culture plates containing Kanamycin. A single colony from the plate was selected and used to inoculate into liquid medium in a shaker flask. The flasks were placed in an incubator shaker for overnight culture and grown to the desired OD. The bacterial culture was then mixed with glycerol solution. The mixed solution was aliquoted into vials to make RCB.

Petitioner Merck Ex-1015, 1149

[0448] To generate the MCB, RCB was inoculated into liquid medium in shaker flasks and grown to the desired OD. The bacterial culture was then brought to 15% glycerol, mixed and aliquoted into vials. The MCB vials were then placed at -80°C (-70°C to -90°C) and selected vials were taken for quality assurance testing.

Manufacturing Process Overview

[0449] The CP1 fusion protein was expressed in *E. coli*. The expressed CP1 fusion protein was released from *E. coli* cells and purified in a series of chromatographic and filtration steps. The following section details the 300 L culture scale CP1 manufacturing process.

[0450] In summary, the process was initiated by thawing and inoculating cells from a MCB vial into cell medium. Initial cell expansion was performed in flask and then the bacterial culture was transferred to a 300 L fermenter.

[0451] Bacteria were harvested by centrifugation. The recovered cell paste was resuspended in a lysis buffer and fluidized by a microfluidizer. Bulk CP1 fusion protein in the fluidized cell lysate was purified by precipitation and chromatography steps. The final process stream was concentration and buffer exchange into 20 mM Tris, 150 mM NaCl at pH 8.0 ± 0.1 via ultrafiltration membranes. Lastly, the CP1 fusion protein was 0.22 μm filtered immediately prior to bottling and stored at -80°C.

[0452] No raw materials contained animal- or human-derived components. For description of the cell, see above.

Upstream Process

[0453] A flow chart of the upstream process for CP1 is provided in Figure 5 and details for the individual steps follow.

[0454] The cell culture utilized chemically defined media and components depending on the manufacturing step. The media used were cell media, production media, feed 1 media and feed 2 media. These media were used in inoculum expansion, production culture and feeding, respectively.

Step 1 - 1: Thawing of Cells

Petitioner Merck Ex-1015, 1150

Sufficient cell medium was prepared and transferred into an appropriate culture flask and then frozen MCB is thawed.

Step 1 - 2: Flask Expansion

Thawed MCB was inoculated and grown in a 2.5 L flask. Cell growth was monitored by a spectrophotometer at OD600.

Step 1 - 3: Production Culture

The production culture was run in fed-batch mode. To support product generation and to prolong the cell culture production period, feed 1 and feed 2 media were added after appropriate cell growth. Once the fermenter temperature reached the set point, isopropyl β-D-1-thiogalactopyranoside was added to induce CP1 fusion protein expression.

After approximately 18 hours from induction, harvest was begun by centrifugation.

Step 1 - 4: Harvest

Bacterial culture was harvested by continuous flow centrifugation.

Step 1 - 5: Cell Lysis and Clarification

The recovered cell paste was resuspended in lysis buffer and fluidized by a microfluidizer. The fluidized cell lysate, including bulk CP1 fusion protein, was centrifuged and the supernatant was recovered.

Downstream Process

[0455] After harvest and cell lysis, CP1 fusion protein was purified by several chromatography steps. A flow chart of CP1 purification process is provided in Figure 6. The details for the individual steps follow.

Step 2 - 1: Ammonium Sulfate Precipitation/Centrifugation and Filtration

Ammonium sulfate solution was added to the clarified lysate and adjusted to a final concentration of 0.8 M. After mixing at room temperature, the clarified lysate with ammonium sulfate was centrifuged and the supernatant was recovered. The recovered supernatant was filtered through 0.22 μm filter and collected in an appropriate container.

Step 2 - 2: Hydrophobic Interaction Chromatography

The purpose of this step is to remove process-related contaminants. CP1 fusion protein bound to the resin was washed with high concentration ammonium sulfate buffer then eluted

Petitioner Merck Ex-1015, 1151

with elution buffer (the concentration of NaCl in the buffer was gradually reduced). A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed with each fraction to confirm containing the CP1 fusion protein.

Step 2 - 3: Tangential Flow Filtration Concentration/Filtration

The elution pool was concentrated and then buffer exchanged with several-fold volumes of Tris-buffered saline using a 30 kDa filtration membrane. The filtrated tangential flow filtration (TFF) retentate was filtered by 0.22 μm filter.

Step 2 - 4: Anion Exchange Chromatography

The purpose of this step is to remove process-related contaminants. CP1 fusion protein bound to the resin was washed with NaCl wash buffer then eluted with NaCl elution buffer (the concentration of NaCl in the buffer was gradually increased). SDS-PAGE analysis was performed with each fraction to confirm containing the CP1 fusion protein.

Step 2 - 5: Type 1 Chromatography

The purpose of this step is to further remove process-related contaminants. CP1 fusion protein bound to the resin was washed with phosphate wash buffer then eluted with phosphate elution buffer (the concentration of phosphate in the buffer was gradually increased). SDS-PAGE analysis was performed with each fraction to confirm containing the CP1 fusion protein.

Step 2 - 6: TFF Formulation

The Type 1 chromatography fraction pool was concentrated and then buffer exchanged with several-fold volumes of formulation buffer (20 mM Tris, 150 mM sodium chloride, pH 8.0 ± 0.1) using a 30 kDa filtration membrane. The formulated CP1 fusion protein was filtered through 0.22 μm filter.

Container Closure System

[0456] CP1 fusion protein was stored at -80°C (-70°C to -90°C) in sterile polyethylene terephthalate bottles.

Example 3: Preparation of MAPS Immunogenic Complexes

Overview

[0457] The MAPS platform takes advantage of the high affinity (dissociation constant

Petitioner Merck Ex-1015, 1152

[KD] ≈ 10^{-15}M), non-covalent binding between biotin and rhizavidin, a biotin-binding protein that has no significant predicted homology with human proteins. Rhizavidin, a naturally occurring dimeric protein in the avidin protein family, was first discovered in *Rhizobium etli*, a symbiotic bacterium of the common bean. Rhizavidin has only a 22% amino acid identity with chicken avidin, a protein commonly found in eggs, but with high conservation of amino acid residues involved in biotin binding. No cross-reactivity to rhizavidin was observed in human serum samples obtained from subjects exposed to avidin [Helppolainen et al, 2007], suggesting that rhizavidin antibodies may not cross-react with chicken avidin. Biotin conjugates have been used in several clinical applications without any reported adverse events [Buller et al, 2014; Paty et al, 2010; Lazzeri et al, 2004]. The biotinylation of the PS and MAPS immunogenic complexing process in MAPS24 were optimized to consistently show no free biotin, thus reducing the potential for generating anti-biotin antibodies.

[0458] MAPS vaccine candidates comprise genetically constructed fusion proteins of rhizavidin, or a biotin-binding domain or biotin-binding fragment thereof, and the protein antigens of interest, which are then complexed with biotinylated PS of interest, leading to a specific assembly into integrated macromolecular immunogenic complexes that, when processed by the immune system, result in the activation of protective B- and T-cell immune responses, as shown schematically in Figure 1.

[0459] MAPS24 is a novel pneumococcal vaccine candidate based on the proprietary MAPS platform. MAPS24 is a multi-valent MAPS vaccine containing 24 pneumococcal capsular polysaccharides of serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F, individually biotinylated and complexed with CP1 fusion protein. MAPS15 is a multi-valent MAPS vaccine containing 15 pneumococcal capsular PS of serotypes 1, 3, 4, 5, 6A, 6B, 7F 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F), individually biotinylated and complexed with CP1 fusion protein. MAPS9 is a multi-valent MAPS vaccine containing 9 pneumococcal capsular PS of serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B, individually biotinylated and complexed with CP1 fusion protein. CP1 comprises a genetic fusion construct of truncated rhizavidin and 2 pneumococcal proteins (SP1500 and SP0785) joined by linkers, as shown in Figure 2. No available vaccine contains

Petitioner Merck Ex-1015, 1153

this unique combination of PS and polypeptide antigens which are capable of eliciting an immune response against such a broad range of pneumococcal serotypes.

Assembly of MAPS Immunogenic Complexes

[0460] Drug substance (MAPS immunogenic complex) comprises PS and CP1 fusion protein. Since PS is biotinylated and CP1 fusion protein has a rhizavidin biotin-binding domain, they are linked by the high affinity biotin-rhizavidin interaction. MAPS immunogenic complexation was conducted individually for each PS serotype.

[0461] The MAPS immunogenic complex was made from 2 key intermediates: PS of 24, 15, or 9 *S. pneumoniae* serotypes, and rhizavidin fusion protein CP1. The PS was activated by creation of a cyanate ester and then biotinylated. The biotinylated PS was mixed with the CP1 fusion protein to create a MAPS immunogenic complex, which is linked by the high affinity biotin-rhizavidin interaction.

[0462] The MAPS immunogenic complex was formulated with 150 mM sodium chloride and surfactant buffer, then 0.2 μm filtered immediately prior to bottling and storing at 2°C to 8°C. This formulated MAPS immunogenic complex is referred to as MAPS drug substance.

[0463] No raw materials contained animal- or human-derived components.

[0464] A flow chart of MAPS Complexation Process is provided in Figure 7 and details for the individual steps follows.

Step 1: Clean-up of Polysaccharide

[0465] The purpose of this step is to remove process residuals. Dissolved PS was purified by filtration then exchanged with

several-fold volumes of water for injection, followed by ultrafiltration to concentrate the PS. The cleaned-up PS was filtered by a 0.22 μm filter membrane.

Step 2: Biotinylation and Purification of Polysaccharide

[0466] The hydroxyl group on the PS was activated with a 1-cyano-4-dimetylamino-pyridinium tetrafluoroborate (CDAP) to create a highly active cyanoester. The cyanoesters were reacted with amine-PEG3-biotin and unreacted cyanoesters were capped with glycine. The

Petitioner Merck Ex-1015, 1154

biotinylated PS was buffer exchanged into 1 mM PBS in order to remove unreacted CDAP, amine-PEG3-biotin, glycine and residuals. After buffer exchange, the biotinylated PS was filtered with a 0.22 μm filter.

Step 3: MAPS immunogenic complexation

[0467] The biotinylated PS was mixed with the CP1 fusion protein in order to create a MAPS immunogenic complex, which is linked by a high affinity biotin-rhizavidin interaction. The MAPS immunogenic complex was purified to remove uncomplexed PS and protein by using a filtration membrane. After purification, the MAPS immunogenic complex was filtered with a 0.22 μm filter and stored at 2°C to 8°C.

Specifications

[0468] Exemplary MAPS immunogenic complex/MAPS drug substance specifications are set forth in Table 3.

Petitioner Merck Ex-1015, 1155

Table 3. Exemplary MAPS Drug Substance Specifications

Serotype (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F)	
Quality Attribute	**Acceptance Criteria**
Appearance	Clear, colorless, no particulate matter
pH	5 - 7.5
PS Identity	Positive
PS Concentration	> 0.25 mg/mL
Protein Concentration	FIO
Free Protein	FIO
Free PS	< 30%
Endotoxin	< $1.25x10^{-2}$ EU/µg PS
Bioburden	≤ 1 CFU/mL

FIO: for information only; MAPS: multiple antigen-presenting system; PS: polysaccharide.

Example 4: MAPS24, MAPS15, and MAPS9 Vaccines

Drug Product

[0469] MAPS24 is a 24-valent MAPS vaccine comprising 24 types of MAPS immunogenic complexes. Each type of complex comprises polysaccharide from one of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F. In exemplary formulations, a MAPS24 vaccine is formulated so that each 0.5-mL dose of MAPS24 drug product comprises 1, 2 or 5 µg of each PS (from each of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F) contributed by each species of MAPS immunogenic complex. MAPS24 was formulated for IM administration with aluminum phosphate adjuvant. The total amount of aluminum per dose was 0.625 mg, which is below the FDA/WHO maximum recommended dose of 0.85 mg to 1.25 mg. This formulated solution exhibited a pH of about 5.8. A pH range of 5.3-6.3 is considered acceptable. MAPS15 and MAPS9 are 15-valent and 9-

Petitioner Merck Ex-1015, 1156

valent MAPS vaccines respectively. Exemplary MAPS15 and MAP9 vaccines were formulated in the same manner as MAPS24 with the appropriate *S. pneumoniae* serotype PS as MAPS immunogenic complexes. The exemplary MAPS15 vaccines comprised 1, 2 or 5 μg of each *S. pneumoniae* serotype 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F PS as MAPS immunogenic complexes. The exemplary MAPS9 comprised 1, 2 or 5 μg of each *S. pneumoniae* serotype 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B as MAPS immunogenic complexes.

Vaccine Manufacturing Process

[0470] Drug products MAPS24, MAPS15 and MAPS9 were formulated by mixing the 24, 15, or 9 MAPS drug substances, compounding by mixing of excipients and buffers, filtering through a sterile filter, adding autoclaved aluminum phosphate and storing in glass vials at 2°C to 8°C. Exemplary manufacturing processes are shown in Panel A and Panel B of Figure 8. Drug product specifications are provided in Table 4.

Petitioner Merck Ex-1015, 1157

Table 4. Exemplary Drug Product Specifications (per PS)

Quality Attribute	1 µg Dose Specifications	2 µg Dose Specifications	5 µg Dose Specifications
Appearance	Grey/white homogeneous suspension	Grey/white homogeneous suspension	Grey/white homogeneous suspension
pH	5.3 - 6.3	5.3 - 6.3	5.3 - 6.3
PS Identity	Positive for each of the 24 serotypes	Positive for each of the 24 serotypes	Positive for each of the 24 serotypes
PS conc.	2.0 ± 0.6 µg/mL	4.0 ± 1.2 µg/mL	10.0 ± 3.0 µg/mL
Total protein conc.	Report Result	Report Result	Report Result
Aluminum content	1.25 ± 0.25 mg/mL	1.25 ± 0.25 mg/mL	1.25 ± 0.25 mg/mL
Osmolality	200 - 400 mOsm/kg	200 - 400 mOsm/kg	200 - 400 mOsm/kg
Extractable volume	> 0.5 mL	> 0.5 mL	> 0.5 mL
Endotoxin	< 40 EU/dose	< 40 EU/dose	< 40 EU/dose
Sterility	Sterile	Sterile	Sterile

Conc.: concentration; PS: polysaccharide.

Example 5: Immunogenicity of the Multivalent Pneumococcal Vaccines

Study 1: Evaluation of Serotype-specific PS Antibody Titers Following Immunization with 24-valent and 15-valent MAPS Drug Product

Methods

[0471] MAPS immunogenic complexes were assembled by combining biotinylated polysaccharide with fusion protein CP1 in the presence of 20 mM Tris, pH 8.0, 150 mM sodium chloride and incubating under stirring conditions at room temperature for 16 ± 4 hours. Aggregates formed during the reaction were removed by filtration through a 1.2 µm filter. The MAPS immunogenic complexes were then purified by diafiltration into 150 mM sodium chloride and sterile filtered using a 0.2 µm filter.

Petitioner Merck Ex-1015, 1158

[0472] Individual MAPS immunogenic complexes were combined to create starting material at full human dose (2.2 µg of each PS per dose) for 24-valent (MAPS24) and 15-valent (MAPS15) drug product. 1.25 mg/ml (based on elemental aluminum content) of adjuvant, either p-$Al(OH)_3$ or $AlPO_4$ were added to these MAPS immunogenic complex mixtures (either 24v or 15v) in formulation buffer and incubated with end-over-end rotation for 18 hours at 4°C to allow the MAPS immunogenic complexes to adsorb onto the adjuvants.

[0473] Six formulations were prepared at full human dose using 24-valent and 15-valent MAPS monovalents. These include: non-adjuvanted MAPS24 (Group A), p-$Al(OH)_3$ adjuvanted MAPS24 (Group B), $AlPO_4$ adjuvanted MAPS24 (Group C), non-adjuvanted MAPS15 (Group E), p-$Al(OH)_3$ adjuvanted MAPS15 (Group F) and $AlPO_4$ adjuvanted MAPS15 (Group G). Prevnar 13 (Group D), was used as control in this study. Prior to study start, Groups A-G above were diluted to 1/10 human dose (0.22 µg of each PS per dose except for PS 6B 0.44 µg PS per dose) with appropriate dilution buffers.

[0474] Six formulations were prepared at full human dose using 24-valent and 15-valent MAPS monovalents. These include: Non-adjuvanted MAPS24 (Group A), p-$Al(OH)_3$ adjuvanted MAPS24 (Group B), $AlPO_4$ adjuvanted MAPS24 (Group C), Non-adjuvanted MAPS15 (Group E), p-$Al(OH)_3$ adjuvanted MAPS15 (Group F) and $AlPO_4$ adjuvanted MAPS15 (Group G). Prevnar 13 (Group D), was used as control in this study. Prior to study start, Groups A-G above were diluted to 1/10 human dose (0.22 µg of each PS per dose except for PS 6B in Prevnar 13 with a 0.44 µg PS per dose) with appropriate dilution buffers (Table 5).

Table 5. Dilutions to Create Doses Used in Study

Groups	Antigen	PS/Dose	Protein/Dose	Adjuvant
A	MAPS24, Non-adjuvanted	0.22 µg each	20.2 µg	NA
B	MAPS24, P-$Al(OH)_3$	0.22 µg each	20.2 µg	P-$Al(OH)_3$
C	MAPS24, $AlPO_4$	0.22 µg each	20.2 µg	$AlPO_4$
D	Prevnar 13 (Pfizer)	0.22 µg each (0.44 µg 6B)	3.4 µg	$AlPO_4$
E	MAPS15, Non-adjuvanted	0.22 µg each	13.3 µg	NA
F	MAPS15, P-$Al(OH)_3$	0.22 µg each	13.3 µg	P-$Al(OH)_3$
G	MAPS15, $AlPO_4$	0.22 µg each	13.3 µg	$AlPO_4$

NA, not applicable

Petitioner Merck Ex-1015, 1159

Rabbit Immunization Protocol

[0475] As summarized in Table 6, New Zealand White (NZW) rabbits (AFV1110-1162) were immunized intramuscularly at a single site (in the thigh; 0.5 mL/site) on day 0 and at the alternate thigh on day 14 with one-tenth human dose (0.22 μg) of each PS for Group A-C, MAPS24; Group E-G, MAPS15, and Group D Prevnar 13 (except for PS 6B 0.44 μg PS per dose). Sera were collected before the first immunization (referred to as P0), second immunization (referred to as P1) and two weeks after the last immunization on day 28 (referred to as P2).

Table 6. Rabbit Immunization Study Groups

Group	Antigen	Dose	Rabbit No	Rabbits	Vaccine Vol.	Admin Route	Immunization Schedule	Sera Collection
A	MAPS24, Non-adjuvanted	1/10 (0.22 μg)	1100-1108	9	0.5 mL	IM	0, 14	0, 14, 28
B	MAPS24, P-Al(OH)$_3$	1/10 (0.22 μg)	1109-1117	9	0.5 mL	IM	0, 14	0, 14, 28
C	MAPS24, $AlPO_4$	1/10 (0.22 μg)	1118-1126	9	0.5 mL	IM	0, 14	0, 14, 28
D	Prevnar 13 (Pfizer)	1/10 *(0.22 μg)	1127-1135	9	0.5 mL	IM	0, 14	0, 14, 28
E	MAPS15, Non-adjuvanted	1/10 (0.22 μg)	1136-1144	9	0.5 mL	IM	0, 14	0, 14, 28
F	MAPS15, P-Al(OH)$_3$	1/10 (0.22 μg)	1145-1153	9	0.5 mL	IM	0, 14	0, 14, 28
G	MAPS15, $AlPO_4$	1/10 (0.22 μg)	1154-1162	9	0.5 mL	IM	0, 14	0, 14, 28

*6B dose was 0.44 μg for 1/10 human dose.

Immunogenicity: Evaluation of serotype-specific polysaccharide antibody titers

[0476] To evaluate serotype-specific polysaccharide antibody titers in immunized rabbits, electrochemiluminescent based Meso Scale Discovery Immunoassay (MSD) was performed with U-plex plates coated with *Streptococcus pneumoniae* polysaccharides (PnPS) (serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 22F, 23F, and 33F). The existing 13v serum standard (BCH14) and 13v internal control serum (BCH13), were spiked with 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20A, 22F, and 33F specific antisera to generate Modified-BCH14 and Modified-BCH13 respectively (to facilitate the quantitation of the serotypes in the MAPS vaccine immunized rabbits). Briefly, 5-fold serial

Petitioner Merck Ex-1015, 1160

dilutions were created starting with a 1:200 dilution for the Modified-BCH14 using PBS-T, 1% BSA, and 5 μg/mL CWPS. The Modified-BCH13 (internal control serum) and the experimental test samples were diluted 1000-fold, 5000-fold, and 25000-fold with PBS-T, 1% BSA, and 5 μg/mL CWPS, respectively. Plates were washed with 1X PBS-T prior to adding the reference standard, control serum and the experimental test sera. After incubating at room temperature for one hour, plates were washed with PBS-T and secondary anti-rabbit SULFO-conjugated antibody was added. The plates were washed with PBS-T and 1X Read Buffer was added and the plates were read using an MSD Meso QuickPlex SQ 120 Model No. 1300.

[0477] Prevnar 13-immunized rabbit sera were used as control in this study.

Results and Conclusion

[0478] Results are shown in Figures 9-13. Overall, equivalent or higher serotype-specific polysaccharide antibody responses were observed in groups that received 24-valent or 15-valent MAPS formulations at both P1 and P2 compared to Prevnar 13 group (represented as dotted line at GMT ratio of 1 in Figures 9-12). In general, higher titers were observed in the non-adjuvanted and the $AlPO_4$ adjuvanted groups, compared to p-$Al(OH)_3$ adjuvanted groups for all serotypes.

[0479] In Figures 9-12, the 24-valent or 15-valent MAPS formulation groups were compared to Prevnar 13 group (dotted line at GMT ratio of 1). If GMT ratio > 1, then anti-PS titer for MAPS formulation groups is higher. If GMT ratio < 1, then anti-PS titer for MAPS formulation groups is lower.

[0480] In Figure 13, the antibody titer against PS of a representative individual serotype of *S. pneumoniae* (shown: serotype 1) in 24-valent or 15-valent MAPS formulation groups was compared to the titer against corresponding PS in the Prevnar 13 group.

Study 2: Evaluation of Serotype-specific PS Antibody Titers Following Immunization with 24-valent MAPS Drug Product in Comparison to PCV13

[0481] One *in vivo* pharmacodynamics study was performed to evaluate the immunogenicity profile of MAPS24 in comparison to PCV13 at one-tenth of PCV13 human dose in rabbits. Test substance evaluated was MAPS24 formulated with $AlPO_4$ adjuvant. The

Petitioner Merck Ex-1015, 1161

MAPS24 test vaccine used in the nonclinical pharmacology study contained 2.2 μg of each PS per dose, corresponding to the amount of each PS found in a dose of PCV13 (except for 6B, 4.4 μg in PCV13). MAPS24 and PCV13 were diluted to one-tenth (0.22 μg of each PS, except for 6B, 0.44 μg in PCV13) in buffer, prior to administration (0.5 mL/dose) in rabbits. Overall, higher immune response was observed with the MAPS24 group in comparison to the PCV13 group for the majority of common serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F), as shown in Figure 14. Moreover, robust immune response was observed with MAPS24 group for additional serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20B, 22F and 33F), as shown in Figure 15.

[0482] Refer to Study 1 above for detailed methods.

Example 6: Immunogenicity and Protection Against Colonization

Study 1: Immunogenic Responses and Protective Effect of 12V and 6V MAPS with Immunogenic Complexes on CP1

[0483] One pharmacodynamic study was conducted to see if T_H17 responses and a protective effect against a pneumococci strain was induced by MAPS immunogenic complexes with CP1. In this study, 12-valent MAPS with CP1 (12V MAPS CP1; serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F and 23F) and 6-valent MAPS with CP1 (6V MAPS CP1; serotypes 1, 3, 4, 5, 14 and 23F) were evaluated. A 6-valent MAPS with an alternate fusion protein (6V MAPS CP2a) and an inactivated whole-cell *S. pneumoniae* vaccine (WCB) were also evaluated. *S. pneumoniae* serotype 6B was used in the mouse challenge studies as the serotype was excluded from the MAPS immunogenic complexes.

[0484] T_H17 responses were evaluated after three immunizations. Peripheral blood samples were taken 2 weeks after the last immunization for *ex vivo* stimulation in the presence of the antigens as stimulant. The amount of interleukin 17A (IL-17A) secreted into media was then assessed after 6 days by ELISA. One to 2 weeks after bleed, mice were challenged with 10^7 colony forming unit (CFU) of type 6B pneumococci (603 strain). Nasopharyngeal wash for each mouse was conducted 10 days post-infection and pneumococci CFU were calculated based on growth on blood agar.

Petitioner Merck Ex-1015, 1162

[0485] The immunization of 12V MAPS CP1 and 6V MAPS CP1 induced T_H17 response that was detected by stimulation with pneumococcal whole cell antigen (WCA), as well as SP1500 and SP0785 proteins, as shown in Figure 16. Interestingly, as shown in Figure 17, significant protection was demonstrated from nasopharyngeal colonization with a *S. pneumoniae* serotype 6B, a serotype that is not incorporated into the PS component of the multivalent MAPS with CP1, suggesting that protection is mediated through a CP1-directed immune response.

Study 2: Immunogenic Responses and Protective Effect of MAPS24 with Immunogenic Complexes on CP1

[0486] A pharmacodynamic study was conducted to see if a Th17 response to CP1 and a protective effect against a pneumococcal strain are induced by MAPS24. Mice were immunized with an exemplary formulation of a 24-valent MAPS vaccine (MAPS24)with adjuvant (MAPS24: serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F), or with an exemplary formulation of a 23-valent MAPS vaccine (MAPS23) lacking the challenge serotype (*S. pneumoniae* serotype 6B) with adjuvant (MAPS23: serotypes 1, 2, 3, 4, 5, 6A,7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F and 33F). Mice were immunized with adjuvant only as a control.

[0487] Th17 responses were evaluated after two immunizations. Splenocytes or peripheral blood collected 2 weeks after the last immunization were subjected to *ex vivo* stimulation in the presence of fused SP1500 and SP0785 proteins of CP1 as stimulant. The amount of interleukin 17A (IL-17A) secreted into the media was assessed 3 to 6 days after culture by electro-chemiluminescent based Meso Scale Immunoassay (MSD). Statistically significant increase in IL-17A was demonstrated for the 24-valent MAPS vaccine with adjuvant (*e.g.*, passivated aluminum hydroxide (p-AlOH) or aluminum phosphate ($AlPO_4$)), compared to the control group immunized with adjuvant (*e.g.*, passivated aluminum hydroxide) alone (Figure 18).

[0488] Mice immunized with the 23-valent MAPS vaccine (lacking serotype 6B) and adjuvant control were challenged with 10^5 to 10^7 colony forming unit (CFU) of serotype 6B pneumococci (603 strain). Nasopharyngeal wash from each mouse was conducted 10 days after infection, and pneumococci CFU were calculated based on growth on blood agar. The group immunized with 23-valent MAPS vaccine and passivated aluminum hydroxide (p-AlOH)) had

Petitioner Merck Ex-1015, 1163

statistically significant reduction in the pneumococcal nasopharyngeal colonization, and the group immunized with 23-valent MAPS vaccine and aluminum phosphate ($AlPO_4$) demonstrated a trend of reduced pneumococcal nasal colonization (Figure 19). Without wishing to be bound by theories, significant protection from nasopharyngeal colonization with *S. pneumoniae* serotype 6B, the polysaccharide component of which is not included in the tested 23-valent MAPS vaccine, indicates that protection may be mediated through immune response to the CP1 protein component of the vaccine, or to polysaccharide components of the vaccine with cross-reactivity to NVT *S. pneumoniae* strains, or to a combination of both.

Example 7. Antibodies from Sera Obtained from Animals Immunized with an Exemplary Formulationof MAPS24 Vaccine Bind to Non-Vaccine Type S. pneumoniae

Methods

[0489] Exemplary formulations comprising MAPS24 and rabbit immunization protocol used in this Example are described in Study 1 of Example 5. New Zealand White rabbits were immunized i.m. on days 0 and 14 with either an exemplary formulation of the 24-valent MAPS24 vaccine or with Prevnar 13, both adjuvanted with aluminum phosphate. Sera were collected prior to first immunization (P0) and two weeks after the second immunization (P2). To assess the binding capacity of antibodies from MAPS24-immunized rabbits for *S. pneumoniae* with capsular polysaccharide serotypes not included in MAPS24 or Prevnar13 (non-vaccine types, NVTs), an ELISA with whole-cell *S. pneumoniae* as the coating antigen was utilized. Such an exemplary assay detects rabbit IgG that bind to whole-cell *S. pneumoniae* coated on the ELISA plate through surface antigens such as protein or polysaccharide. To reduce binding and signal from non-immunized rabbit sera, the *S. pneumoniae*-coated plate was blocked with non-fat dry milk. In addition, rabbit sera were pre-incubated with both non-fat dry milk and *S. pneumoniae* cell-wall polysaccharide to adsorb any cell-wall polysaccharide-specific and any non-specific antibodies that could produce signal in the assay. Sera from rabbits immunized with MAPS24 (0.22 μg dose per polysaccharide) or Prevnar 13 (0.22 μg dose per polysaccharide, except 6B 0.44 μg dose) and corresponding pre-immunization sera were assayed for binding to six different serotypes of *S. pneumoniae* with capsular polysaccharide not

Petitioner Merck Ex-1015, 1164

incorporated into either vaccine. Whole-cell binding IgG titers were expressed as arbitrary units/ml.

Results and Conclusions

[0490] As shown in Figure 20, a statistically significant increase between pre-immunization (P0) and post-immunization (P2) IgG binding levels for MAPS24 sera was observed for all six serotypes ($p<0.01$ for all serotypes, two-tailed, Wilcoxon matched pairs test). There was no statistically significant difference between the binding IgG levels for P0 and P2 for Prevnar 13 sera for four of the six serotypes assayed and lower significance than MAPS24 for two serotypes.

[0491] The induction ratios (fold-change) of the P2 IgG titers relative to the P0 IgG titers for each serum sample from both immunization groups were calculated, and the geometric mean induction ratio determined for antibody binding to each whole-cell NVT *S. pneumoniae* strain. As shown in Figure 21, the induction ratio for all six serotypes was significantly higher for MAPS24-immunized rabbits than for Prevnar 13-immunized rabbits ($p<0.001$ for five serotypes and $p<0.01$ for one serotype, two tailed Mann-Whitney test).

Example 8: Opsonophagocytic Killing of Non-Vaccine Types of S. pneumoniae: MAPS 24

Methods

[0492] Exemplary formulations comprising MAPS24 and rabbit immunization protocol used in this Example are described in Study 1 of Example 5. New Zealand White rabbits were immunized i.m. on days 0 and 14 with an exemplary formulation of MAPS24 adjuvanted with aluminum phosphate. Sera were collected prior to first immunization (P0) and two weeks after the second immunization (P2).

[0493] To demonstrate presence of functional antibodies against non-vaccine types (NVTs) of *S. pneumoniae*, polysaccharides of which are not incorporated in the 24-valent MAPS vaccine, a modified concentrated opsonophagocytic assay (COPA) with sequential incubation steps was established. In such an exemplary assay, the presence of functional antibodies is shown by killing of NVT *S. pneumoniae* strains following incubation with immune sera. Briefly,

Petitioner Merck Ex-1015, 1165

frozen stocks of *S. pneumoniae* were thawed and resuspended at 2 x 10^5 CFU/ml in assay buffer (Hank's buffered saline with 10 % heat-inactivated FBS). To each well of a 96-well plate, 20 µl of heat-inactivated rabbit serum diluted in assay buffer was added, followed by 10 µl of bacterial suspension in assay buffer. The bacteria and rabbit sera were incubated at room temperature for 30 minutes with shaking at 650 rpm. To each well, 10 µl of baby rabbit complement (Pel-Freeze Biologicals), neat or appropriately diluted in assay buffer, was added followed by incubation at room temperature for 30 min with shaking at 650 rpm. Differentiated HL60 cells (ATCC) were washed with assay buffer and resuspended to 1 x 10^7 cells/ml in assay buffer. To each well, 40 µl of this HL60 suspension was added (200 to 1, HL60 to bacteria ratio) followed by incubation at 37 °C with 5 % CO_2 and shaking at 650 rpm for 1 hour. Each plate was transferred to ice and incubated for 20 minutes. Contents of each well were diluted in water 1/5 and 1/25, and each dilution was then plated on 5 % blood agar plates. After overnight incubation at 37 °C with 5 % CO_2, the CFU were counted for each sample and dilution, discarding data points with colony counts greater than 250 CFU.

Results and Conclusions

[0494] Sera from rabbits immunized with MAPS24 were assayed at various dilutions, for example, ranging from 1:2 to 1:54, in comparison to matched naïve sera collected prior to immunization (pre-immune sera). Six different NVT *S. pneumoniae* strains were assayed separately (15A, 16F, 23A, 31, 35B and 35F). Colony forming units (CFU) were enumerated on blood agar plates. Killing activity was expressed as the percent reduction in CFU following incubation with immune sera compared to incubation with matched pre-immune sera, where the percent reduction is determined by [1 - (CFU/ml for immune sera / CFU/ml for pre-immune sera)] * 100. Without wishing to be bound by theory, in some embodiments, the killing activity of immune sera was dependent on both HL60 cells and active complement. As shown in Figure 22, reduction of CFU for the immune sera at different dilutions over the matched pre-immune sera was observed for at least one or more (*e.g.*, 1, 2, 3, 4, 5, or 6) non-vaccine serotypes. For example, reduction of CFU for the immune sera at 1/18 dilution or lower over the matched pre-immune sera was observed for all six non-vaccine serotypes. Titration profiles for killing activity vary with the bacterial serotype. Without wishing to be bound by theories, in some embodiments, the functional antibodies against one or more non-vaccine serotypes (NVT) of *S.*

Petitioner Merck Ex-1015, 1166

pneumoniae generated by MAPS24 immunization may be directed to the CP1 protein component of the vaccine, or to the polysaccharide components of the vaccine with cross-reactivity to NVT *S. pneumoniae* strains, or to a combination of both.

Example 9: Opsonophagocytic Killing of Non-Vaccine Types of S. pneumoniae: MAPS24 and Prevnar 13

Methods

[0495] Exemplary formulations comprising MAPS24 and rabbit immunization protocol used in this Example are described in Study 1 of Example 5. New Zealand White rabbits were immunized i.m. on days 0 and 14 with either an exemplary formulation of MAPS24 or with Prevnar 13, both adjuvanted with aluminum phosphate. Sera were collected prior to first immunization (P0) and two weeks after the second immunization (P2). To demonstrate presence of functional antibodies against NVTs of *S. pneumoniae,* polysaccharides of which are not incorporated in the 24-valent MAPS vaccine or Prevnar13, a modified concentrated opsonophagocytic assay (COPA) with sequential incubation steps was performed as described in Example 8. In such an exemplary assay, the presence of functional antibodies is shown by killing of NVT *S. pneumoniae* strains following incubation with immune sera.

Results and Conclusions

[0496] Sera from rabbits immunized with either MAPS24 or Prevnar 13 were assayed at various dilutions, for example, ranging from 1:2 to 1:54, in comparison to matched naïve sera collected prior to immunization (pre-immune sera). Two different NVT *S. pneumoniae* strains (15A and 16F) were assayed separately. Colony forming units (CFU) were enumerated on blood agar plates. Results were expressed as the percent survival of bacteria incubated with immune sera compared to incubation with matched pre-immune sera.

[0497] Figure 23 shows that NVT *S. pneumoniae* serotype 16F incubated with sera from MAPS24-immunized rabbits displayed reduced survival, as compared to bacteria incubated with sera from Prevnar13-immunized rabbits. Panel A of Figure 23 shows a statistically significant reduction in survival of the NVT *S. pneumoniae* serotype 16F for three of the four tested dilutions of MAPS24-immunized rabbit sera when compared to pre-immune sera. In contrast,

Petitioner Merck Ex-1015, 1167

Panel B of Figure 23 shows that the Prevnar 13-immunized rabbit sera did not result in significant decreases in bacterial survival at any dilution.

[0498] Figure 24 shows that NVT *S. pneumoniae* serotype 15A incubated with sera from MAPS24-immunized rabbits displayed reduced survival, as compared to bacteria incubated with sera from Prevnar13-immunized rabbits. Panel A of Figure 24 shows a statistically significant reduction in survival of the NVT *S. pneumoniae* serotype 15A for three of the four tested dilutions of MAPS24-immunized rabbit sera when compared to pre-immune sera. In contrast, Panel B of Figure 24 shows that the Prevnar 13-immunized rabbit sera did not result in significant decreases in bacterial survival at any dilution.

[0499] Without wishing to be bound by theories, in some embodiments, the functional antibodies against one or more non-vaccine serotypes (NVT) of *S. pneumoniae* generated by MAPS24 immunization may be directed to the CP1 protein component of the vaccine, or to the polysaccharide components of the vaccine with cross-reactivity to NVT *S. pneumoniae* strains, or to a combination of both.

Example 10: Inhibition of Opsonophagocytic Killing of Non-Vaccine Type Serotype of S. pneumoniae with CP1

[0500] Examples 8 and 9 demonstrate the presence of functional antibodies by opsonophagocytic killing of non-vaccine type serotype(s) (NVTs) of *S. pneumoniae* using exemplary formulations of MAPS (*e.g.*, MAPS24). To demonstrate specificity of functional antibodies against NVTs of *S. pneumoniae*, an inhibition variant of the modified concentrated opsonophagocytic assay (iCOPA) was established. In such an exemplary assay, the presence of functional antibodies is shown by killing of NVT *S. pneumoniae* serotypes following incubation with immune sera; the specificity of these functional antibodies is assayed by pre-incubation of the immune sera with a specific inhibitory reagent (*e.g.*, a purified protein, *e.g.*, fusion protein CP1) that inhibits or competes with binding of the functional antibodies to a target antigen and subsequent incubation with NVT *S. pneumoniae* serotypes, to determine if killing has been inhibited. Sera from rabbits immunized with MAPS24 were assayed at various dilutions, for example, ranging from 1:2 to 1:54, in comparison to matched naïve sera collected prior to immunization (pre-immune sera). Killing of the NVT *S. pneumoniae* 16F strain was assessed

Petitioner Merck Ex-1015, 1168

using immune sera with and without pre-incubation with the purified CP1 component of MAPS24. Colony forming units (CFU) were enumerated on blood agar plates. Killing activity was expressed as the percent reduction in CFU following incubation with immune sera compared to incubation with matched pre-immune sera, where the percent reduction is determined by [1 - (CFU/ml for immune sera / CFU/ml for pre-immune sera)] * 100. As shown in Figure 25, the presence of functional antibodies to NVT *S. pneumoniae* 16F in immune sera, in the absence of a specific inhibitory reagent, is shown by percent killing activity for the immune sera over the pre-immune sera. Pre-incubation of the immune sera with purified CP1 protein, under conditions favoring competitive binding of functional antibodies to CP1 protein, resulted in lower percent killing activity. Without wishing to be bound by theory, this result indicates that functional antibodies against non-vaccine type serotype (*e.g.*, serotype 16F) of *S. pneumoniae* generated by MAPS24 immunization may be directed to the CP1 protein component of the vaccine.

Example 11: Opsonophagocytic Killing of Non-Vaccine Type Serotype of S. pneumoniae: SP1500 and SP0785 polypeptides

[0501] This Example demonstrates the presence of functional antibodies against a representative NVT of *S. pneumoniae* in sera collected from animals immunized with SP1500 or SP0785 polypeptides (immune sera).

Methods

[0502] Exemplary rabbit immunization protocols used in this Example are described in Study 1 of Example 5. New Zealand White rabbits (n = 3) were immunized i.m. separately on days 0, 14, and 28 with 100 μg of SP1500 or SP0785 polypeptides, adjuvanted with aluminum phosphate. Sera were collected prior to first immunization (P0) and two weeks after the third immunization (P3). Sera collected from the 3 rabbits at each timepoint were combined to form a pool and stored at -80°C. To demonstrate presence of functional antibodies against NVT *S. pneumoniae* 16F, polysaccharides of which are not incorporated in the SP1500- or SP0785-comprising formulations , a modified concentrated opsonophagocytic assay (COPA) with sequential incubation steps was performed as described in Example 8. In such an exemplary

Petitioner Merck Ex-1015, 1169

assay, the presence of functional antibodies is shown by killing of NVT *S. pneumoniae* 16F following incubation with immune sera.

[0503] Results. Sera from rabbits immunized with either SP1500 or SP0785 polypeptides were assayed at various dilutions, for example, ranging from 1:2 to 1:54, in comparison to matched naïve sera collected prior to immunization (pre-immune sera). Colony forming units (CFU) were enumerated on blood agar plates. Killing activity was expressed as the percent reduction in CFU following incubation with immune sera compared to incubation with matched pre-immune sera, where the percent reduction is determined by [1 - (CFU/ml for immune sera / CFU/ml for pre-immune sera)] * 100.

[0504] Figure 26 shows that incubation with immune sera from rabbits separately immunized with SP1500 and SP0785 polypeptides resulted in killing activity against NVT *S. pneumoniae* serotype 16F for three of the four tested dilutions when compared to pre-immune sera. Without wishing to be bound by theory, this result indicates that functional antibodies against one or more non-vaccine serotypes (NVT) of *S. pneumoniae* may be generated by immunization with SP1500 or SP0785 polypeptides.

[0505] A pre-clinical study demonstrates that multivalent MAPS vaccines as described herein (*e.g.*, MAPS24, MAPS15 and MAPS9 vaccines) are highly immunogenic in animal models and induce serotype-specific responses. MAPS24 confers clinically meaningful advantages over the current PCV13 vaccine at least by adding significant additional coverage to the target populations with an additional 11 serotypes. Further, MAPS24 generated functional antibodies against one or more non-vaccine serotypes. In addition, the MAPS24 vaccine simplifies the current complex pneumococcal immunization regimen in high-risk and older adults by providing protection against all included serotypes in a single dose instead of two doses of two different vaccines administered according to complex schedules.

Petitioner Merck Ex-1015, 1170

Sequence Listing

[0506] SEQ ID NO:1, rhizavidin protein, full-length [amino acids 1-179]:

MIITSLYATFGTIADGRRTSGGKTMIRTNAVAALVFAVATSALAFDASNFKDFSSIASASSSWQ
NQSGSTMIIQVDSFGNVSGQYVNRAQGTGCQNSPYPLTGRVNGTFIAFSVGWNNSTENCNSATG
WTGYAQVNGNNTEIVTSWNLAYEGGSGPAIEQGQDTFQYVPTTENKSLLKD

[0507] SEQ ID NO:2, truncated rhizavidin protein, denoted Rhavi [amino acids 45-179]:

FDASNFKDFSSIASASSSWQNQSGSTMIIQVDSFGNVSGQYVNRAQGTGCQNSPYPLTGRVNGT
FIAFSVGWNNSTENCNSATGWTGYAQVNGNNTEIVTSWNLAYEGGSGPAIEQGQDTFQYVPTTE
NKSLLKD

[0508] SEQ ID NO:3, linker sequence [7 amino acids]:

GGGGSSS

[0509] SEQ ID NO:4, SP0785 protein lacking signal sequence and transmembrane domain [amino acids 33-399]:

Note: One T394A mismatch with SP0785 NCBI Sequences ABJ54007.1 and YP816180

FRQPSQTALKDEPTHLVVAKEGSVASSVLLSGTVTAKNEQYVYFDASKGDLDEILVSVGDKVSE
GQALVKYSSSEAQAAYDSASRAVARADRHINELNQARNEAASAPAPQLPAPVGGEDATVQSPTP
VAGNSVASIDAQLGDARDARADAAAQLSKAQSQLDATTVLSTLEGTVVEVNSNVSKSPTGASQV
MVHIVSNENLQVKGELSEYNLANLSVGQEVSFTSKVYPDKKWTGKLSYISDYPKNNGEAASPAA
GNNTGSKYPYTIDVTGEVGDLKQGFSVNIEVKSKTKAILVPVSSLVMDDSKNYVWIVDEQQKAK
KVEVSLGNADAENQEITSGLTNGAKVISNPTSSLEEGKEVKADEATN

[0510] SEQ ID NO:5, SP1500 protein lacking signal sequence [amino acids 27-278]:

TSGDNWSKYQSNKSITIGFDSTFVPMGFAQKDGSYAGFDIDLATAVFEKYGITVNWQPIDWDLK
EAELTKGTIDLIWNGYSATDERREKVAFSNSYMKNEQVLVTKKSSGITTAKDMTGKTLGAQAGS
SGYADFEANPEILKNIVANKEANQYQTFNEALIDLKNDRIDGLLIDRVYANYYLEAEGVLNDYN
VFTVGLETEAFAVGARKEDTNLVKKINEAFSSLYKDGKFQEISQKWFGEDVATKEVKEGQ

[0511] SEQ ID NO:6, fusion protein CP1:

Petitioner Merck Ex-1015, 1171

MFDASNFKDFSSIASASSSWQNQSGSTMIIQVDSFGNVSGQYVNRAQGTGCQNSPYP
LTGRVNGTFIAFSVGWNNSTENCNSATGWTGYAQVNGNNTEIVTSWNLAYEGGSGP
AIEQGQDTFQYVPTTENKSLLKDGGGGSSSTSGDNWSKYQSNKSITIGFDSTFVPMGF
AQKDGSYAGFDIDLATAVFEKYGITVNWQPIDWDLKEAELTKGTIDLIWNGYSATDE
RREKVAFSNSYMKNEQVLVTKKSSGITTAKDMTGKTLGAQAGSSGYADFEANPEILK
NIVANKEANQYQTFNEALIDLKNDRIDGLLIDRVYANYYLEAEGVLNDYNVFTVGLE
TEAFAVGARKEDTNLVKKINEAFSSLYKDGKFQEISQKWFGEDVATKEVKEGQAAAF
RQPSQTALKDEPTHLVVAKEGSVASSVLLSGTVTAKNEQYVYFDASKGDLDEILVSV
GDKVSEGQALVKYSSSEAQAAYDSASRAVARADRHINELNQARNEAASAPAPQLPAP
VGGEDATVQSPTPVAGNSVASIDAQLGDARDARADAAAQLSKAQSQLDATTVLSTLE
GTVVEVNSNVSKSPTGASQVMVHIVSNENLQVKGELSEYNLANLSVGQEVSFTSKV
YPDKKWTGKLSYISDYPKNNGEAASPAAGNNTGSKYPYTIDVTGEVGDLKQGFSVN
IEVKSKTKAILVPVSSLVMDDSKNYVWIVDEQQKAKKVEVSLGNADAENQEITSGLT
NGAKVISNPTSSLEEGKEVKADEATN

[0512] SEQ ID NO:7, SP0785 gene encoding SP0785 protein lacking signal sequence [amino acids 33-399]:

TTTAGACAACCTTCTCAGACTGCTCTAAAAGATGAGCCTACTCATCTTGTTGTTGCCAAGGAAG
GAAGCGTGGCCTCCTCTGTTTTATTGTCAGGGACAGTAACAGCAAAAAATGAACAATATGTTTA
TTTTGATGCTAGTAAGGGTGATTTAGATGAAATCCTTGTTTCTGTGGGCGATAAGGTCAGCGAA
GGGCAGGCTTTAGTCAAGTACAGTAGTTCAGAAGCGCAGGCGGCCTATGATTCAGCTAGTCGAG
CAGTAGCTAGGGCAGATCGTCATATCAATGAACTCAATCAAGCACGAAATGAAGCCGCTTCAGC
TCCGGCTCCACAGTTACCAGCGCCAGTAGGAGGAGAAGATGCAACGGTGCAAAGCCCAACTCCA
GTGGCTGGAAATTCTGTTGCTTCTATTGACGCTCAATTGGGTGATGCCCGTGATGCGCGTGCAG
ATGCTGCGGCGCAATTAAGCAAGGCTCAAAGTCAATTGGATGCAACAACTGTTCTCAGTACCCT
AGAGGGAACTGTGGTCGAAGTCAATAGCAATGTTTCTAAATCTCCAACAGGGGCGAGTCAAGTT
ATGGTTCATATTGTCAGCAATGAAAATTTACAAGTCAAGGGAGAATTGTCTGAGTACAATCTAG
CCAACCTTTCTGTAGGTCAAGAAGTAAGCTTTACTTCTAAAGTGTATCCTGATAAAAAATGGAC
TGGGAAATTAAGCTATATTTCTGACTATCCTAAAAACAATGGTGAAGCAGCTAGTCCAGCAGCC
GGGAATAATACAGGTTCTAAATACCCTTATACTATTGATGTGACAGGCGAGGTTGGTGATTTGA
AACAAGGTTTTTCTGTCAACATTGAGGTTAAAAGCAAAACTAAGGCTATTCTTGTTCCTGTTAG

Petitioner Merck Ex-1015, 1172

CAGTCTAGTAATGGATGATAGTAAAAATTATGTCTGGATTGTGGATGAACAACAAAAGGCTAAA
AAAGTTGAGGTTTCATTGGGAAATGCTGACGCAGAAAATCAAGAAATCACTTCTGGTTTAACGA
ACGGTGCTAAGGTCATCAGTAATCCAACATCTTCCTTGGAAGAAGGAAAAGAGGTGAAGGCTGA
TGAAGCAACTAAT

[0513] SEQ ID NO:8, SP1500 gene encoding SP1500 protein lacking signal sequence [amino acids 27-278]:

ACTAGTGGAGATAATTGGTCAAAGTACCAGTCTAACAAGTCTATTACTATTGGATTTGATAGTA
CTTTTGTTCCAATGGGATTTGCTCAGAAAGATGGTTCTTATGCAGGATTTGATATTGATTTAGC
TACAGCTGTTTTTTGAAAAATACGGAATCACGGTAAATTGGCAACCGATTGATTGGGATTTGAAA
GAAGCTGAATTGACAAAAGGAACGATTGATCTGATTTGGAATGGCTATTCCGCTACAGACGAAC
GCCGTGAAAAGGTGGCTTTCAGTAACTCATATATGAAGAATGAGCAGGTATTGGTTACGAAGAA
ATCATCTGGTATCACGACTGCAAAGGATATGACTGGAAAGACATTAGGAGCTCAAGCTGGTTCA
TCTGGTTATGCGGACTTTGAAGCAAATCCAGAAATTTTGAAGAATATTGTCGCTAATAAGGAAG
CGAATCAATACCAAACCTTTAATGAAGCCTTGATTGATTTGAAAAACGATCGAATTGATGGTCT
ATTGATTGACCGTGTCTATGCAAACTATTATTTAGAAGCAGAAGGTGTTTTAAACGATTATAAT
GTCTTTACAGTTGGACTAGAAACAGAAGCTTTTGCGGTTGGAGCCCGTAAGGAAGATACAAACT
TGGTTAAGAAGATAAATGAAGCTTTTTCTAGTCTTTACAAGGACGGCAAGTTCCAAGAAATCAG
CCAAAAATGGTTTGGAGAAGATGTAGCAACCAAAGAAGTAAAAGAAGGACAG

[0514] SEQ ID NO:9, codon-optimized sequence encoding fusion protein CP1 [nucleic acid]:

ATGTTCGACGCATCCAACTTTAAAGACTTTAGCAGCATCGCGTCCGCAAGCTCTAGCTGGCAGA
ATCAATCTGGTAGCACCATGATTATCCAAGTGGACAGCTTTGGTAACGTCAGCGGTCAATATGT
TAATCGTGCACAGGGTACGGGTTGTCAGAATTCTCCGTACCCGCTGACCGGTCGTGTTAACGGC
ACGTTCATCGCTTTCAGCGTCGGTTGGAACAATTCTACTGAAAATTGCAACAGCGCGACCGGTT
GGACGGGCTATGCACAAGTGAATGGCAATAACACCGAAATCGTCACGTCCTGGAATCTGGCGTA
TGAGGGTGGCAGCGGTCCGGCTATTGAACAGGGCCAGGATACCTTCCAATACGTCCCTACGACC
GAGAATAAGTCCCTTCTGAAAGACGGCGGTGGCGGTTCGAGCTCGACCAGCGGCGACAATTGGT
CCAAATACCAGAGCAACAAGAGCATCACGATCGGCTTCGACAGCACTTTTGTGCCGATGGGTTT
CGCGCAAAAAGACGGTAGCTACGCGGGTTTCGATATTGACCTGGCGACCGCTGTCTTTGAGAAA
TACGGCATTACGGTTAATTGGCAGCCGATTGATTGGGACCTGAAAGAGGCCGAACTCACCAAAG

Petitioner Merck Ex-1015, 1173

GCACCATCGACCTGATCTGGAATGGTTACTCCGCAACCGATGAGCGTCGCGAAAAAGTTGCCTT
CAGCAACAGCTATATGAAGAATGAACAAGTGTTGGTAACCAAGAAATCTAGCGGCATTACGACC
GCGAAAGACATGACCGGTAAGACGCTGGGTGCGCAGGCCGGTAGCTCTGGCTATGCGGATTTCG
AGGCGAATCCTGAGATTCTGAAAAACATCGTTGCGAATAAAGAGGCGAACCAGTACCAGACCTT
TAACGAAGCACTGATCGACCTGAAAAACGATCGCATTGACGGTCTGCTGATCGATCGTGTGTAC
GCGAACTATTATCTGGAAGCCGAGGGCGTTCTGAACGATTATAATGTTTTTACCGTGGGTCTGG
AGACTGAGGCATTCGCGGTTGGTGCGCGCAAGGAAGATACCAACCTGGTTAAAAAGATTAATGA
GGCATTTAGCTCACTGTACAAGGACGGCAAGTTCCAAGAAATTAGCCAGAAGTGGTTCGGTGAA
GATGTTGCGACGAAAGAGGTTAAAGAGGGCCAAGCGGCCGCATTTCGCCAACCGAGCCAGACTG
CGTTGAAAGATGAGCCGACCCATCTGGTTGTTGCGAAAGAGGGCAGCGTGGCATCGAGCGTGCT
GCTGAGCGGTACGGTTACTGCCAAAAACGAACAATACGTGTACTTCGATGCTAGCAAGGGTGAT
CTGGATGAAATTCTGGTGAGCGTGGGTGACAAAGTTAGCGAAGGCCAGGCACTGGTGAAGTATT
CATCCTCCGAGGCACAGGCAGCGTACGACAGCGCAAGCCGCGCAGTGGCGCGTGCCGACCGTCA
CATTAACGAATTGAACCAAGCGCGTAACGAGGCCGCAAGCGCGCCAGCACCGCAGCTGCCGGCT
CCGGTGGGTGGCGAAGATGCGACGGTGCAGAGCCCGACCCCGGTTGCGGGTAATTCGGTCGCCA
GCATCGATGCGCAGCTGGGTGACGCGCGTGATGCCCGTGCGGATGCGGCTGCTCAACTGAGCAA
GGCTCAGAGCCAACTGGACGCGACGACGGTGCTGAGCACCTTGGAGGGTACCGTTGTCGAAGTC
AACAGCAATGTGAGCAAGAGCCCAACGGGTGCGAGCCAGGTTATGGTCCACATTGTGAGCAATG
AAAACTTACAGGTCAAGGGTGAGCTGAGCGAGTATAACCTGGCGAATCTGAGCGTTGGTCAAGA
GGTCAGCTTTACCAGCAAGGTCTACCCGGATAAGAAATGGACCGGCAAGTTGAGCTACATCAGC
GACTACCCGAAGAACAATGGCGAGGCAGCCTCCCCGGCAGCCGGCAACAATACCGGCTCTAAGT
ATCCGTACACCATCGACGTAACCGGTGAGGTCGGCGACCTGAAACAGGGTTTTAGCGTGAATAT
CGAAGTGAAGTCCAAGACCAAGGCAATTTTGGTTCCGGTTAGCTCCCTGGTGATGGACGATAGC
AAGAATTATGTGTGGATTGTCGACGAGCAACAGAAAGCGAAAAAAGTTGAAGTGAGCCTGGGCA
ATGCTGATGCCGAGAACCAAGAAATCACGTCTGGTCTGACCAACGGTGCGAAAGTTATTAGCAA
CCCGACCAGCAGCCTGGAAGAGGGTAAAGAGGTCAAAGCCGACGAAGCTACGAAC

[0515] SEQ ID NO:10, SP0785 protein, full-length [amino acids 1-399], TIGR4 strain:

MKKKNGKAKKWQLYAAIGAASVVVLGAGGILLFRQPSQTALKDEPTHLVV
AKEGSVASSVLLSGTVTAKNEQYVYFDASKGDLDEILVSVGDKVSEGQAL
VKYSSSEAQAAYDSASRAVARADRHINELNQARNEAASAPAPQLPAPVGG

Petitioner Merck Ex-1015, 1174

EDATVQSPTPVAGNSVASIDAQLGDARDARADAAAQLSKAQSQLDATTVL
STLEGTVVEVNSNVSKSPTGASQVMVHIVSNENLQVKGELSEYNLANLSV
GQEVSFTSKVYPDKKWTGKLSYISDYPKNNGEAASPAAGNNTGSKYPYTI
DVTGEVGDLKQGFSVNIEVKSKTKAILVPVSSLVMDDSKNYVWIVDEQQK

AKKVEVSLGNADAENQEITSGLTNGAKVISNPTSSLEEGKEVKADEATN

[0516] SEQ ID NO:11, SP0785 gene encoding SP0785 protein, full-length [amino acids 1-399], TIGR4 strain:

ATGAAGAAAAAGAATGGTAAAGCTAAAAAGTGGCAACTGTATGCAGCAAT

CGGTGCTGCGAGTGTAGTTGTATTGGGTGCTGGGGGGATTTTACTCTTTA
GACAACCTTCTCAGACTGCTCTAAAAGATGAGCCTACTCATCTTGTTGTT
GCCAAGGAAGGAAGCGTGGCCTCCTCTGTTTTATTGTCAGGGACAGTAAC
AGCAAAAAATGAACAATATGTTTATTTTGATGCTAGTAAGGGTGATTTAG
ATGAAATCCTTGTTTCTGTGGGCGATAAGGTCAGCGAAGGGCAGGCTTTA
GTCAAGTACAGTAGTTCAGAAGCGCAGGCGGCCTATGATTCAGCTAGTCG
AGCAGTAGCTAGGGCAGATCGTCATATCAATGAACTCAATCAAGCACGAA
ATGAAGCCGCTTCAGCTCCGGCTCCACAGTTACCAGCGCCAGTAGGAGGA
GAAGATGCAACGGTGCAAAGCCCAACTCCAGTGGCTGGAAATTCTGTTGC
TTCTATTGACGCTCAATTGGGTGATGCCCGTGATGCGCGTGCAGATGCTG
CGGCGCAATTAAGCAAGGCTCAAAGTCAATTGGATGCAACAACTGTTCTC
AGTACCCTAGAGGGAACTGTGGTCGAAGTCAATAGCAATGTTTCTAAATC
TCCAACAGGGGCGAGTCAAGTTATGGTTCATATTGTCAGCAATGAAAATT
TACAAGTCAAGGGAGAATTGTCTGAGTACAATCTAGCCAACCTTTCTGTA
GGTCAAGAAGTAAGCTTTACTTCTAAAGTGTATCCTGATAAAAAATGGAC
TGGGAAATTAAGCTATATTTCTGACTATCCTAAAAACAATGGTGAAGCAG
CTAGTCCAGCAGCCGGGAATAATACAGGTTCTAAATACCCTTATACTATT
GATGTGACAGGCGAGGTTGGTGATTTGAAACAAGGTTTTTCTGTCAACAT
TGAGGTTAAAAGCAAAACTAAGGCTATTCTTGTTCCTGTTAGCAGTCTAG
TAATGGATGATAGTAAAAATTATGTCTGGATTGTGGATGAACAACAAAAG
GCTAAAAAAGTTGAGGTTTCATTGGGAAATGCTGACGCAGAAAATCAAGA
AATCACTTCTGGTTTAACGAACGGTGCTAAGGTCATCAGTAATCCAACAT

Petitioner Merck Ex-1015, 1175

CTTCCTTGGAAGAAGGAAAAGAGGTGAAGGCTGATGAAGCAACTAAT

[0517] SEQ ID NO:12, SP1500 protein, full-length [amino acids 1-278], TIGR4 strain:

MKKWMLVLVSLMTALFLVACGKNSSETSGDNWSKYQSNKSITIGFDSTFV
PMGFAQKDGSYAGFDIDLATAVFEKYGITVNWQPIDWDLKEAELTKGTID
LIWNGYSATDERREKVAFSNSYMKNEQVLVTKKSSGITTAKDMTGKTLGA
QAGSSGYADFEANPEILKNIVANKEANQYQTFNEALIDLKNDRIDGLLID
RVYANYYLEAEGVLNDYNVFTVGLETEAFAVGARKEDTNLVKKINEAFSS
LYKDGKFQEISQKWFGEDVATKEVKEGQ

[0518] SEQ ID NO:13, SP1500 gene encoding SP1500 protein, full-length [amino acids 1-278], TIGR4 strain:

ATGAAAAAATGGATGCTTGTATTAGTCAGTCTGATGACTGCTTTGTTCTT
AGTAGCTTGTGGGAAAAATTCTAGCGAAACTAGTGGAGATAATTGGTCAA
AGTACCAGTCTAACAAGTCTATTACTATTGGATTTGATAGTACTTTTGTT
CCAATGGGATTTGCTCAGAAAGATGGTTCTTATGCAGGATTTGATATTGA
TTTAGCTACAGCTGTTTTTGAAAAATACGGAATCACGGTAAATTGGCAAC
CGATTGATTGGGATTTGAAAGAAGCTGAATTGACAAAAGGAACGATTGAT
CTGATTTGGAATGGCTATTCCGCTACAGACGAACGCCGTGAAAAGGTGGC
TTTCAGTAACTCATATATGAAGAATGAGCAGGTATTGGTTACGAAGAAAT
CATCTGGTATCACGACTGCAAAGGATATGACTGGAAAGACATTAGGAGCT
CAAGCTGGTTCATCTGGTTATGCGGACTTTGAAGCAAATCCAGAAATTTT
GAAGAATATTGTCGCTAATAAGGAAGCGAATCAATACCAAACCTTTAATG
AAGCCTTGATTGATTTGAAAAACGATCGAATTGATGGTCTATTGATTGAC
CGTGTCTATGCAAACTATTATTTAGAAGCAGAAGGTGTTTTAAACGATTA
TAATGTCTTTACAGTTGGACTAGAAACAGAAGCTTTTGCGGTTGGAGCCC
GTAAGGAAGATACAAACTTGGTTAAGAAGATAAATGAAGCTTTTTCTAGT
CTTTACAAGGACGGCAAGTTCCAAGAAATCAGCCAAAAATGGTTTGGAGA
AGATGTAGCAACCAAAGAAGTAAAAGAAGGACAG

Petitioner Merck Ex-1015, 1176

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Petitioner Merck Ex-1015, 1177

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Petitioner Merck Ex-1015, 1180

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Equivalents

[0519] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

Petitioner Merck Ex-1015, 1181

Claims

We claim:

1. A vaccine comprising an immunogenic complex, wherein the immunogenic complex comprises:

(a) a biotinylated polysaccharide antigen; and

(b) a fusion protein comprising:

(i) a biotin-binding moiety; and

(ii) at least one polypeptide antigen;

wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex.

2. The vaccine of claim 1, wherein the fusion protein comprises:
an SP1500 polypeptide or antigenic fragment thereof; or
an SP0785 polypeptide or antigenic fragment thereof.

3. The vaccine of claim 1 or claim 2, wherein the fusion protein comprises:

(a) a first polypeptide antigen comprising an SP1500 polypeptide or antigenic fragment thereof; and

(b) a second polypeptide antigen comprising an SP0785 polypeptide or antigenic fragment thereof.

4. The vaccine of any one of the preceding claims, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae.*

5. The vaccine of any one of the preceding claims, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A,

Petitioner Merck Ex-1015, 1182

23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

6. The vaccine of any one of claims 1-5, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F.

7. The vaccine of any one of claims 1-6, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

8. The vaccine of any one of claims 1-6, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B.

9. A vaccine comprising:

a plurality of immunogenic complexes comprising:

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

Petitioner Merck Ex-1015, 1183

95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

10. A vaccine comprising:

a plurality of immunogenic complexes comprising:

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

11. A vaccine comprising:

Petitioner Merck Ex-1015, 1184

a plurality of immunogenic complexes comprising:

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

12. A vaccine comprising:

a plurality of immunogenic complexes comprising:

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

Petitioner Merck Ex-1015, 1185

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

13. A vaccine comprising:

a plurality of immunogenic complexes comprising:

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

14. A vaccine comprising:

a plurality of immunogenic complexes comprising:

Petitioner Merck Ex-1015, 1186

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the first plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

15. A vaccine comprising:

a plurality of immunogenic complexes comprising:

(a) a plurality of biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B; and

(b) a plurality of fusion proteins, each fusion protein comprising

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

Petitioner Merck Ex-1015, 1187

95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

wherein each of the plurality of biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of fusion proteins to form an immunogenic complex.

16. The vaccine of any one of the preceding claims, wherein the fusion protein, or each of the plurality of fusion proteins, is or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:6.

17. The vaccine of any one of the preceding claims, wherein the biotin-binding moiety is a polypeptide comprising (i) an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:1 or a biotin-binding fragment thereof; or (ii) a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:2 or a biotin-binding fragment thereof.

18. The vaccine of any one of the preceding claims, wherein the fusion protein is SEQ ID NO: 6.

19. The vaccine of any one of the preceding claims, wherein the plurality of immunogenic complexes comprises one species of immunogenic complexes, wherein the species comprises a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype.

20. The vaccine of any one of the preceding claims, wherein the plurality of immunogenic complexes comprises two or more different species of immunogenic complexes, wherein each species comprises a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype.

Petitioner Merck Ex-1015, 1188

21. The vaccine of any one of the preceding claims, wherein the plurality of immunogenic complexes comprises nine or more different species of immunogenic complexes, wherein each species comprises a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype.

22. The vaccine of any one of the preceding claims, wherein the plurality of immunogenic complexes comprises fifteen or more different species of immunogenic complexes, wherein each species comprises a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype.

23. The vaccine of any one of the preceding claims, wherein the plurality of immunogenic complexes comprises twenty four or more different species of immunogenic complexes, wherein each species comprises a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype.

24. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with a fusion protein;

Petitioner Merck Ex-1015, 1189

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with a fusion protein; and

Petitioner Merck Ex-1015, 1190

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

and wherein each of the biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of at least one fusion protein to form an immunogenic complex.

25. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with a fusion protein;

Petitioner Merck Ex-1015, 1191

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with a fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

and wherein each of the biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of at least one fusion protein to form an immunogenic complex.

Petitioner Merck Ex-1015, 1192

26. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with a biotin-binding fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with a fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

Petitioner Merck Ex-1015, 1193

least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof;

and wherein each of the biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of at least one fusion protein to form an immunogenic complex.

27. The vaccine of any one of claims 19-26, wherein the vaccine comprises a stoichiometrically equal ratio, by weight, of each of the polysaccharide antigens of the different species.

28. The vaccine of any one of claims 19-26, wherein the vaccine comprises at least one of the polysaccharide antigens of the different species at a stoichiometrically different ratio, by weight.

29. The vaccine of any one of claims 19-26, wherein the vaccine comprises a stoichiometrically different ratio, by weight, of each of the polysaccharide antigens of the different species.

30. The vaccine of any one of the preceding claims, wherein the biotin-binding moiety is (i) a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:1 or a biotin-binding fragment thereof; (ii) or a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:2 or a biotin-binding fragment thereof.

31. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* non-covalently associated with a fusion protein, wherein the fusion protein comprises a biotin-binding moiety and at least one polypeptide antigen.

32. The immunogenic complex of claim 31, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or

Petitioner Merck Ex-1015, 1194

more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

33. The immunogenic complex of claim 31 or claim 32, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F.

34. The immunogenic complex of any one of claims 31-33, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

35. The immunogenic complex of any one of claims 31-33, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B.

36. The immunogenic complex of any one of claims 31-35, wherein the fusion protein comprises:

an SP1500 polypeptide or antigenic fragment thereof;

an SP0785 polypeptide or antigenic fragment thereof;

or a combination thereof.

37. The immunogenic complex of any one of claims 31-36, wherein the fusion protein comprises:

(a) a first polypeptide antigen comprising an SP1500 polypeptide or antigenic fragment thereof; and

Petitioner Merck Ex-1015, 1195

(b) a second polypeptide antigen comprising an SP0785 polypeptide or antigenic fragment thereof.

38. The immunogenic complex of claim 37, wherein the fusion protein comprises:

(a) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(b) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

39. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

40. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

Petitioner Merck Ex-1015, 1196

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

41. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

42. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

Petitioner Merck Ex-1015, 1197

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

43. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

44. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

Petitioner Merck Ex-1015, 1198

45. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

46. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

47. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently associated with a fusion protein, wherein the fusion protein comprises

Petitioner Merck Ex-1015, 1199

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

48. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

49. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

Petitioner Merck Ex-1015, 1200

least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

50. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

51. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

Petitioner Merck Ex-1015, 1201

least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

52. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

53. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

Petitioner Merck Ex-1015, 1202

54. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

55. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

56. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

Petitioner Merck Ex-1015, 1203

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

57. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

58. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

Petitioner Merck Ex-1015, 1204

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

59. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

60. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

Petitioner Merck Ex-1015, 1205

61. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

62. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently associated with a fusion protein, wherein the fusion protein comprises

(a) a biotin-binding moiety;

(b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:5 or an antigenic fragment thereof; and

(c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:4 or an antigenic fragment thereof.

63. The immunogenic complex of any one of claims 31-62, wherein the biotin-binding moiety is a polypeptide comprising (i) an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least

Petitioner Merck Ex-1015, 1206

99%, or at least 99.5% identical to SEQ ID NO:1 or a biotin-binding fragment thereof; or (ii) an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO:2 or a biotin-binding fragment thereof.

64. The immunogenic complex of any one of claims 31-63, comprising a ratio of fusion protein to polysaccharide antigen of about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1, by weight.

65. A vaccine comprising one or more immunogenic complexes of any one of claims 31-64.

66. A pharmaceutical composition comprising the vaccine of any one of claims 1-30 and 65, and a pharmaceutically acceptable carrier.

67. A pharmaceutical composition comprising the immunogenic complex of any one of claims 31-64, and a pharmaceutically acceptable carrier.

68. The pharmaceutical composition of claim 66 or claim 67, further comprising one or more adjuvants.

69. The pharmaceutical composition of claim 68, wherein the one or more adjuvants is or comprises a co-stimulation factor.

70. The pharmaceutical composition of claim 68 or claim 69, wherein the one or more adjuvants are selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphated aluminum hydroxide.

71. The pharmaceutical composition of any one of claims 68-70, wherein the one or more adjuvants is or comprises aluminum phosphate.

Petitioner Merck Ex-1015, 1207

72. The pharmaceutical composition of any one of claims 66-71, wherein the pharmaceutical composition is formulated for injection.

73. The pharmaceutical composition of any one of claims 66-72, wherein upon administration to a subject, the pharmaceutical composition induces an immune response.

74. The pharmaceutical composition of claim 73, wherein the immune response comprises an antibody and/or B cell response.

75. The pharmaceutical composition of claim 73 or claim 74, wherein the immune response comprises a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response); a CD8+ T cell response; a CD4+ and CD8+ T cell response; or a CD4-/CD8- T cell response.

76. The pharmaceutical composition of any one of claims 73-75, wherein the immune response comprises (i) an antibody or B cell response and (ii) a T cell response.

77. The pharmaceutical composition of any one of claims 73-76, wherein the immune response is to (i) at least one polysaccharide antigen of the vaccine or immunogenic complex, and/or (ii) at least one polypeptide antigen of the vaccine or immunogenic complex.

78. The pharmaceutical composition of any one of claims 73-77, wherein the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen of the vaccine or immunogenic complex, and (ii) a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response to at least one polypeptide antigen of the vaccine or immunogenic complex.

79. The pharmaceutical composition of any one of claims 73-78, wherein the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen of the vaccine or immunogenic complex, and (ii) an antibody or B cell response to at least one polypeptide antigen of the vaccine or immunogenic complex.

Petitioner Merck Ex-1015, 1208

80. The pharmaceutical composition of any one of claims 73-79, wherein the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen of the vaccine or immunogenic complex, and (ii) an antibody or B cell response; and a CD4+ T cell response (including T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response to at least one polypeptide antigen of the vaccine or immunogenic complex.

81. The pharmaceutical composition of any one of claims 66-80, wherein upon administration to a subject, the pharmaceutical composition induces an opsonic/bactericidal response against one or more serotypes of *Streptococcus pneumoniae.*

82. The pharmaceutical composition of any one of claims 66-81, wherein upon administration to a subject, the pharmaceutical composition reduces or inhibits transmission of one or more serotypes of *Streptococcus pneumoniae* from the subject to another subject.

83. The pharmaceutical composition of any one of claims 66-82, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of occurrence of, Invasive Pneumonoccal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

84. The pharmaceutical composition of any one of claims 66-83, wherein upon administration to a subject, the pharmaceutical composition reduces the severity of Invasive Pneumonoccal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

85. The pharmaceutical composition of any one of claims 66-84, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of occurrence of, bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

Petitioner Merck Ex-1015, 1209

86. The pharmaceutical composition of any one of claims 66-85, wherein upon administration to a subject, the pharmaceutical composition reduces the severity of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

87. The pharmaceutical composition of any one of claims 66-86, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of occurrence of, organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

88. The pharmaceutical composition of any one of claims 66-87, wherein upon administration to a subject, the pharmaceutical composition reduces the severity of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

89. The pharmaceutical composition of any one of claims 66-88, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of occurrence of, pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

90. The pharmaceutical composition of any one of claims 66-89, wherein upon administration to a subject, the pharmaceutical composition reduces the severity of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

91. The pharmaceutical composition of any one of claims 66-90, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of occurrence of, otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

Petitioner Merck Ex-1015, 1210

92. The pharmaceutical composition of any one of claims 66-91, wherein upon administration to a subject, the pharmaceutical composition reduces the severity of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

93. The pharmaceutical composition of any one of claims 66-92, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of occurrence of, sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

94. The pharmaceutical composition of any one of claims 66-93, wherein upon administration to a subject, the pharmaceutical composition reduces the severity of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

95. The pharmaceutical composition of any one of claims 66-94, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of, colonization of mucosal surfaces by one or more serotypes of *Streptococcus pneumoniae*.

96. The pharmaceutical composition of any one of claims 66-95, wherein upon administration to a subject, the pharmaceutical composition inhibits, or reduces the rate of, colonization of the nasopharynx by one or more serotypes of *Streptococcus pneumoniae*.

97. A method of making a vaccine, comprising non-covalently complexing a plurality of biotinylated polysaccharide antigens with a plurality of fusion proteins, wherein each fusion protein comprises at least one polypeptide antigen selected from:

an SP1500 polypeptide or antigenic fragment thereof;

an SP0785 polypeptide or antigenic fragment thereof;

and a combination thereof.

98. The method of claim 97, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Streptococcus pneumoniae* serotypes selected from 1,

Petitioner Merck Ex-1015, 1211

2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

99. The method of claim 97 or claim 98, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Streptococcus pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F.

100. The method of any one of claims 97-99, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Streptococcus pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F.

101. The method of any one of claims 97-100, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Streptococcus pneumoniae* serotypes selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

102. The method of any one of claims 97-101, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

103. The method of any one of claims 97-100, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Streptococcus pneumoniae* serotypes selected from 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B.

104. The method of any one of claims 97-100 and 103, wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of *Streptococcus pneumoniae* serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B.

Petitioner Merck Ex-1015, 1212

105. A method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization comprising administering to the subject an immunologically effective amount of the vaccine of any one of claims 1-30 and 65.

106. A method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization comprising administering to the subject an immunologically effective amount of the immunogenic complex of any one of claims 31-64.

107. A method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization comprising administering to the subject an immunologically effective amount of the pharmaceutical composition of any one of claims 66-96.

108. The method of any one of claims 105-107, wherein the vaccine, immunogenic composition, or pharmaceutical composition induces an immune response.

109. The method of claim 108, wherein the immune response comprises an antibody or B cell response.

110. The method of any one of claims 108-109, wherein the immune response comprises a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response.

111. The method of any one of claims 108-110, wherein the immune response comprises (i) an antibody or B cell response and (ii) a T cell response.

112. The method of any one of claims 108-111, wherein the immune response is to at least one polysaccharide antigen or at least one polypeptide of a fusion protein.

Petitioner Merck Ex-1015, 1213

113. The method of any one of claims 108-112, wherein the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response to at least one polypeptide of a fusion protein.

114. The method of any one of claims 108-113, wherein the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response to at least one polypeptide of a fusion protein.

115. The method of any one of claims 108-114, wherein the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response and a CD4+ T cell response, including T_H1, T_H2, or T_H17 response, or a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response to at least one polypeptide of a fusion protein.

116. The method of any one of claims 105-115, wherein the vaccine induces an opsonic/bactericidal response against one or more serotypes of *Streptococcus pneumoniae.*

117. The method of any one of claims 105-116, wherein the vaccine inhibits transmission of one or more serotypes of *Streptococcus pneumoniae* from the subject to another subject.

118. The method of any one of claims 105-117, wherein the vaccine inhibits or reduces the rate of occurrence of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

119. The method of any one of claims 105-118, wherein the vaccine reduces the severity of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

Petitioner Merck Ex-1015, 1214

120. The method of any one of claims 105-119, wherein the vaccine inhibits or reduces the rate of occurrence of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

121. The method of any one of claims 105-120, wherein the vaccine reduces the severity of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

122. The method of any one of claims 105-121, wherein the vaccine inhibits or reduces the rate of occurrence of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

123. The method of any one of claims 105-122, wherein the vaccine reduces the severity of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

124. The method of any one of claims 105-123, wherein the vaccine inhibits or reduces the rate of occurrence of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

125. The method of any one of claims 105-124, wherein the vaccine reduces the severity of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

126. The method of any one of claims 105-125, wherein the vaccine inhibits or reduces the rate of occurrence of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

127. The method of any one of claims 105-126, wherein the vaccine reduces the severity of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

Petitioner Merck Ex-1015, 1215

128. The method of any one of claims 105-127, wherein the vaccine inhibits or reduces the rate of occurrence of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

129. The method of any one of claims 105-128, wherein the vaccine reduces the severity of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*.

130. The method of any one of claims 105-129, wherein the vaccine inhibits colonization of mucosal surfaces by one or more serotypes of *Streptococcus pneumoniae*.

131. The method of any one of claims 105-130, wherein the vaccine inhibits colonization of the nasopharynx by one or more serotypes of *Streptococcus pneumoniae*.

132. The method of any one of claims 105-131, wherein the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

133. The method of any one of claims 105-132, wherein the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F.

134. The method of any one of claims 105-133, wherein the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

Petitioner Merck Ex-1015, 1216

135. The method of any one of claims 105-133, wherein the *Streptococcus pneumoniae* has a serotype selected from one or more of 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, and 20B.

136. The method of any one of claims 105-135, wherein the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with one dose of a vaccine.

137. The method of any one of claims 105-135, wherein the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with two doses of a vaccine.

138. The method of any one of claims 105-135, wherein the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with three doses of a vaccine.

Petitioner Merck Ex-1015, 1217

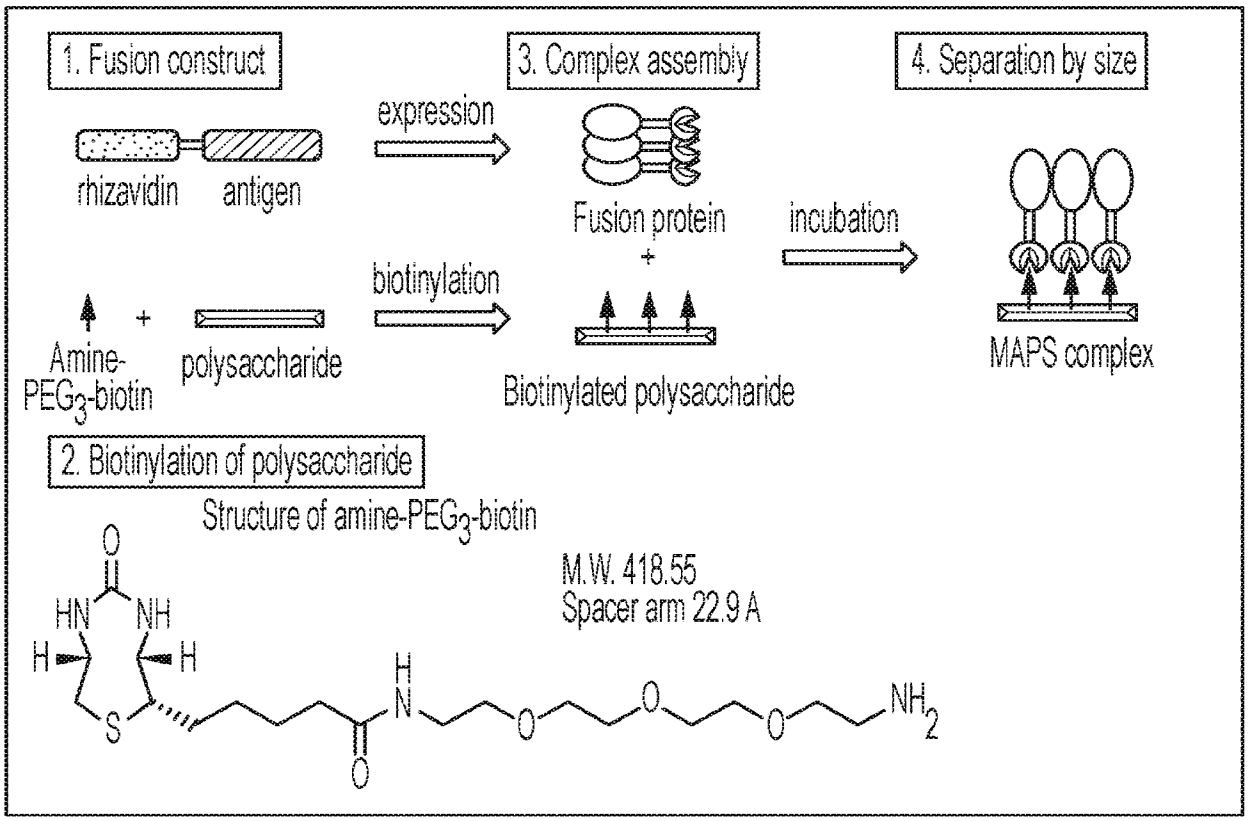


FIG. 1

Petitioner Merck Ex-1015, 1218

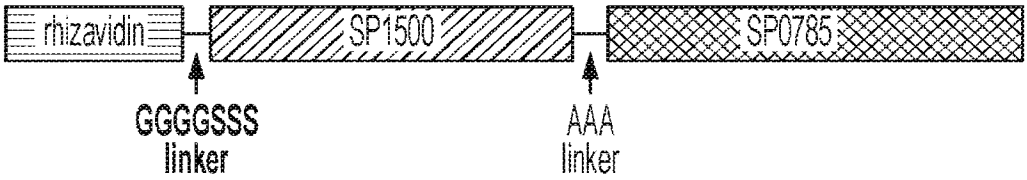


FIG. 2

Petitioner Merck Ex-1015, 1219

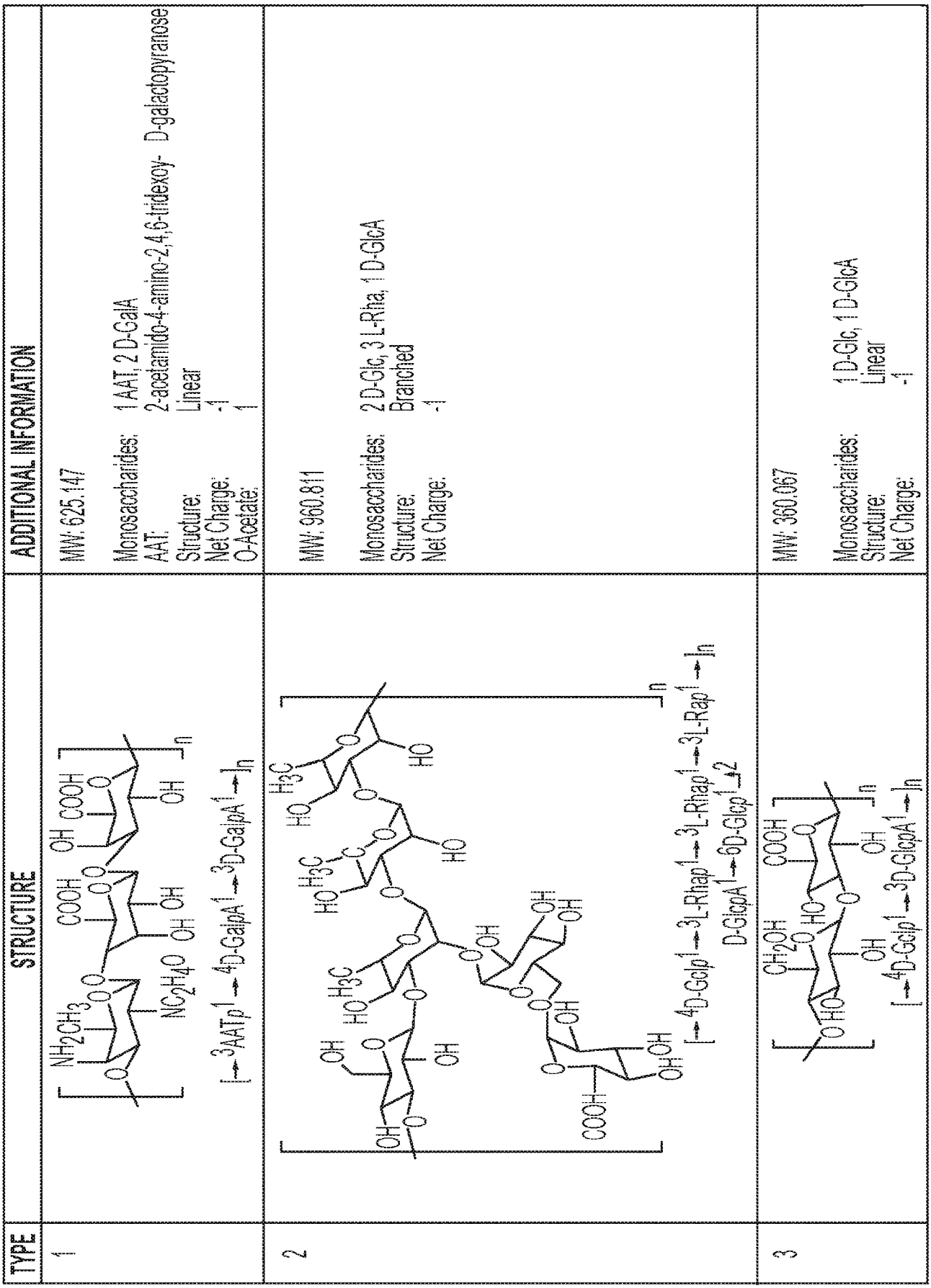


FIG. 3

Petitioner Merck Ex-1015, 1220

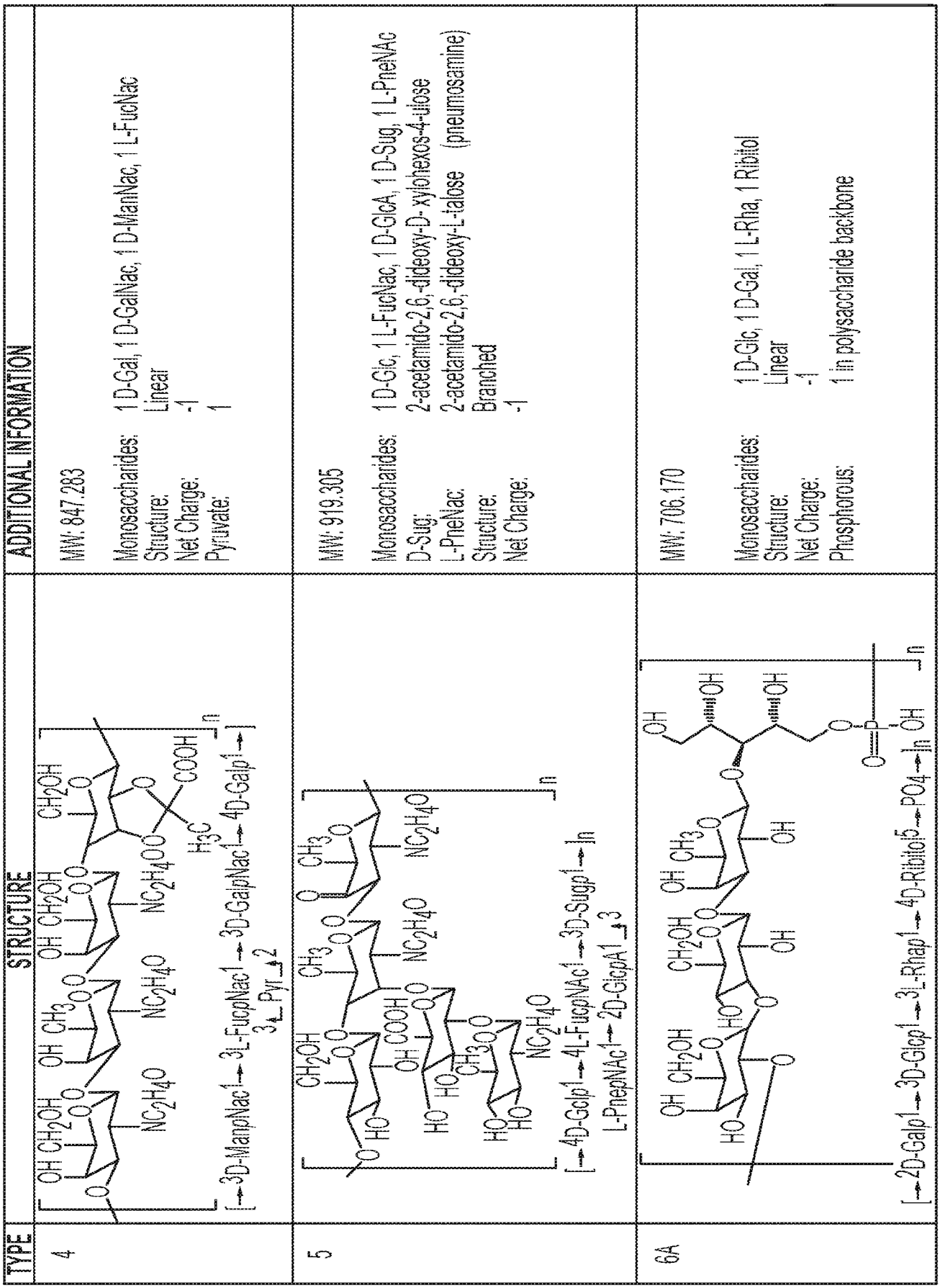


FIG. 3
Continued

Petitioner Merck Ex-1015, 1221

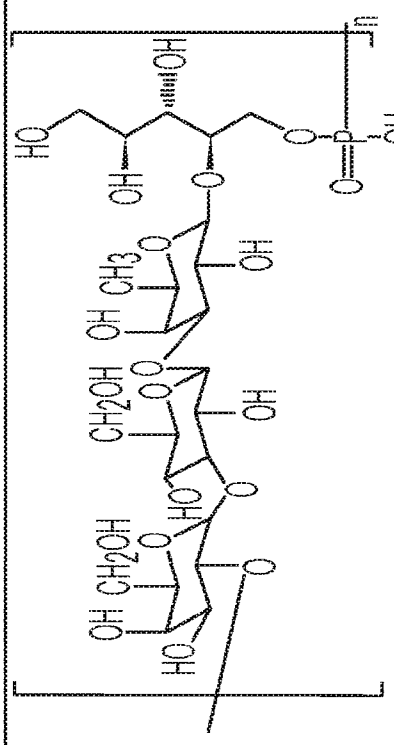
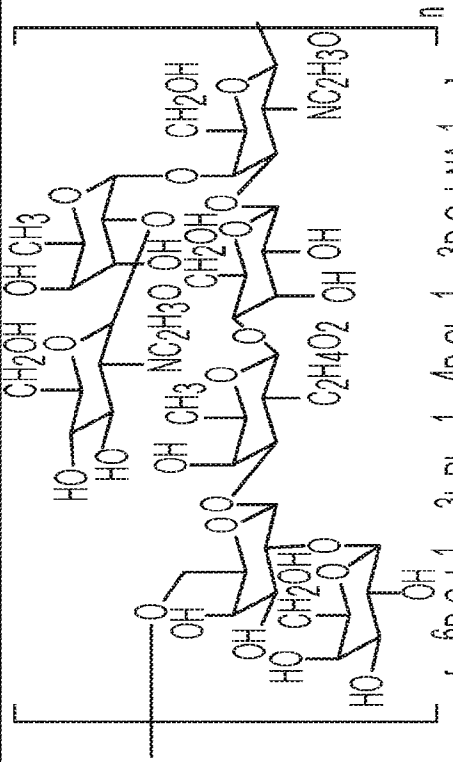
TYPE	STRUCTURE	ADDITIONAL INFORMATION
6B		MW: 706.170 Monosaccharides: 1 D-Glc, 1 D-Gal, 1 L-Rha, 1 Ribitol Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone
7F		Monosaccharides: 1 D-Glc, 2 D-Gal, 2 L-Rha, 1 D-GlcNac, 1 D-GalNac Structure: Branched Net Charge: 0 O-Acetate: 1 MW: 1,226.444
8		MW: 684.528 Monosaccharides: 2 D-Glc, 1 D-Gal, 1 D-GlcA Structure: Linear Net Charge: -1

FIG. 3
Continued

Petitioner Merck Ex-1015, 1222

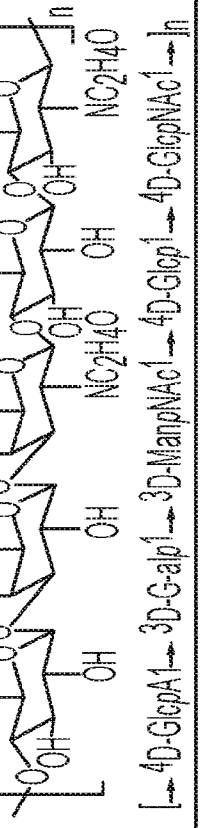
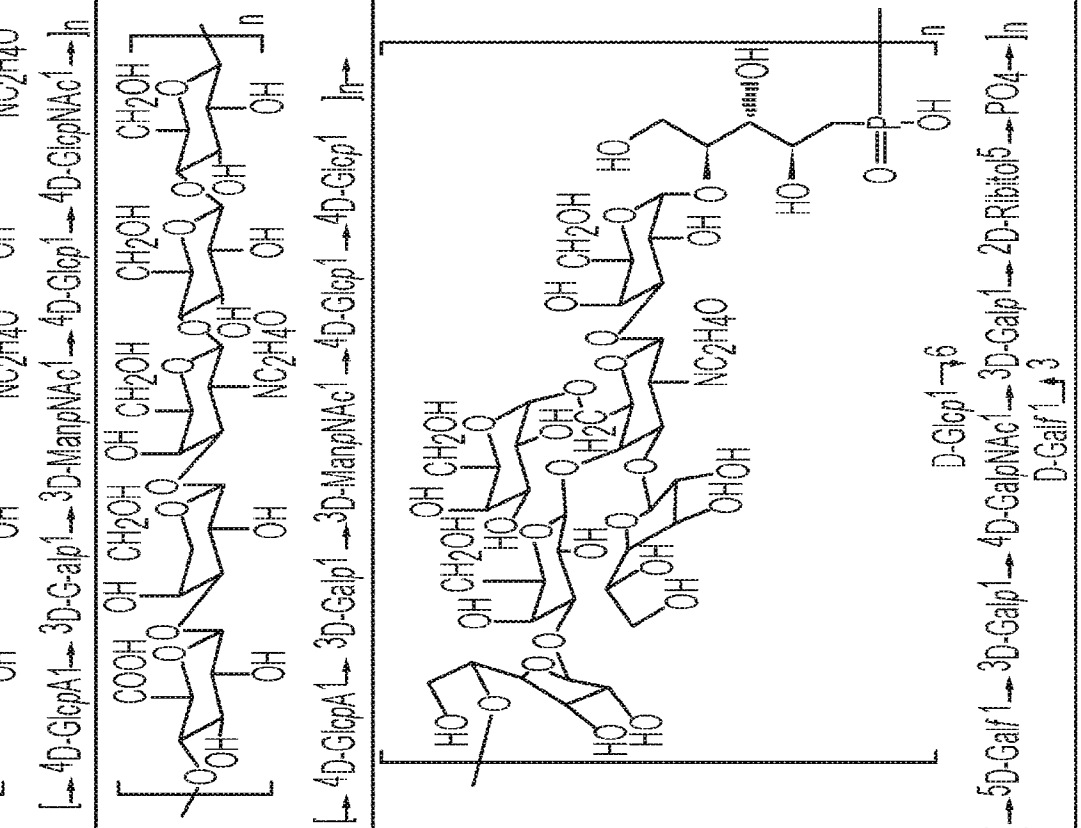
TYPE	STRUCTURE	ADDITIONAL INFORMATION
9N		MW: 928.772 Monosaccharides: 1 D-Glc, 1 D-Gal, 1D-GlcNac, 1 D-ManNac, 1 D-GlcA Structure: Linear Net Charge: -1
9V		MW: 971.794 Monosaccharides: 2 D-Glc, 1 D-Gal, 1 D-ManNac, 1 D-GlcA Structure: Linear Net Charge: -1 O-Acetate: 2
10A		MW: 1,249.989 Monosaccharides: 5 D-Gal, 1 D-GalNac, 1 Ribitol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide backbone

FIG. 3
Continued

Petitioner Merck Ex-1015, 1223

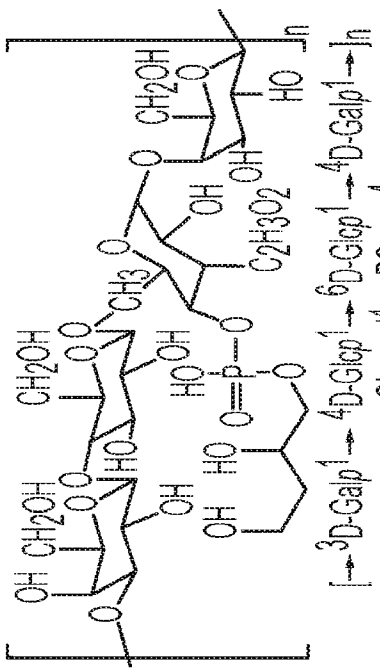
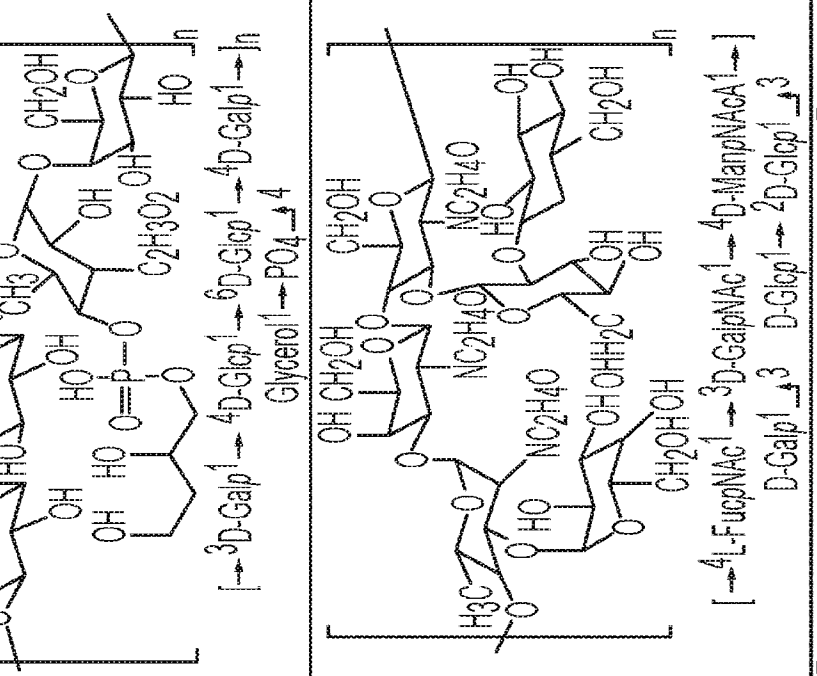
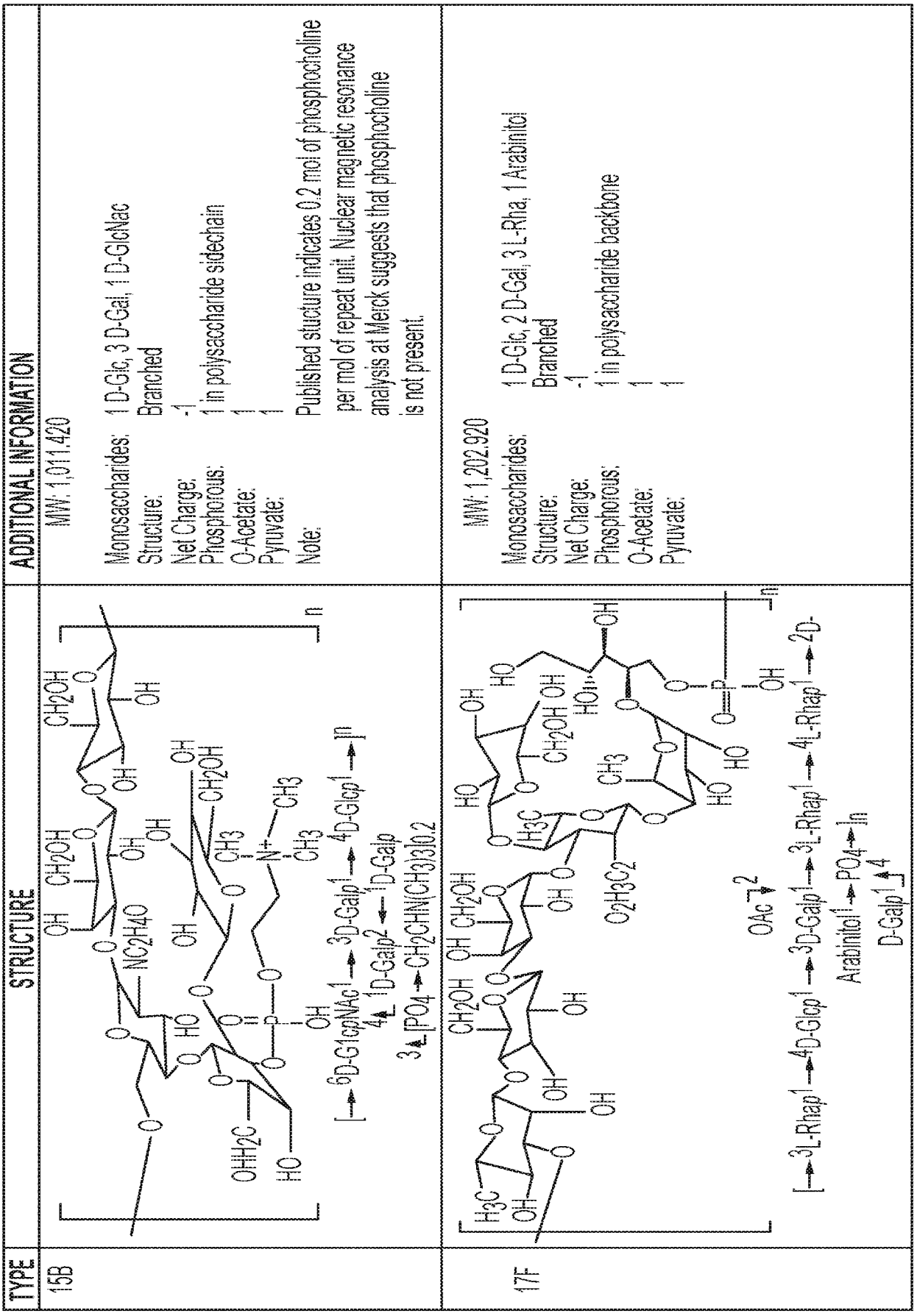
TYPE	STRUCTURE	ADDITIONAL INFORMATION
11A		MW: 933.900 Monosaccharides: 2 D-Glc, 2 D-Gal, 1 Glycerol Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide sidechaine O-Acetate: 1
12F		Monosaccharides: 2 D-Glc, 1 D-Gal, 1 D-GalNac, 1 L- FucNac, 1D-ManNacA Structure: Branched Net Charge: -1 MW: 1,116.02
14		Monosaccharides: 1 D-Glc, 2 D-Gal, 1 D-GlcNac Structure: Branched Net Charge: 0 MW: 689.238

FIG. 3
Continued

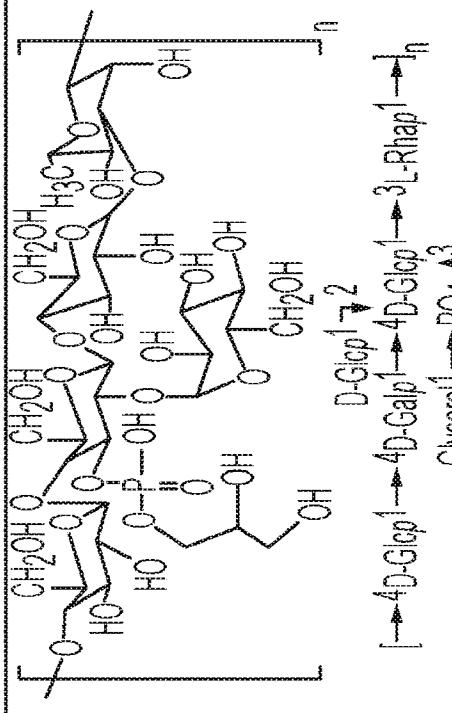
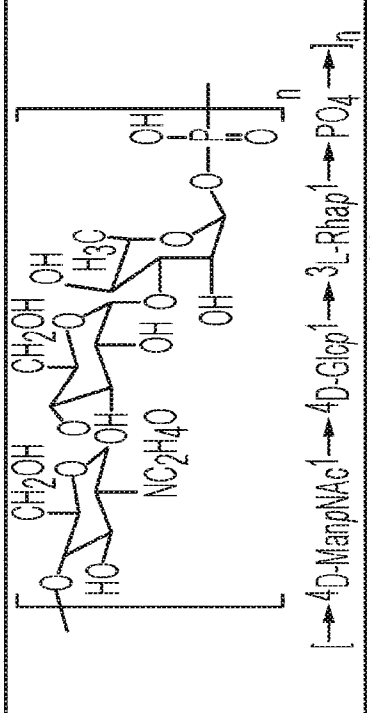
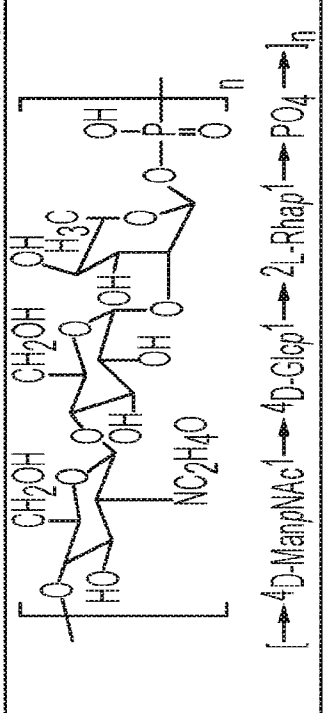
Petitioner Merck Ex-1015, 1224

FIG. 3 cont'd



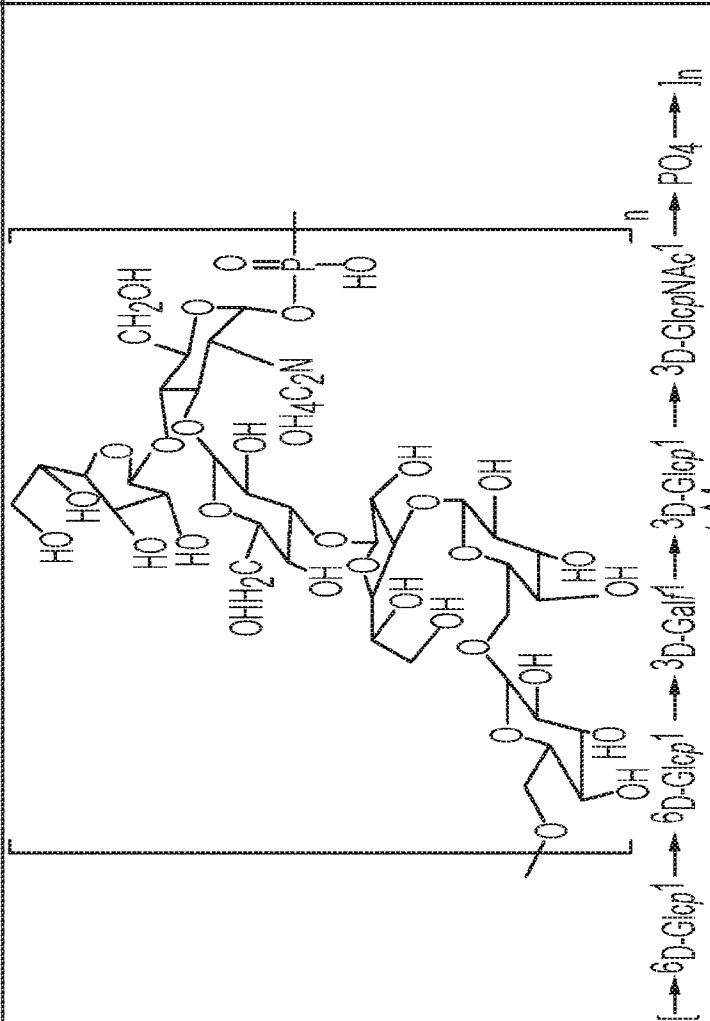
Petitioner Merck Ex-1015, 1225

FIG. 3 cont'd

TYPE	STRUCTURE	ADDITIONAL INFORMATION
18C		MW: 1,012.265 Monosaccharides: 3 D-Glc, 1 D-Gal, 1 L-Rha, 1 Glycerol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide sidechain O-Acetate: 1
19A		MW: 613.138 Monosaccharides: 1 D-Glc, 1 L-Rha 1 D-ManNac Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone
19F		MW: 613.138 Monosaccharides: 1 D-Glc, 1 L-Rha 1 D-ManNac Structure: Linear Net Charge: -1 Phosphorous: 1 in polysaccharide backbone

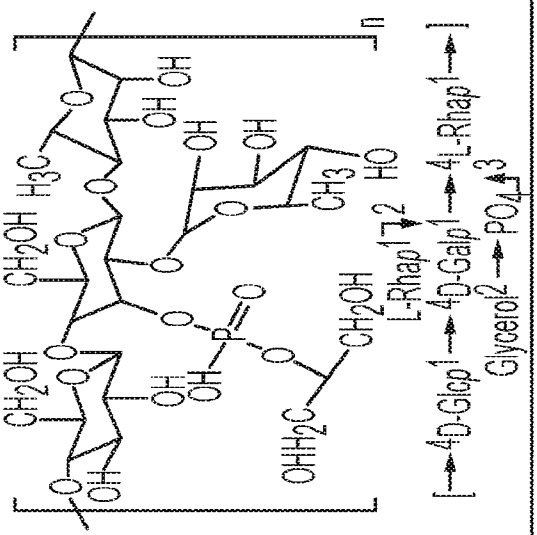
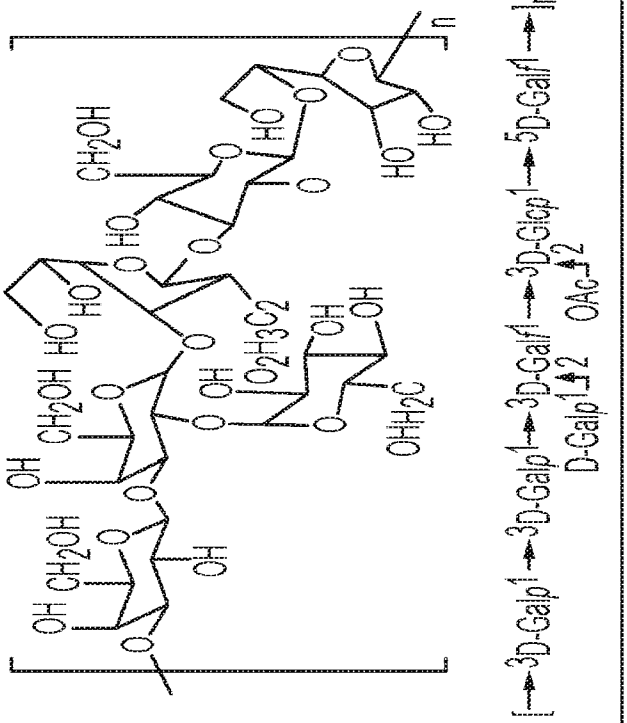
Petitioner Merck Ex-1015, 1226

FIG. 3 cont'd

TYPE	STRUCTURE	ADDITIONAL INFORMATION
18C		MW: 1,391.498 Monosaccharides: 3 D-Glc, 2 D-Gal, 1 D-GlcNac Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide backbone O-Acetate: 1
22F		Monosaccharides: 2 D-Glc, 1 D-Gal, 2 L-Rha, 1 D-GlcA Structure: Branched Net Charge: -1 O-Acetate: 1 MW: 1,010.447

Petitioner Merck Ex-1015, 1227

FIG. 3 cont'd

TYPE	STRUCTURE	ADDITIONAL INFORMATION
23F		MW: 792.207 Monosaccharides: 1 D-Glc, 1 D-Gal, 2 L-Rha, 1 Glycerol Structure: Branched Net Charge: -1 Phosphorous: 1 in polysaccharide sidechain
33F		MW: 993.862 Monosaccharides: 1 D-Glc, 5 D-Gal Structure: Branched Net Charge: 0 O-Acetate: 1

Petitioner Merck Ex-1015, 1228

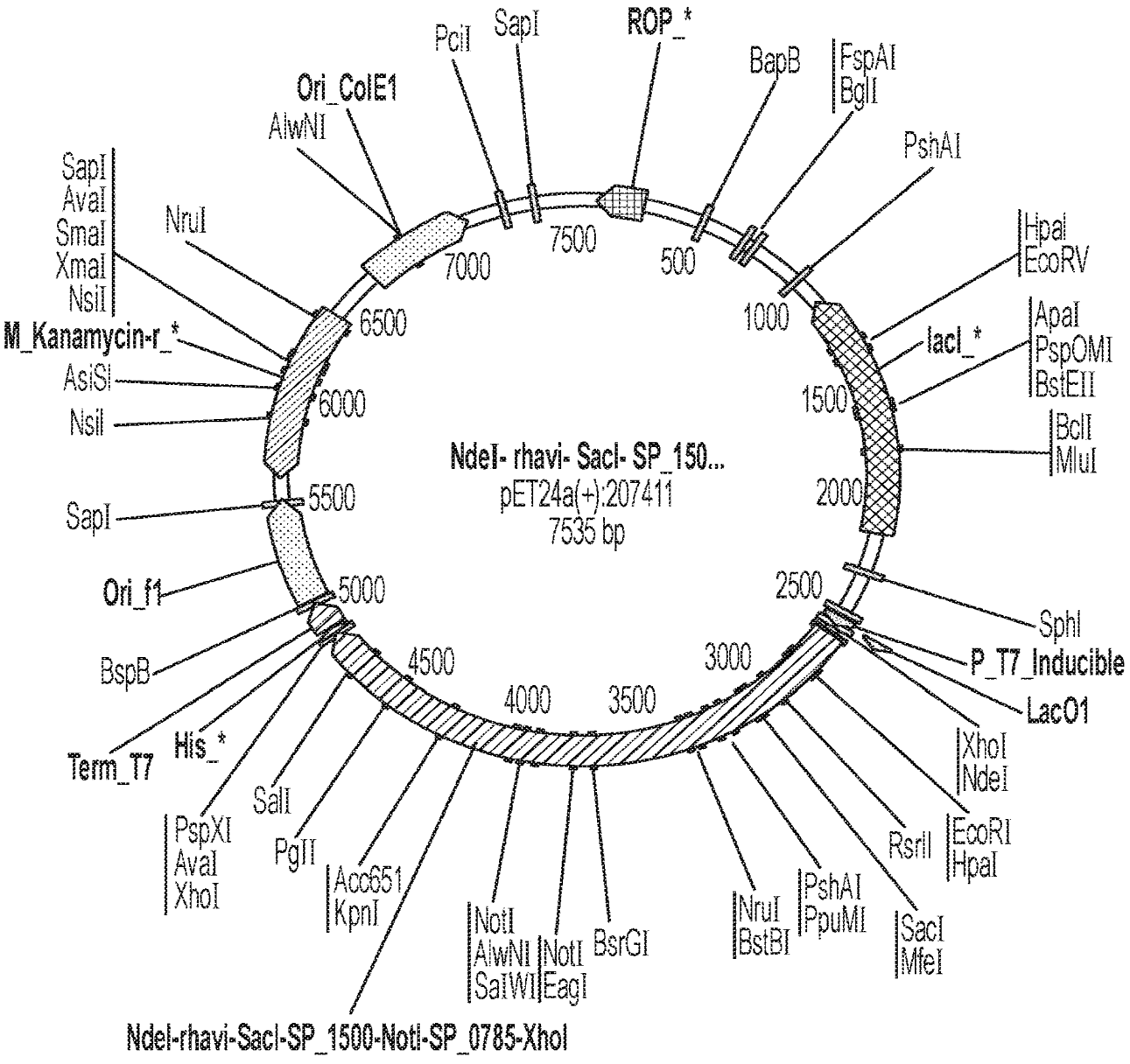


FIG. 4

Petitioner Merck Ex-1015, 1229

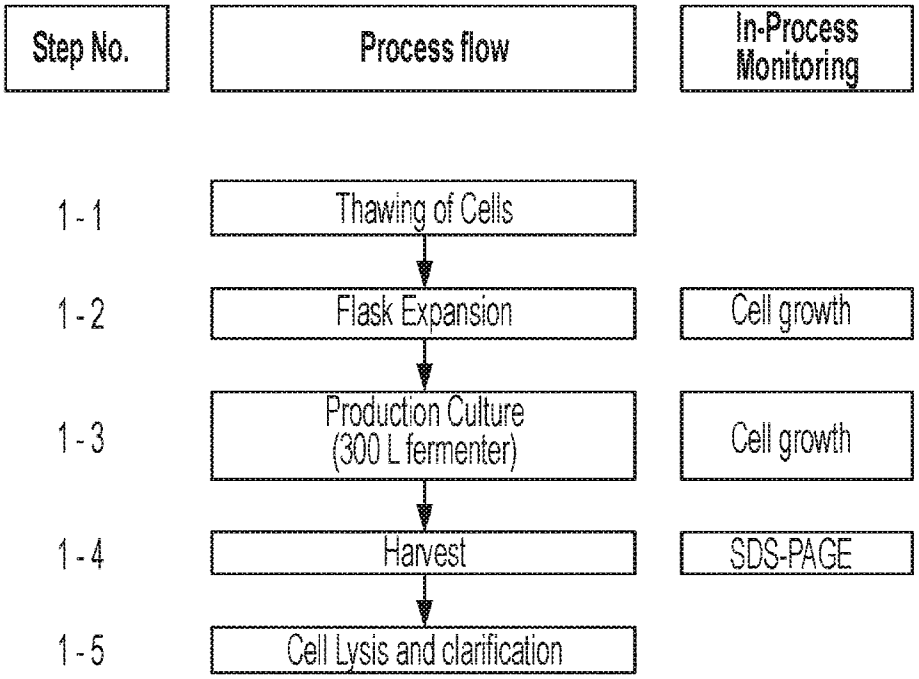


FIG. 5

Petitioner Merck Ex-1015, 1230

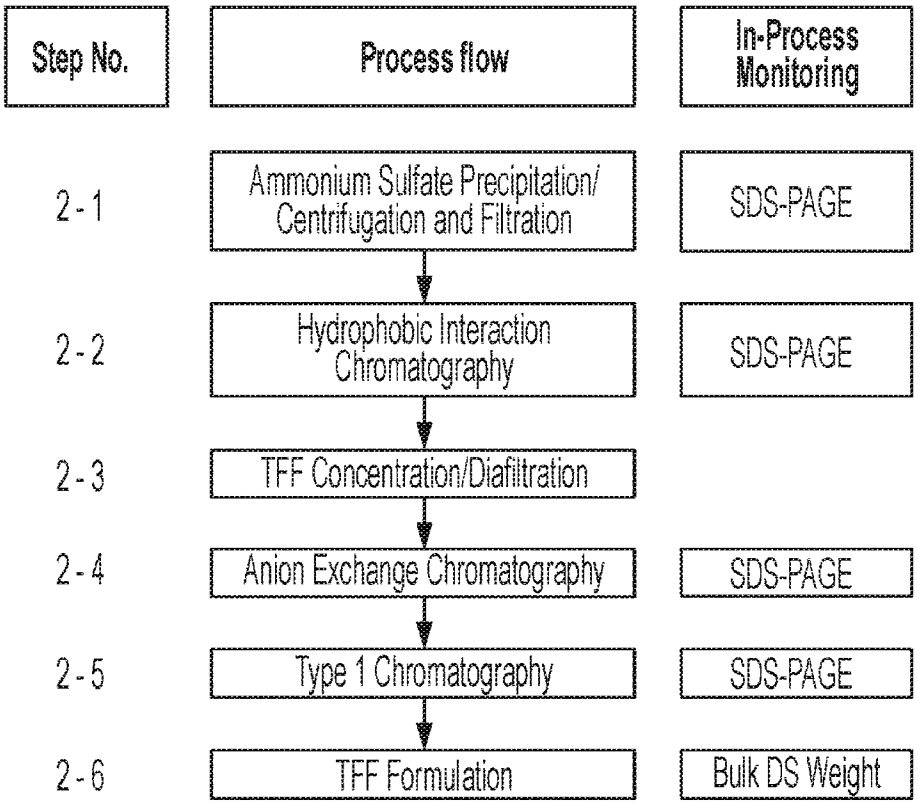


FIG. 6

Petitioner Merck Ex-1015, 1231

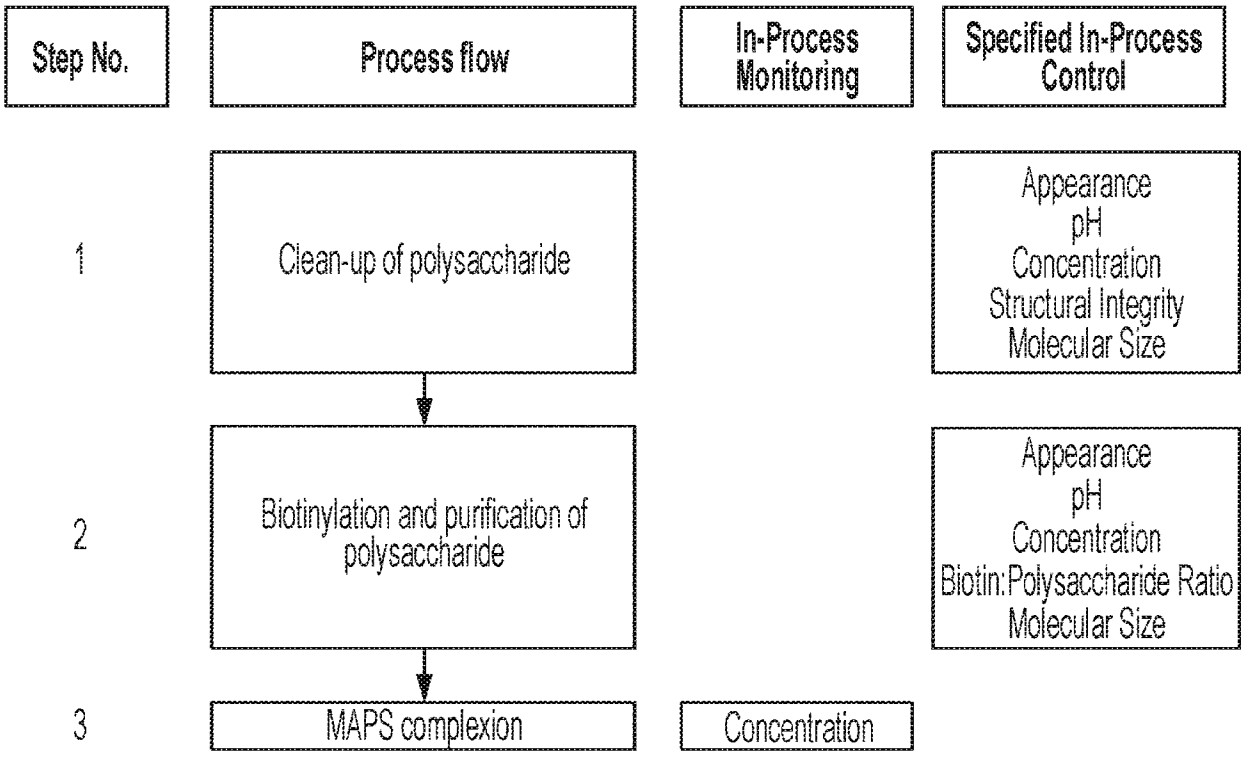


FIG. 7

Petitioner Merck Ex-1015, 1232

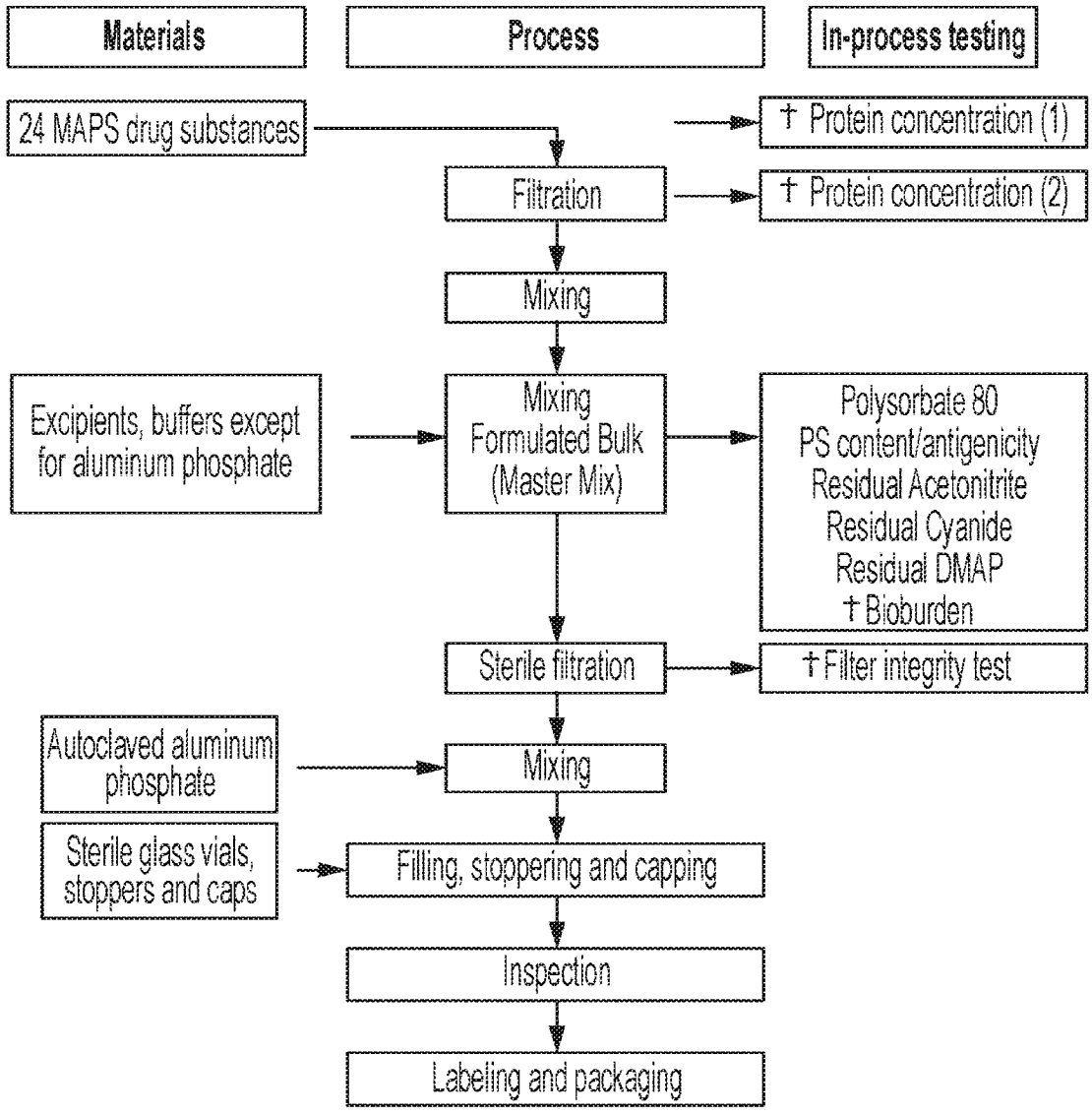


FIG. 8
Panel A

Petitioner Merck Ex-1015, 1233

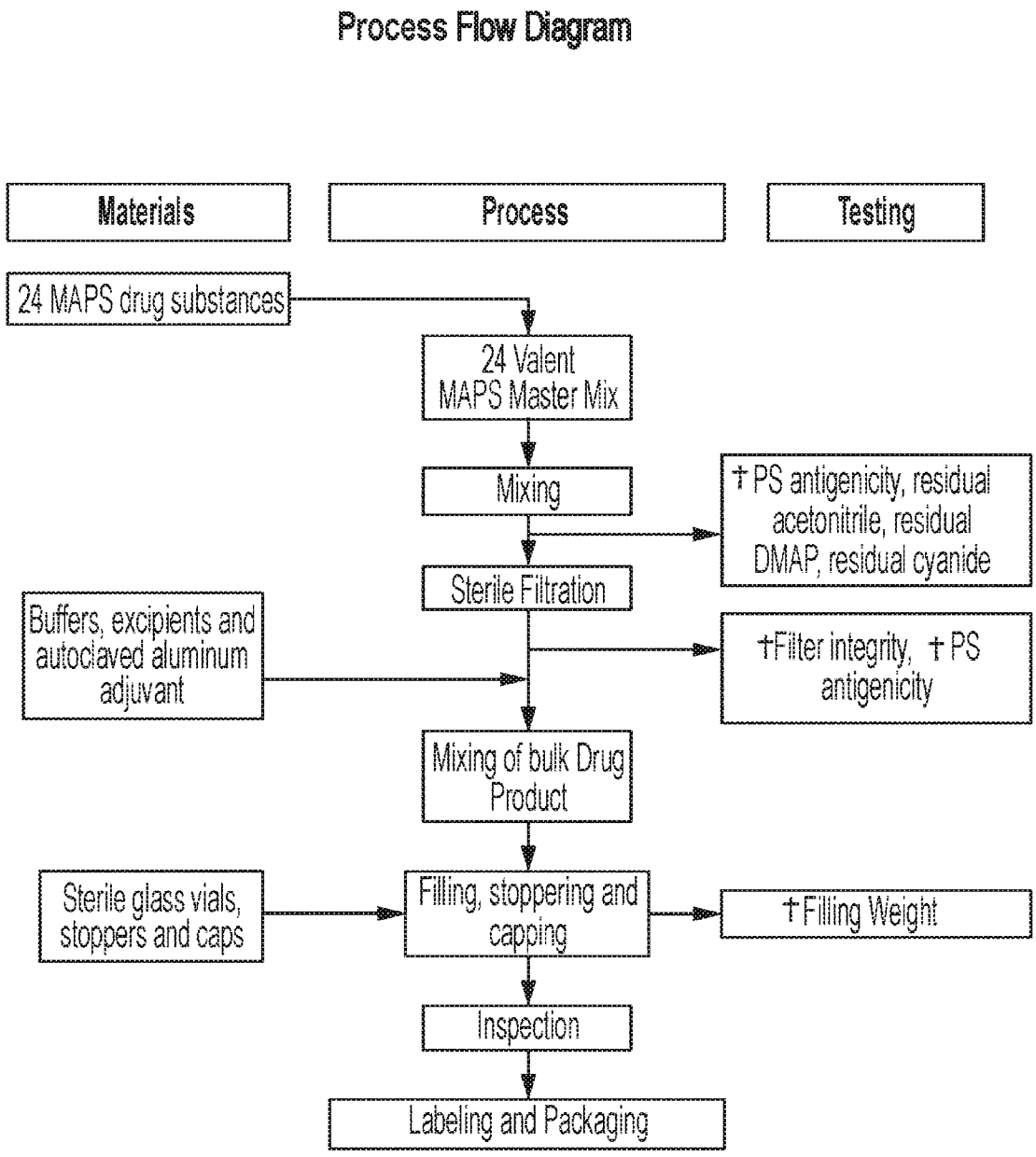


FIG. 8
Panel B

Petitioner Merck Ex-1015, 1234

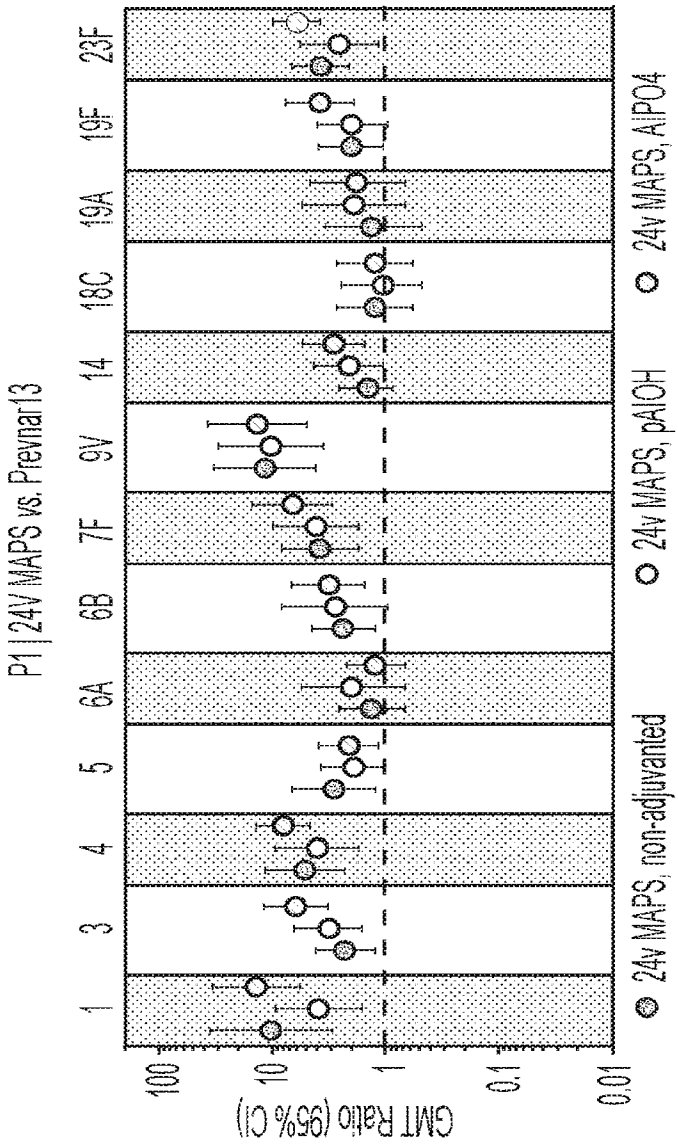


FIG. 9

Petitioner Merck Ex-1015, 1235

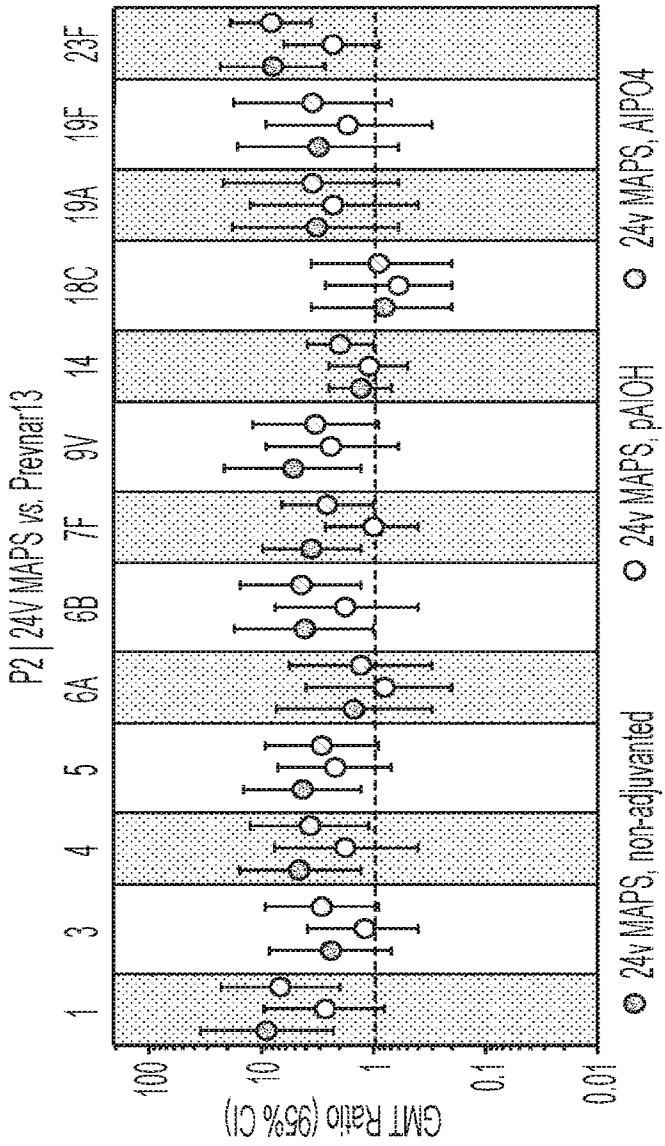


FIG. 10

Petitioner Merck Ex-1015, 1236

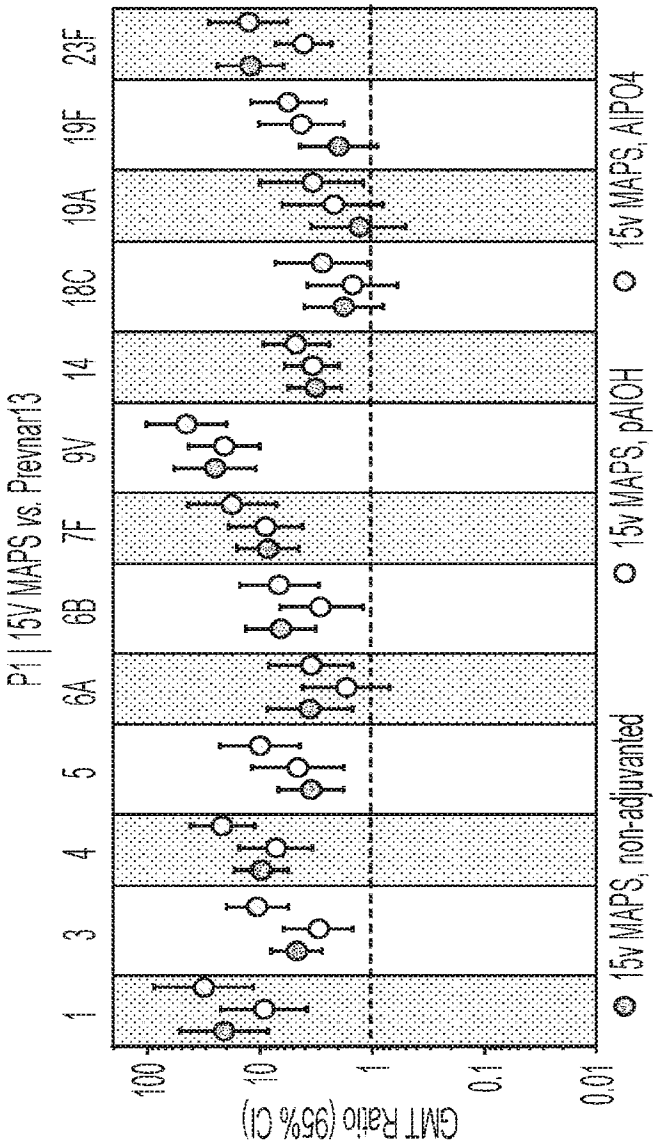


FIG. 11

Petitioner Merck Ex-1015, 1237

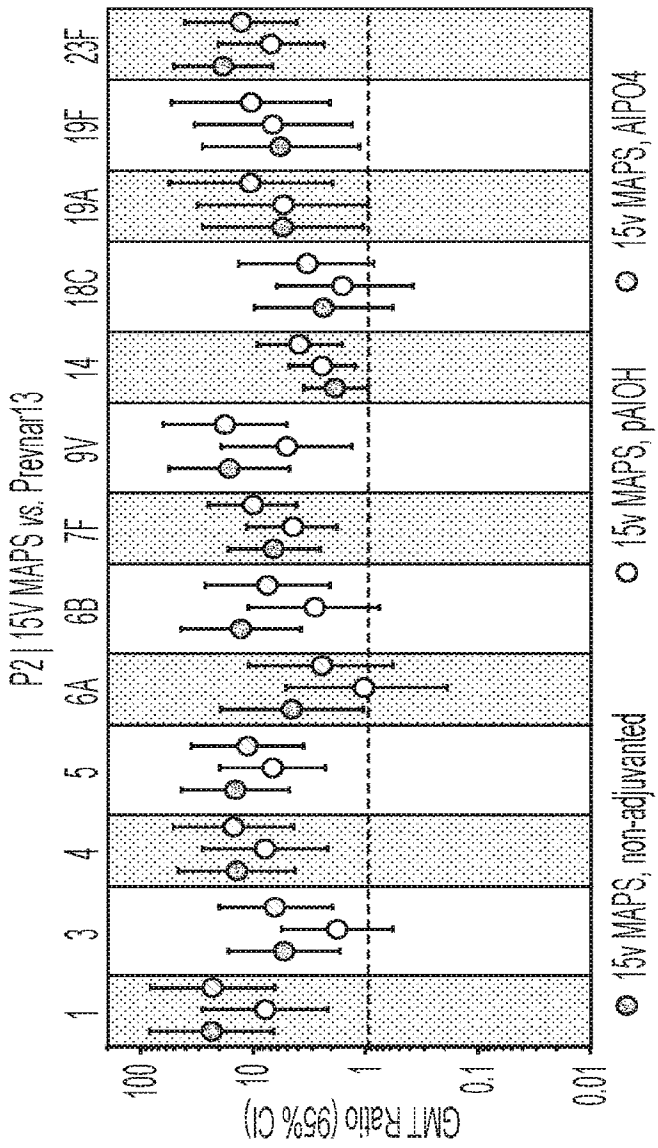


FIG. 12

Petitioner Merck Ex-1015, 1238

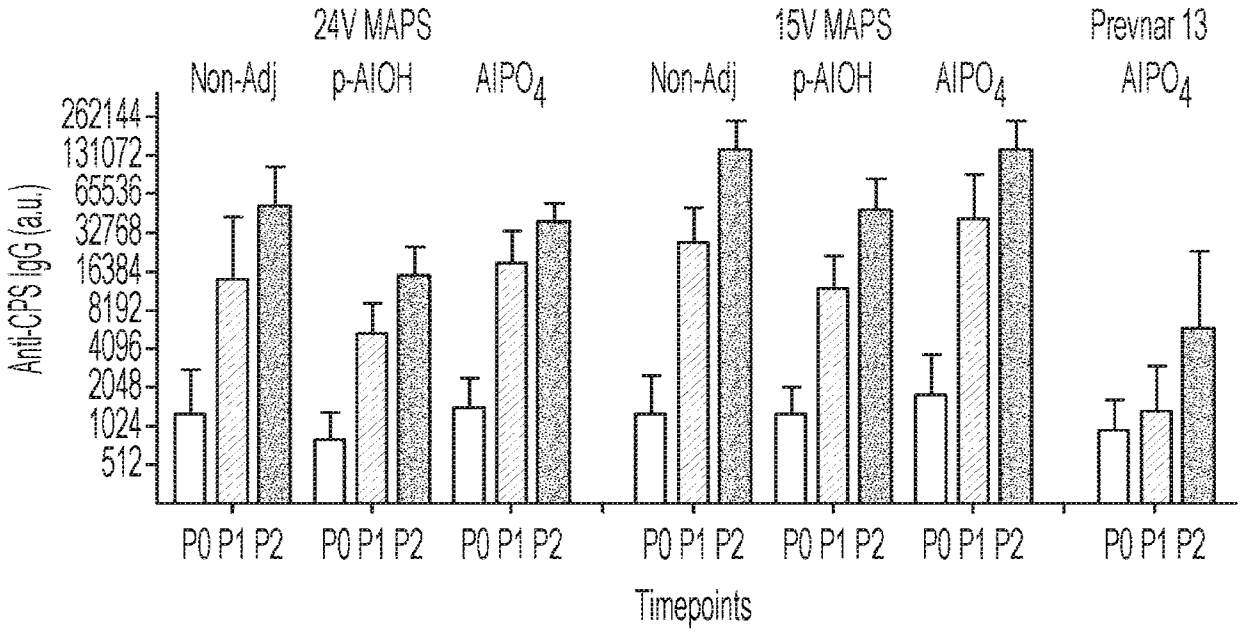


FIG. 13

Petitioner Merck Ex-1015, 1239

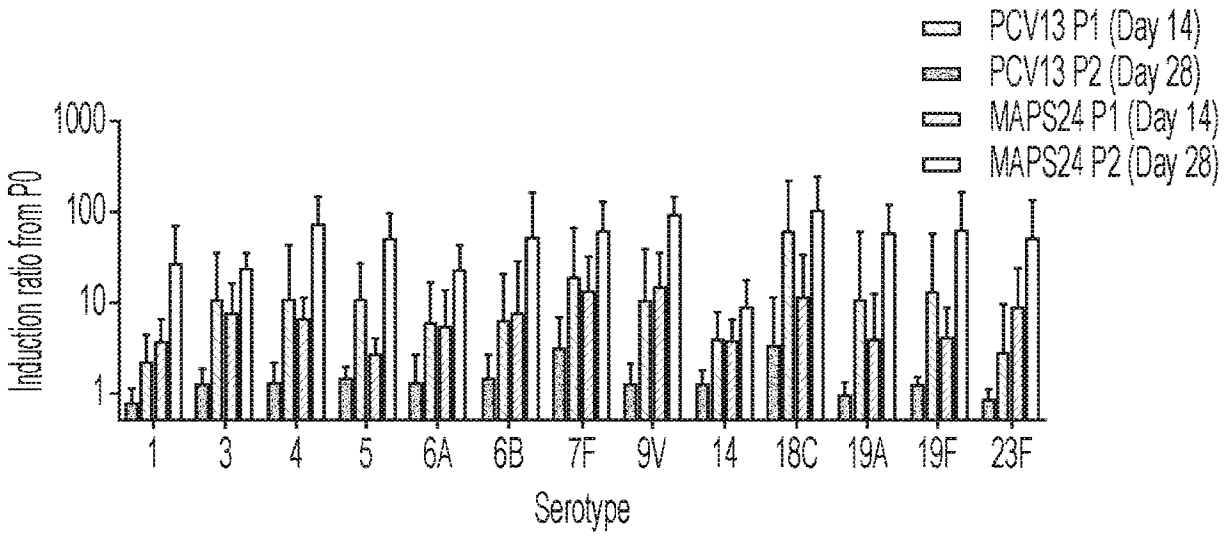


FIG. 14

Petitioner Merck Ex-1015, 1240

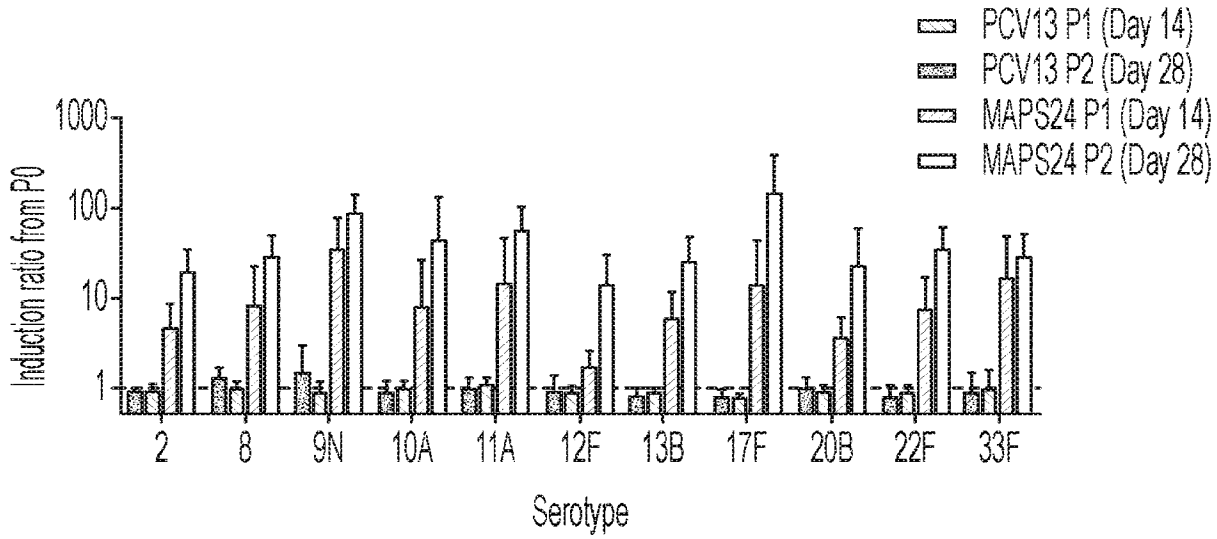


FIG. 15

Petitioner Merck Ex-1015, 1241

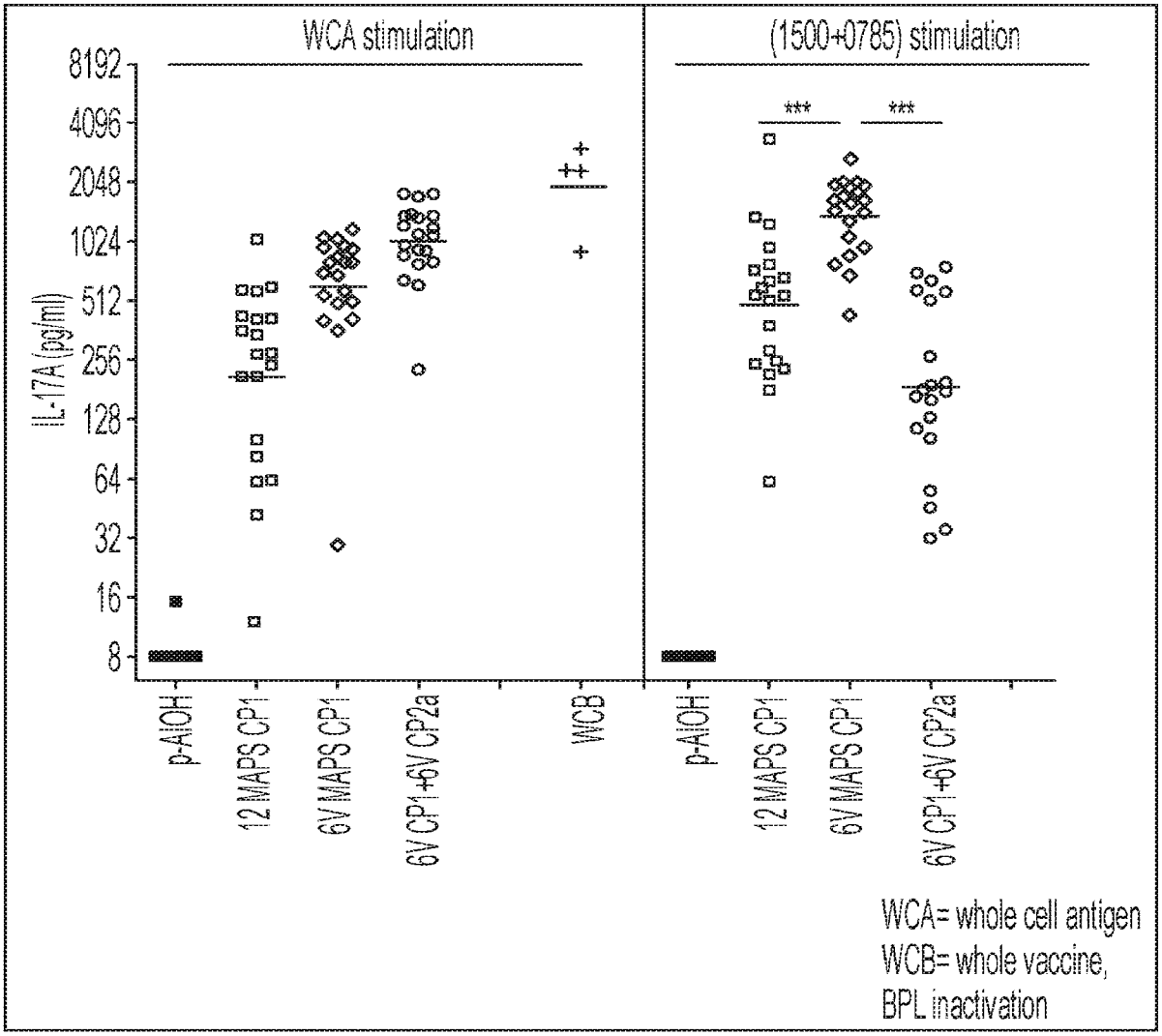


FIG. 16

Petitioner Merck Ex-1015, 1242

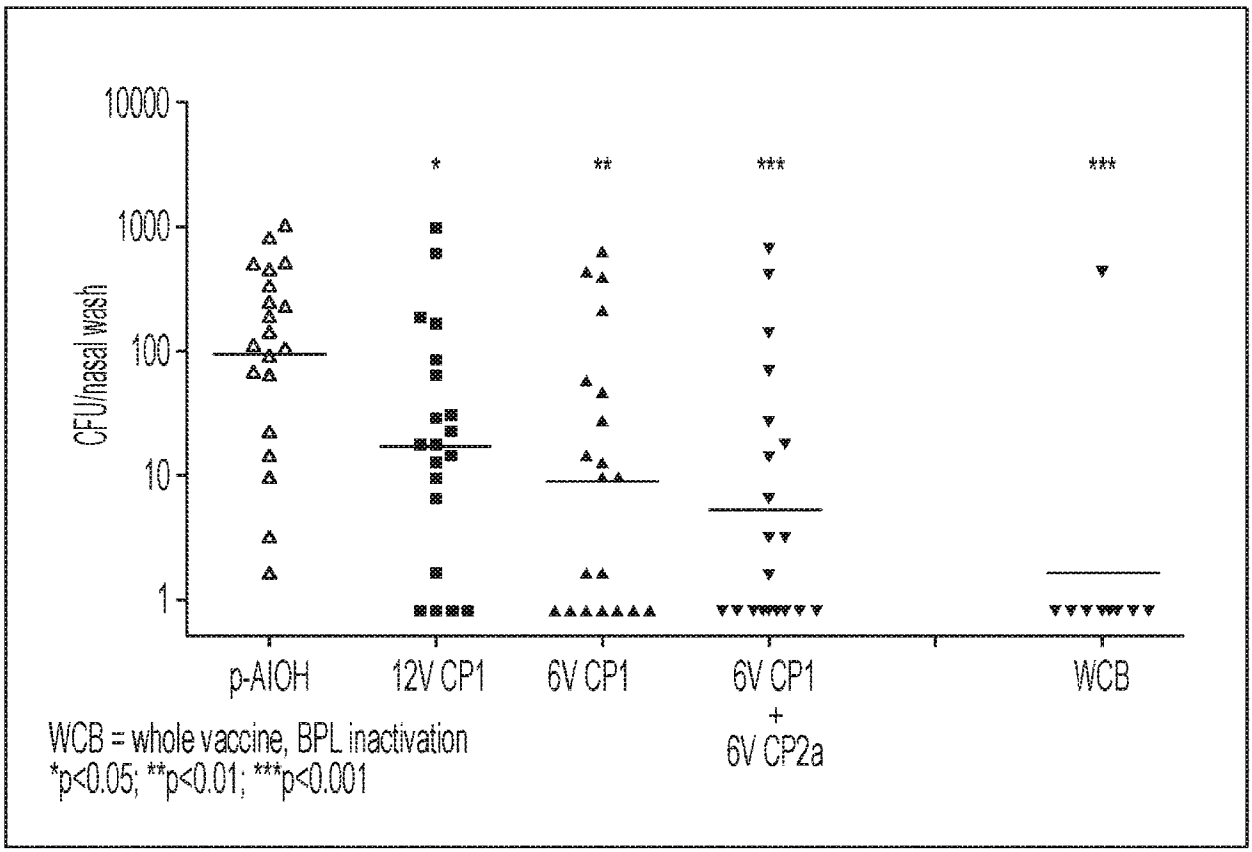


FIG. 17

Petitioner Merck Ex-1015, 1243

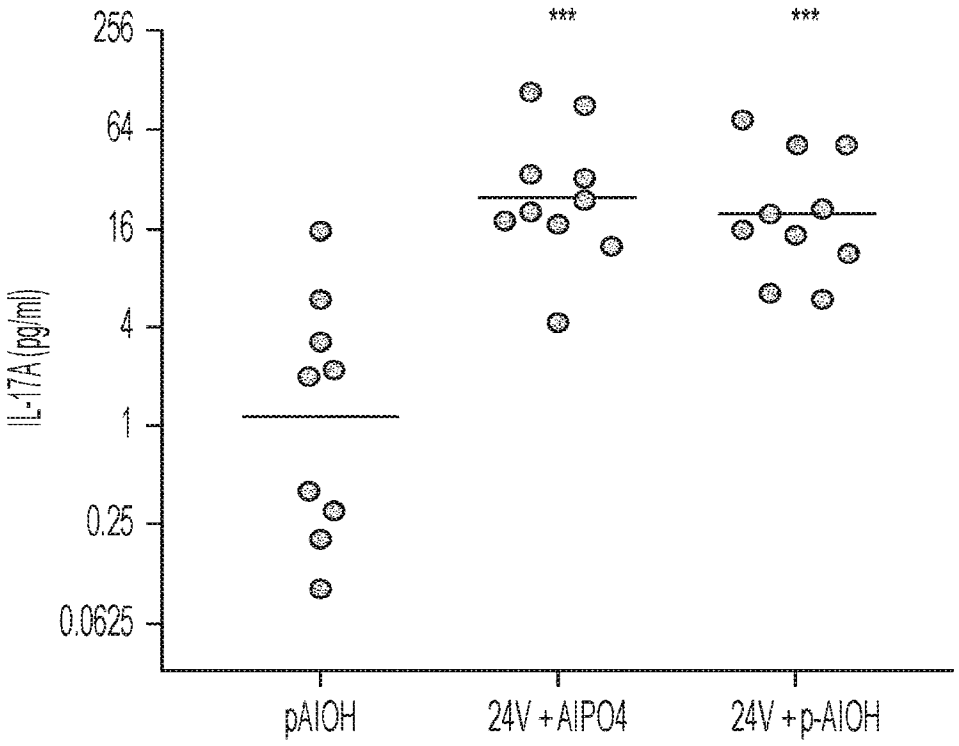


FIG. 18

Petitioner Merck Ex-1015, 1244

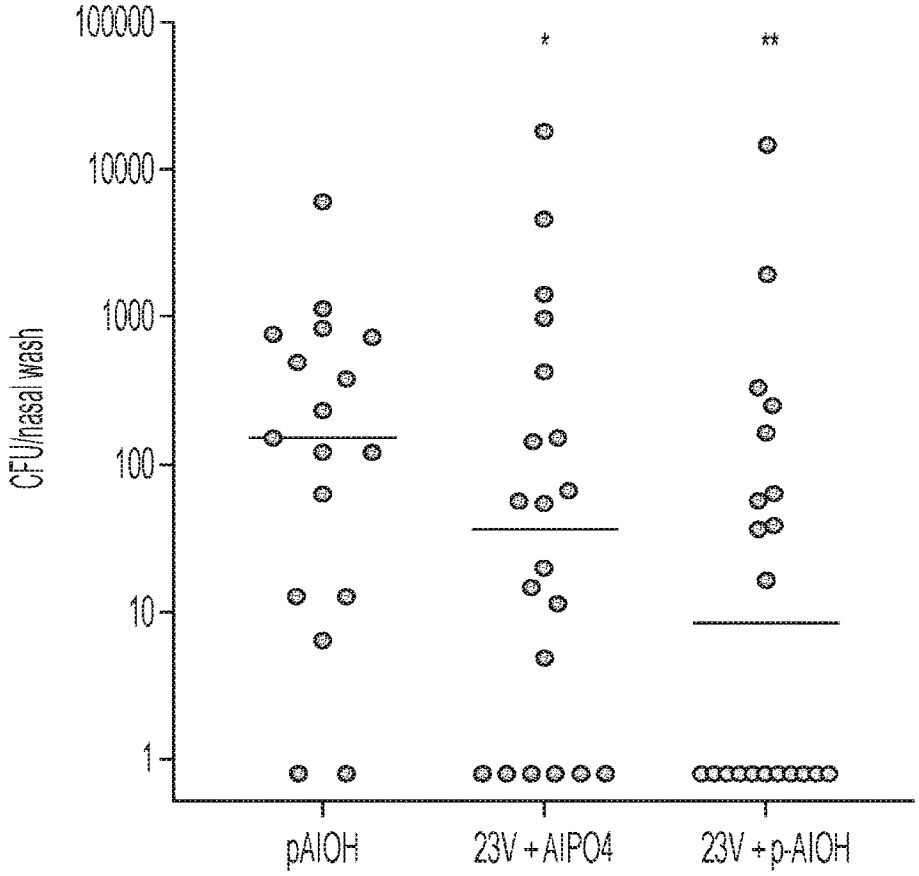


FIG. 19

Petitioner Merck Ex-1015, 1245

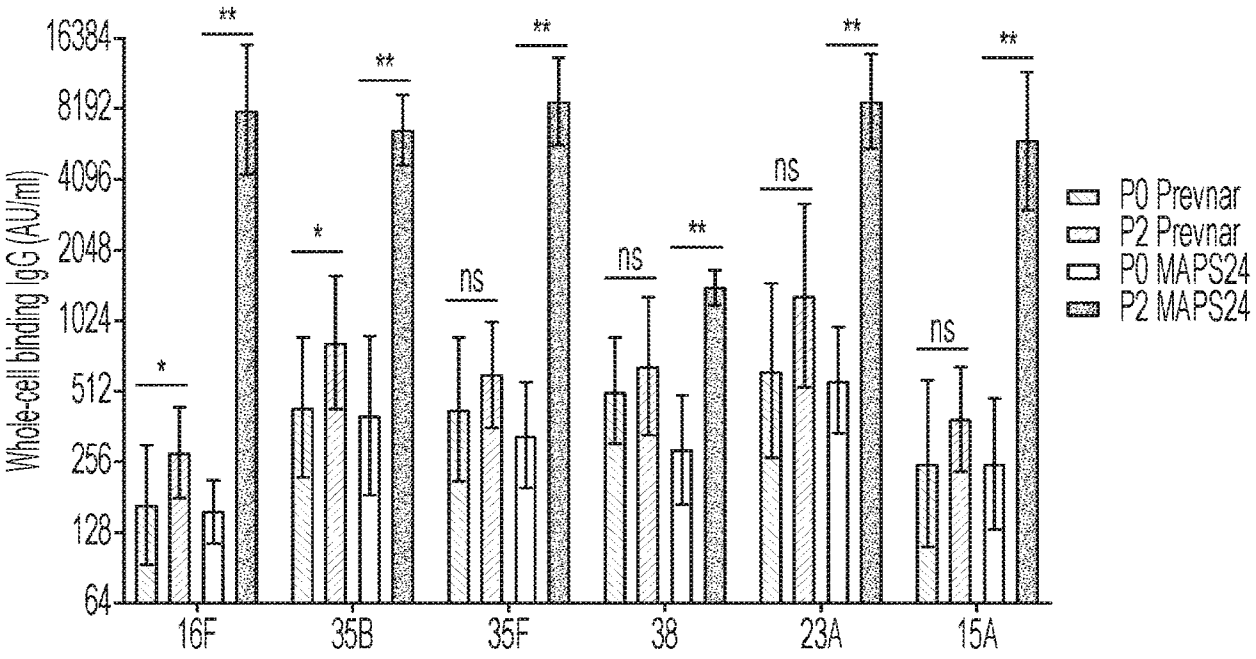


FIG. 20

Petitioner Merck Ex-1015, 1246

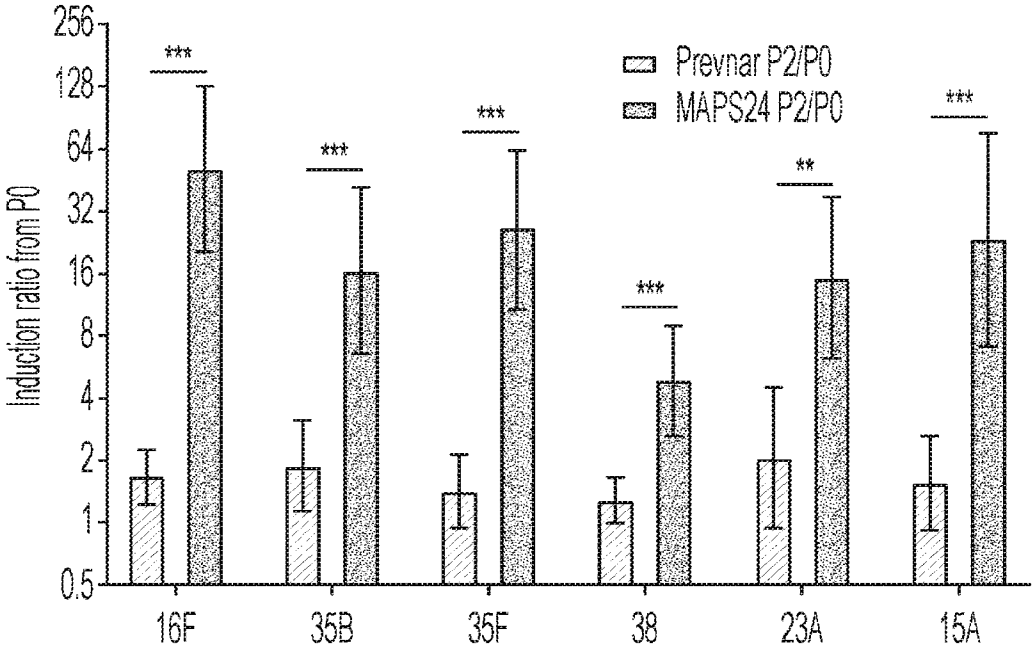


FIG. 21

Petitioner Merck Ex-1015, 1247

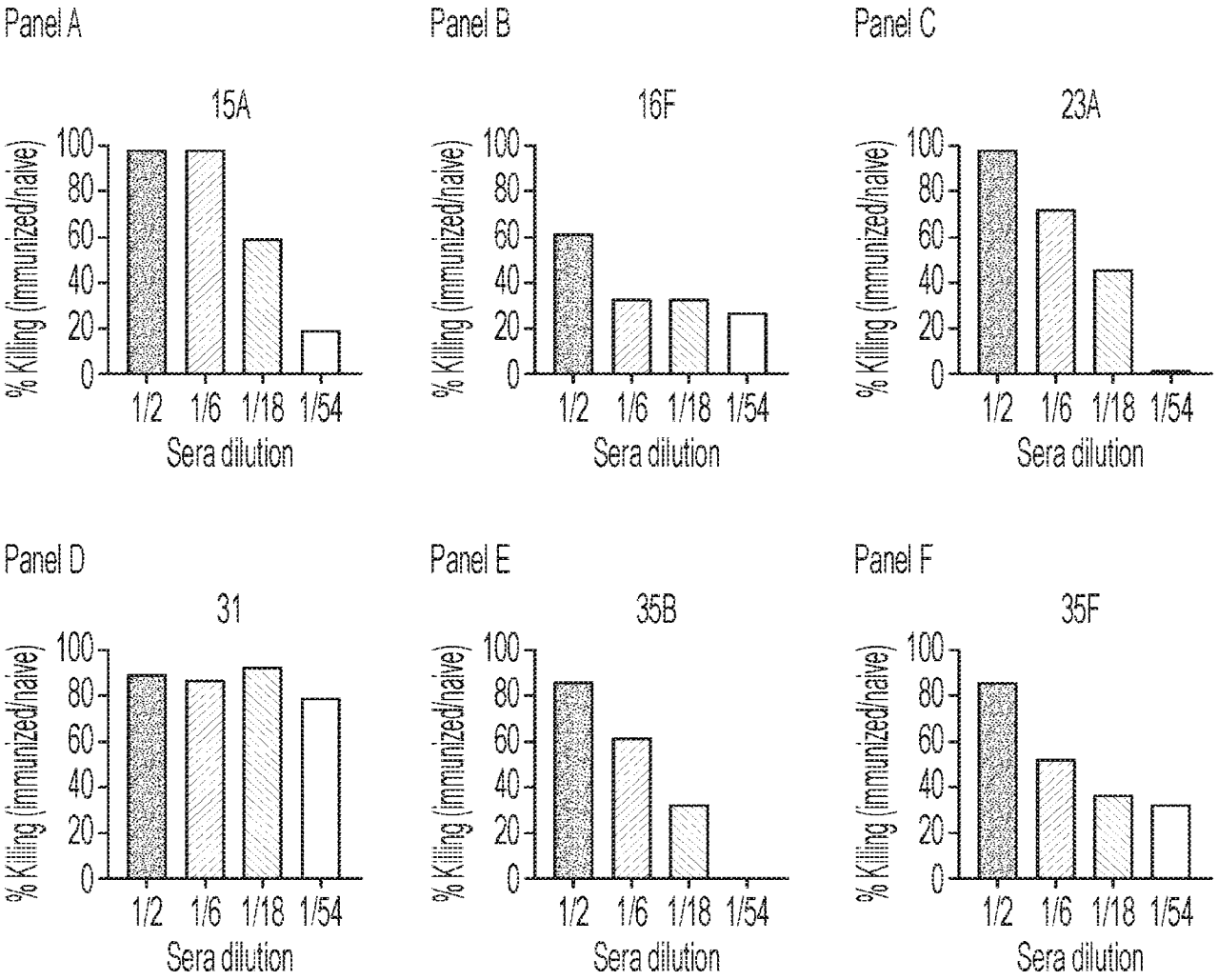


FIG. 22

Petitioner Merck Ex-1015, 1248

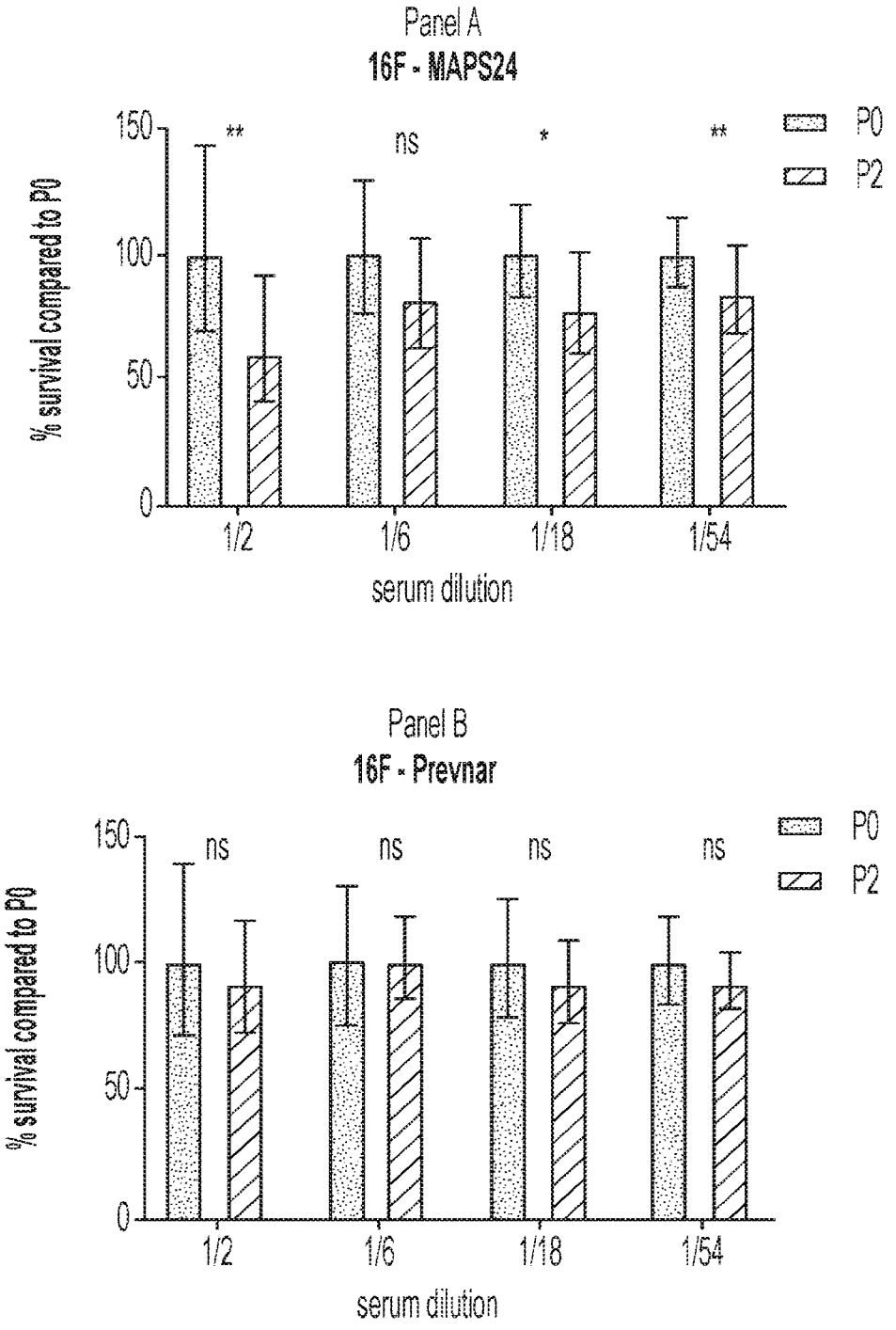


FIG. 23

Petitioner Merck Ex-1015, 1249

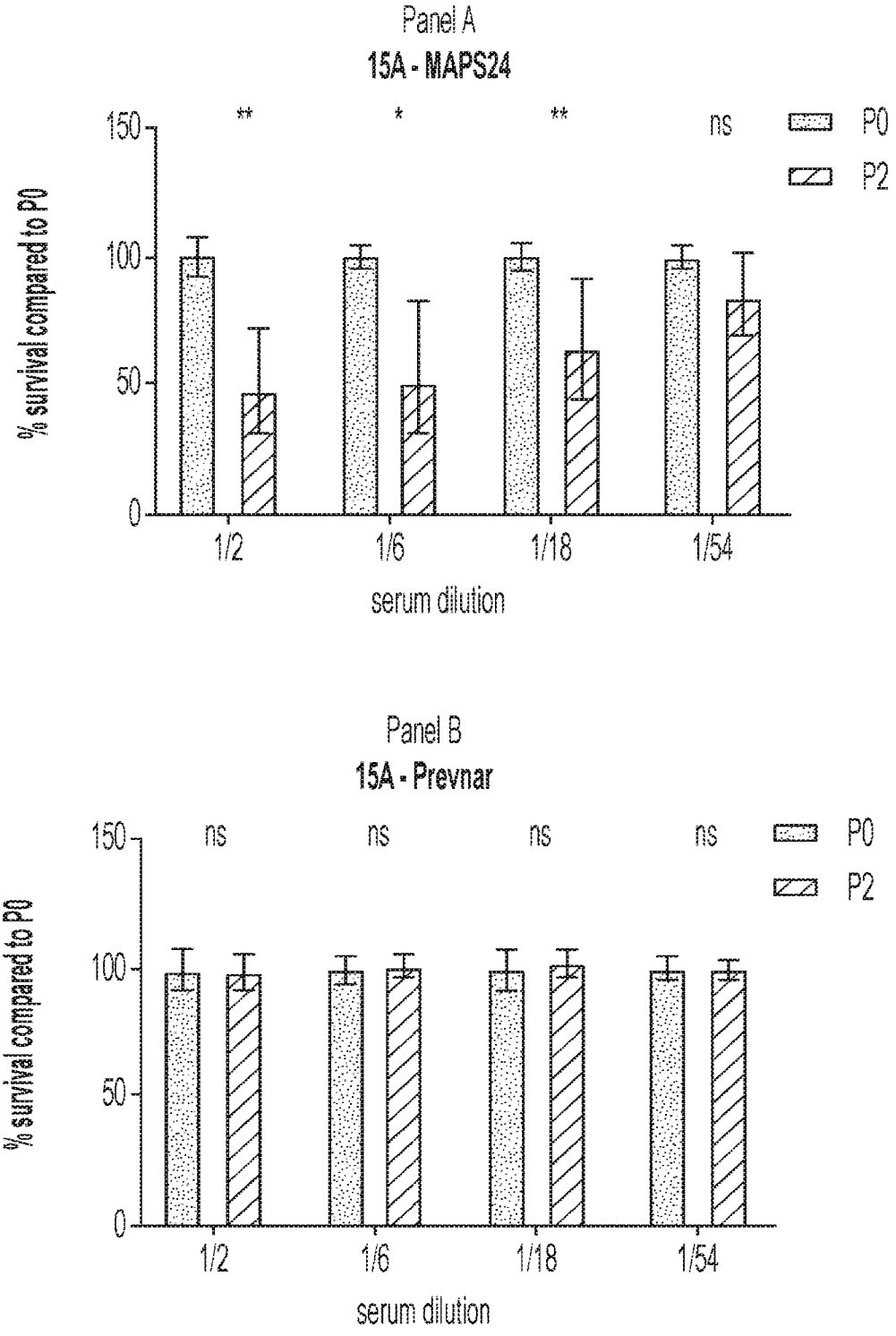


FIG. 24

Petitioner Merck Ex-1015, 1250

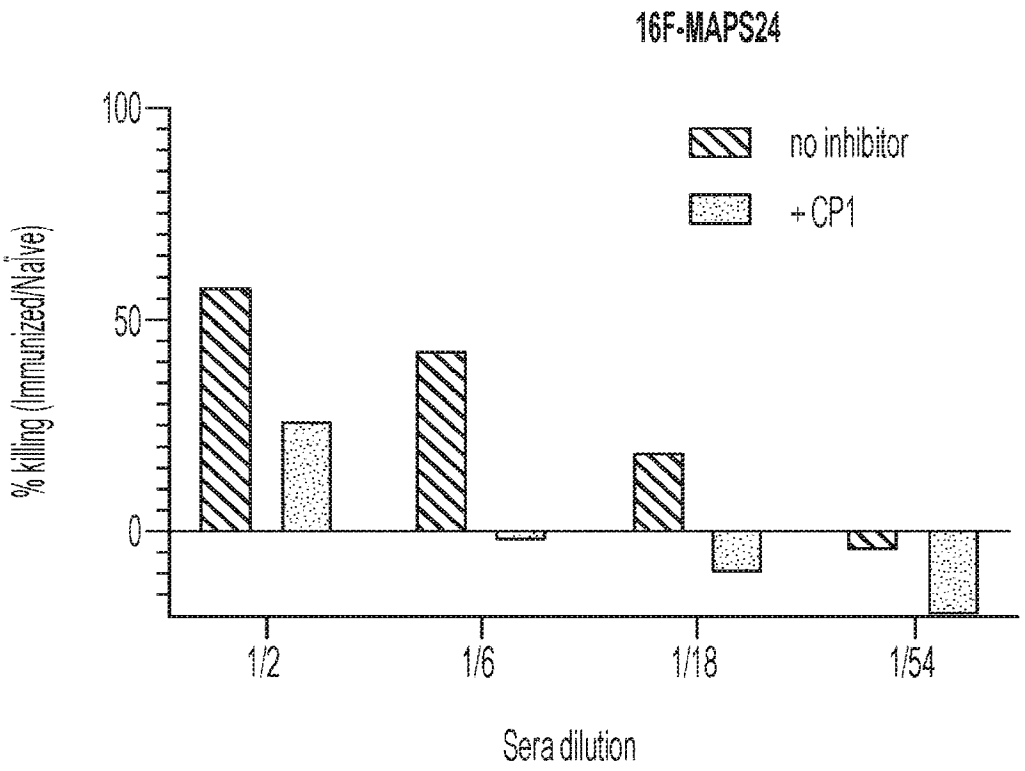


FIG. 25

Petitioner Merck Ex-1015, 1251

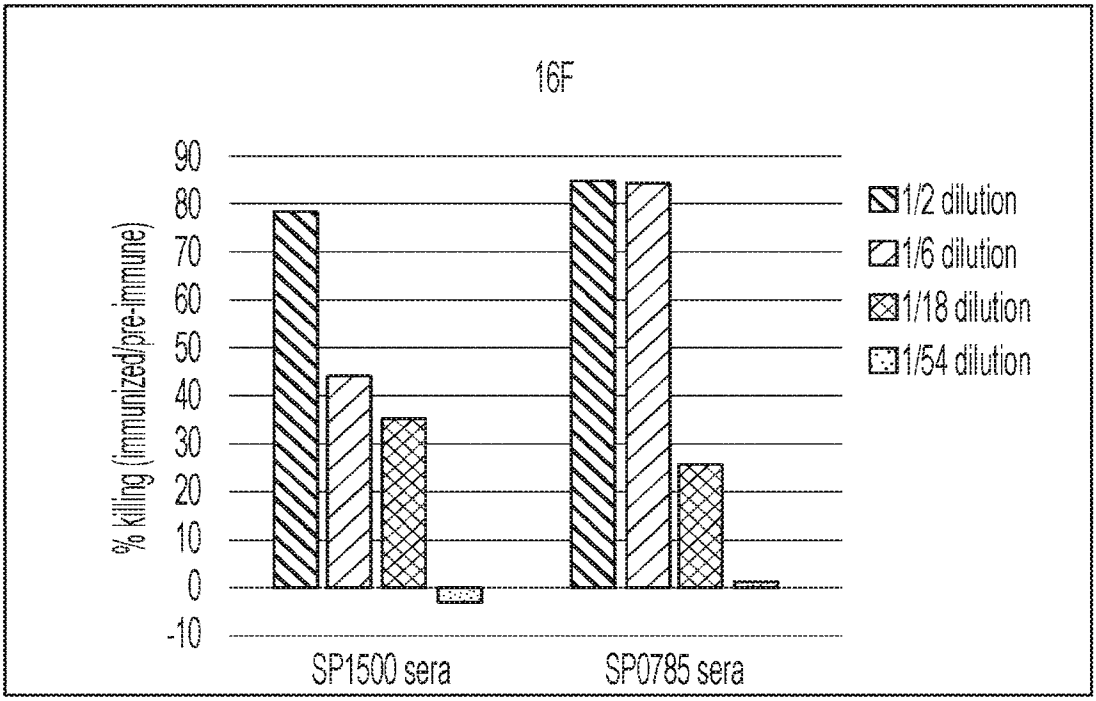


FIG. 26

Petitioner Merck Ex-1015, 1252

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/50907

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/16, 39/09, 47/64; C07K 14/315 (2020.01)

CPC - A61K 38/164, 39/092, 47/646; C07K 14/3156

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0374811 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) 31 December 2015; paragraphs [0006], [0007], [0012], [0192], [0194]-[0195], [0440]	1-2, 31-32, 33/31-32, 97-98, 99/97-98
A	ZHANG, F et al. Multiple antigen-presenting system (MAPS) to induce comprehensive B- and T-cell immunity. 13 August 2013. PNAS. Vol. 110, No. 33, pp. 13564-13569. entire document. DOI: https://doi.org/10.1073/pnas.1307228110	1-2, 31-32, 33/31-32, 97-98, 99/97-98
A	US 2013/0121958 A1 (LECLERC et al.) 16 May 2013; entire document	1-2, 31-32, 33/31-32, 97-98, 99/97-98

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January 2020 (24.01.2020)	10 FEB 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

Petitioner Merck Ex-1015, 1253

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/50907

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-8, 16-23, 27-30, 34-38, 63-96, 100-138
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 2 (in-part), 31, 32 (in-part), 33 (in-part), and 97-99 (each in-part), an SP1500 polypeptide (polypeptide antigen), and a polysaccharide antigen of S. pneumoniae serotype 1 (polysaccharide antigen); Exemplary election: an SP0785 polypeptide (polypeptide antigen)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Petitioner Merck Ex-1015, 1254

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/50907

-***-Continued from Box No. III Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 9-15, 24-26, 31-33, 39-62, 97-99, an SP1500 polypeptide (polypeptide antigen), and a polysaccharide antigen of S. pneumoniae serotype 1 (polysaccharide antigen) are directed toward vaccines or immunogenic complexes, and a method of making the vaccines.

The vaccines, complexes and methods will be searched to the extent they encompass an SP1500 polypeptide (first exemplary polypeptide antigen), and a polysaccharide antigen of S. pneumoniae serotype 1 (first exemplary polysaccharide antigen). Applicant is invited to elect additional polypeptide antigen(s) or fragment(s) thereof, with, where applicable, specified SEQ ID NO: for each, with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and/or specific polysaccharide antigen(s), where available as an option within at least one searchable claim, to be searched. Additional polypeptide antigen(s) and/or polysaccharide antigen(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2 (in-part), 31, 32 (in-part), 33 (in-part), and 97-99 (each in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass an SP1500 polypeptide (polypeptide antigen), and a polysaccharide antigen of S. pneumoniae serotype 1 (polysaccharide antigen). Applicants must specify the searchable claims that encompass any additionally elected polypeptide antigen(s) and/or polysaccharide antigen(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an SP0785 polypeptide (polypeptide antigen).

No technical features are shared between the polypeptide antigens and/or polysaccharide antigens of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a vaccine comprising an immunogenic complex, wherein the immunogenic complex comprises: (a) a biotinylated polysaccharide antigen of one or more Streptococcus pneumoniae serotypes; and (b) a fusion protein comprising: (i) a biotin-binding moiety; and (ii) at least one polypeptide antigen; wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex; an immunogenic complex comprising a biotinylated polysaccharide antigen of Streptococcus pneumoniae non-covalently associated with a fusion protein, wherein the fusion protein comprises a biotin-binding moiety and at least one polypeptide antigen; and a method of making a vaccine, comprising non-covalently complexing a plurality of biotinylated polysaccharide antigens with a plurality of fusion proteins, wherein each fusion protein comprises at least one polypeptide antigen selected from: an SP1500 polypeptide or antigenic fragment thereof; an SP0785 polypeptide or antigenic fragment thereof; and a combination thereof.

However, these shared technical features are previously disclosed by US 2015/0374811 A1 to Childrens' Medical Center Corporation (hereinafter 'CMCC').

CMCC discloses a vaccine (a vaccine; paragraph [0007]) comprising an immunogenic complex (comprising a MAPS (immunogenic) complex; paragraph [0052], [0192]), wherein the immunogenic complex comprises: (a) a biotinylated polysaccharide antigen of one or more Streptococcus pneumoniae serotypes (wherein the MAPS (immunogenic) complex comprises: (a) a biotinylated polysaccharide antigen of one or more Streptococcus pneumoniae serotypes; paragraphs [0052], [0192]); and (b) a fusion protein comprising: (i) a biotin-binding moiety; and (ii) at least one polypeptide antigen (a fusion protein comprising: (i) rhizavidin (a biotin-binding moiety); and (ii) at least one polypeptide antigen; paragraphs [0052], [0192]); wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex (wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex; paragraph [0192]); an immunogenic complex comprising a biotinylated polysaccharide antigen of Streptococcus pneumoniae non-covalently associated with a fusion protein (a MAPS (an immunogenic) complex comprising a biotinylated polysaccharide antigen of Streptococcus pneumoniae non-covalently associated with a fusion protein; paragraphs [0052], [0192]), wherein the fusion protein comprises a biotin-binding moiety and at least one polypeptide antigen (wherein the fusion protein comprises rhizavidin (a biotin-binding moiety) and at least one polypeptide antigen; paragraphs [0052], [0192]); and a method of making a vaccine (a method of making a vaccine; paragraph [0305]), comprising non-covalently complexing a plurality of biotinylated polysaccharide antigens with a plurality of fusion proteins (comprising bringing the biotinylated polysaccharide antigens antigens and plurality of fusion proteins into association; paragraphs [0052], [0192], [0305]), wherein each fusion protein comprises at least one polypeptide antigen (wherein each fusion protein comprises at least one polypeptide antigen; paragraphs [0017], [0052]) selected from: an SP1500 polypeptide or antigenic fragment thereof (selected from SEQ ID NO: 51 [SP1500] (an SP1500 polypeptide or antigenic fragment thereof); paragraph [0017]; wherein residues 27-278 of SEQ ID NO: 51 are 100% identical to Applicants' SQ ID NO: 5); and an SP0785 polypeptide or antigenic fragment thereof (a polypeptide selected from SEQ ID NO: 34 [SP0785](an SP0785 polypeptide or antigenic fragment thereof); paragraph [0017], wherein residues 33-399 of SEQ DI NO: 34 are 100% identical to Applicants' SEQ ID NO: 4).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the CMCC reference, unity of invention is lacking.

Petitioner Merck Ex-1015, 1255

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 1 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		
		4,221,906	A	Querry *et al.*	09-09-1980
		4,242,501	A	Cano *et al.*	12-30-1980
		4,459,286	A	Hilleman *et al.*	07-10-1984
		4,496,538	A	Gordon	01-29-1985
		4,673,574	A	Anderson	06-16-1987
		4,686,102	A	Ritchey *et al.*	08-11-1987
		4,902,506	A	Anderson *et al.*	02-20-1990
		5,097,020	A	Anderson *et al.*	03-17-1992
		5,192,540	A	Kuo *et al.*	03-09-1993
		5,360,897	A	Anderson *et al.*	11-01-1994
		5,445,817	A	Schneerson *et al.*	08-29-1995
		5,623,057	A	Marburg *et al.*	04-22-1997
		5,695,768	A	Malcolm	12-09-1997
		5,847,112	A	Kniskern *et al.*	12-08-1998
		5,965,714	A	Ryall	10-12-1999
		6,224,880	B1	Chan *et al.*	05-01-2001
		6,451,317	B1	Blake *et al.*	09-17-2002
		6,656,472	B1	Chong *et al.*	12-02-2003
		7,018,637	B2	Chong *et al.*	03-28-2006
		7,588,765	B2	Porro	09-15-2009
		7,709,001	B2	Hausdorff *et al.*	05-04-2010
		7,862,823	B1	Leroy	01-04-2011
		7,955,605	B2	Prasad	06-07-2011

EXAMINER:	DATE CONSIDERED:

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

*a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 16/089,040, filed 09-27-2018, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 2 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

		8,029,798	B2	Leroy	10-04-2011
		8,192,746	B2	Caulfield *et al.*	06-05-2012
		8,481,054	B2	Nahm *et al.*	07-09-2013
		8,603,484	B1	Chong *et al.*	12-02-2003
		8,753,645	B2	Biemans *et al.*	06-17-2014
		8,808,707	B2	Siber *et al.*	08-19-2014
		8,808,708	B2	Hausdorff *et al.*	08-19-2014
		8,895,024	B2	Hausdorff *et al.*	11-25-2014
		8,926,985	B2	Capiau *et al.*	01-06-2015
		9,095,567	B2	Khandke *et al.*	08-04-2015
		9,107,872	B2	Biemans *et al.*	08-18-2015
		9,168,313	B2	Capiau *et al.*	10-27-2015
		9,359,400	B2	Usera *et al.*	06-07-2016
		9,399,060	B2	Hausdorff *et al.*	07-26-2016
		9,492,559	B2	Emini *et al.*	11-15-2016
		9,610,340	B2	Biemans *et al.*	04-04-2017
		9,669,084	B2	Siber *et al.*	06-06-2017
		9,764,039	B2	Thanos *et al.*	09-19-2017
		9,884,113	B2	Biemans *et al.*	02-06-2018
		9,981,035	B2	Hausdorff *et al.*	05-29-2018
		10,124,050	B2	Watson *et al.*	11-13-2018
		10,716,848	B2	Hausdorff *et al.*	07-21-2020
		11,058,757	B2	Forrest *et al.*	07-13-2021
		11,116,828	B2	Smith *et al.*	09-14-2021
		11,701,416	B2	Malley *et al.*	07-18-2023
		11,850,278	B2	Smith *et al.*	12-26-2023
		2005-0214329	A1	Laferriere *et al.*	09-29-2005

EXAMINER:	DATE CONSIDERED:

[#] EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

*a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 16/089,040, filed 09-27-2018, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

Petitioner Merck Ex-1015, 1257

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 3 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

		2006-0228380	A1	Hausdorff *et al.*	10-12-2006
		2009-0010959	A1	Biemans *et al.*	01-08-2009
		2015-0344530	A1	Kapre	12-03-2015
		2015-0374811	A1	Malley *et al.*	12-31-2015
		2016-0324949	A1	Han *et al.*	11-10-2016
		2017-0021006	A1	Watson *et al.*	01-26-2017
		2018-0333484	A1	Fairman *et al.*	11-22-2018
		2020-0054739	A1	Fairman *et al.*	02-20-2020
		2020-0113993	A1	Forrest *et al.*	04-16-2020
		2020-0188502	A1	Faridmoayer *et al.*	06-18-2020
		2020-0282040	A1	Porambo *et al.*	09-10-2020
		2020-0282070	A1	Porambo *et al.*	09-10-2020
		2020-0330578	A1	Porambo *et al.*	10-22-2020
		2020-0330579	A1	Forrest *et al.*	10-22-2020
		2020-0360500	A1	Chintala *et al.*	11-19-1020
		2021-0393764	A1	Cooper *et al.*	12-23-2021
		2022-0143167	A1	Forrest *et al.*	05-12-2022

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Examiner's Initials #	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/ Country	Number	Kind Code			
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Petitioner Merck Ex-1015, 1258

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 4 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

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		WO	2020/056202	A1	Affinivax Inc.	03-19-2020	

OTHER ART – NON PATENT LITERATURE DOCUMENTS

Examiner's Initials #	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
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	*	International Search Report and Written Opinion, mailed September 13, 2017, in connection with Application No. PCT/US2017/025621 (P1143.70000WO00).	
	*	International Preliminary Report on Patentability, mailed October 11, 2018, in connection with Application No. PCT/US2017/025621 (P1143.70000WO00).	
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Petitioner Merck Ex-1015, 1259

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 5 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

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Petitioner Merck Ex-1015, 1260

FORM PTO-1449/A and B (modified PTO/SB/08) **INFORMATION DISCLOSURE STATEMENT BY APPLICANT**				APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
				FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
				FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet	6	of	6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	17/347,435-Conf. #4020
Filing Date	June 14, 2021
First Named Inventor	Bruce D. Forrest
Art Unit	1645
Examiner Name	Khatol S. S. Shah
Attorney Docket Number	P1143.70000US05

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
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  - [ ] After Final
  - [ ] Affidavits/declaration(s)
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- [ ] Status Letter
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PTO-1449
Copies of Cited References

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	May 20, 2024	Reg. No.	75,713

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APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
17/347,435	**05/20/2024 05:19:12 PM Z ET**	**P1143.70000US05**

Title of Invention

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4020	FILED BY	Abigail Feldman
PATENT CENTER #	65599825	FILING DATE	06/14/2021
CUSTOMER #	23628	FIRST NAMED INVENTOR	Bruce D. Forrest
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Chelsea Witte-Garcia

Documents

TOTAL DOCUMENTS: 18

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
EP0338265B1_FOR.pdf	25	Foreign Reference	1073 KB
WO03051392A2_FOR.pdf	47	Foreign Reference	2128 KB
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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62/103,630 15 January 2015 (15.01.2015) US

(71) Applicant: **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, New York 10017 (US).

(72) Inventors: **COOPER, David**; 9 Webb Farm Road, Monroe, New York 10950 (US). **JANSEN, Kathrin Ute**; 160 E. 65th Street, Apt. 11C, New York, New York 10065 (US). **PRIDE, Michael William**; 80 Bay Street Landing, Unit 8A, Staten Island, New York 10301 (US).

(74) Agent: **WALDRON, Roy F.**; PFIZER INC., 235 East 42nd Street, MS 235/9/S20, New York, New York 10017 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: IMMUNOGENIC COMPOSITIONS FOR USE IN PNEUMOCOCCAL VACCINES

(57) **Abstract**: An object of the present invention is to provide immunogenic compositions for protection against S. *pneumoniae*, in particular against S. *pneumoniae* serogroup 9, while limiting the number of conjugates. The present invention thereforerelates to new immunogenic compositions for use in pneumococcal vaccines and to vaccination of human subjects, in particular infants and elderly, against pneumoccocal infections using said immunogenic compositions.

WO 2016/113644 A1

Petitioner Merck Ex-1015, 1268

Immunogenic compositions for use in pneumococcal vaccines

Field of the Invention

The present invention relates to new immunogenic compositions for use in pneumococcal vaccines. Immunogenic compositions of the present invention will typically comprise conjugated capsular saccharide antigens (glycoconjugates), wherein the saccharides are derived from serotypes of *Streptococus pneumoniae*. An object of the present invention is to provide immunogenic compositions for protection against *S. pneumoniae* serogroup 9.

Background of the Invention

Infections caused by pneumococci are a major cause of morbidity and mortality all over the world. Pneumonia, febrile bacteraemia and meningitis are the most common manifestations of invasive pneumococcal disease, whereas bacterial spread within the respiratory tract may result in middle-ear infection, sinusitis or recurrent bronchitis. Compared with invasive disease, the non-invasive manifestations are usually less severe, but considerably more common.

The etiological agent of pneumococcal diseases, *Streptococcus pneumoniae* (pneumococcus), is a Gram-positive encapsulated coccus, surrounded by a polysaccharide capsule. Differences in the composition of this capsule permit serological differentiation between about 91 capsular types, some of which are frequently associated with pneumococcal disease, others rarely. Invasive pneumococcal infections include pneumonia, meningitis and febrile bacteremia; among the common non-invasive manifestations are otitis media, sinusitis and bronchitis.

Pneumococcal conjugate vaccines (PCVs) are pneumococcal vaccines used to protect against disease caused by *S. pneumoniae* (pneumococcus). There are currently three PCV vaccines available on the global market: PREVNAR® (called Prevenar in some countries) (heptavalent vaccine), SYNFLORIX® (a decavalent vaccine) and PREVNAR 13® (tridecavalent vaccine).

The specific serotypes causing disease beyond the 13 in PREVNAR 13® vary by region, population, and may change over time due to acquisition of antibiotic resistance, pneumococcal vaccine introduction and secular trends of unknown origin.

Petitioner Merck Ex-1015, 1269

The addition of conjugates to an immunogenic composition is not a straightforward process as the combination of conjugates into a single multivalent injection may result in competition among the different components and may adversely affect the immunogenicity of any individual conjugate.

This phenomenon of interference may limit the number of conjugates which can be included in a multi-valent vaccine. Therefore protection against a high number of serotypes, while limiting the number of conjugates in the composition, maybe very difficult to obtain despite of the significant value.

An object of the present invention is to provide immunogenic compositions for appropriate protection against *S. pneumoniae*, in particular against *S. pneumoniae* serogroup 9, while limiting the number of conjugates.

Streptococcus pneumoniae serogroup 9 consists of four different types, 9V, 9A, 9L, and 9N, each of which produces its own, type-specific capsular polysaccharide (Richards, J.C and M.B. Perry, 1988, In A.M. Wu (ed.), The molecular immunology complex carbohydrates. Plenum, New York, pp. 593-594).

It is an object of the present invention to provide immunogenic compositions for appropriate protection against *S. pneumoniae* serotypes 9V, 9A, 9L, and 9N, with a limited number of conjugates.

Summary of the Invention

The present invention relates to an immunogenic composition comprising at least one glycoconjugate from *S. pneumoniae* serotype 9V for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L. Preferably said composition does not comprise capsular saccharide from *S. pneumoniae* serotypes 9N, 9A and 9L.

In one aspect the present invention relates to the use of an immunogenic composition comprising at least one glycoconjugate from *S. pneumoniae* serotype 9V for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L. Preferably said composition does not comprise capsular saccharide from *S. pneumoniae* serotypes 9N, 9A and 9L.

In one aspect, the above immunogenic compositions further comprise at least one glycoconjugate from *S. pneumoniae* serotypes 4, 6B, 14, 18C, 19F and/or 23F.

In an aspect the above immunogenic compositions further comprise at least one glycoconjugate from *S. pneumoniae* serotype 1, 5 and/or 7F.

Petitioner Merck Ex-1015, 1270

In an aspect the above immunogenic compositions further comprise at least one glycoconjugate from *S. pneumoniae* serotype 6A and/or 19A.

In an aspect the above immunogenic compositions further comprise at least one glycoconjugate from *S. pneumoniae* serotype 3, 15B, 22F, 33F, 12F, 10A, 11A and/or 8.

In a further aspect the above immunogenic compositions further comprise at least one glycoconjugate from *S. pneumoniae* serotype 2, 15C, 17F and/or 20.

In a further aspect the immunogenic compositions is a 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24-valent pneumococcal conjugate composition.

In a further aspect the glycoconjugates of the immunogenic compositions are individually conjugated to CRM197.

In on aspect, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14 and/or 23F of the immunogenic compositions are individually conjugated to PD, and if present, the glycoconjugate from *S. pneumoniae* serotype 18C is conjugated to TT and the glycoconjugate from *S. pneumoniae* serotype 19F is conjugated to DT.

In on aspect, the glycoconjugates are prepared using CDAP chemistry or by reductive amination chemistry.

The immunogenic composition may further comprise antigens from other pathogens, and/or at least one adjuvant such as aluminum phosphate, aluminum sulphate or aluminum hydroxide.

In an aspect the immunogenic compositions are able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9N, 9A and/or 9L polysaccharide at a concentration of at least 0.35 µg/ml as determined by ELISA assay.

In an aspect the immunogenic compositions are able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9N, 9A and/or 9L in at least 50% of the subjects as determined by in vitro opsonophagocytic killing assay (OPA).

In an aspect the immunogenic compositions are able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9N, 9A and/or 9L as compared to the pre-immunized population.

In an aspect the immunogenic compositions are able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9N, 9L and/or 9A as compared to the pre-immunized population.

In an aspect the immunogenic compositions are for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9L and/or 9A.

Petitioner Merck Ex-1015, 1271

In an aspect the immunogenic compositions are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, for use to prevent to prevent serotypes 9N, 9A and/or 9L S. pneumoniae infection in a subject or for use in a method to protect or treat a human susceptible to *S. pneumoniae* serotypes 9N, 9A and/or 9L infection, by means of administering said immunogenic compositions via a systemic or mucosal route.

In one aspect the present invention relates to the use of the immunogenic composition disclosed in the present document for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, for use to prevent to prevent serotypes 9N, 9A and/or 9L *S. pneumoniae* infection in a subject or for use in a method to protect or treat a human susceptible to *S. pneumoniae* serotypes 9N, 9A and/or 9L infection, by means of administering said immunogenic compositions via a systemic or mucosal route.

In an aspect the invention relates to a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of the immunogenic composition of the invention.

In an aspect the invention relates to a method of preventing an infection by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of the immunogenic composition of the invention.

The invention further relates to a kit comprising an immunogenic composition disclosed herein and an information leaflet, wherein said information leaflet mentions the ability of the composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N and process for producing said kit.

WO2013/191459 discloses a 15-valent immunogenic composition comprising glycoconjugates from both *S. pneumoniae* serotypes 9N and 9V. Similarly WO2014/092377 and Chinese patent applications Nos. CN101590224 and CN 103656631 disclose immunogenic compositions combining glycoconjugates from both *S. pneumoniae* serotypes 9N and 9V.

It has been surprisingly found that serotype 9V polysaccharide conjugate beyond eliciting functional reactive antibodies to serogroup 9V, can additionnaly elicit functional, cross-reactive antibodies to the other serotypes within the serogroup 9: 9A, 9L and/or 9N.

Petitioner Merck Ex-1015, 1272

Figures

Figure 1 Cross-Functional OPA Responses. A subset of 59 sera from adults vaccinated with a 13 valent Pneumococcal Conjugate Vaccine (US Study 6115A1-004; ClinicalTrials.gov Identifier: NCT00427895) was assessed in OPAs for the presence of functional antibodies against serotypes 9V, 9A, 9L, and 9N. The percent of samples with OPA positive titer (i.e., ≥1:8) is indicated above each group. Geometric mean titers (GMT) are listed in the x axis below each group.

Figure 2 Cross-Functional OPA Responses of Sixty-six Matched pre/post Sera. A subset of 66 matched pre- and post-vaccinated serum panel from adults vaccinated with a 13 valent Pneumococcal Conjugate Vaccine (study 6115A1-3005; ClinicalTrials.gov Identifier: NCT00546572) were assessed in OPAs for the presence of functional antibodies against serotypes 9V, 9A, 9L, and 9N. The percent of samples with OPA positive titer (i.e., ≥1:8) is indicated above each group. Geometric mean titers (GMT) are listed in the x axis below each group.

Figure 3 Reverse cumulative distribution curves (RCDC) of pre and post Immunization – pneumococcal serotype 9V (Pn9V).

Reverse cumulative distribution curves of OPA titers to serotype 9V from a matched pre- and post-vaccination serum panel (N = 66) vaccinated with a 13 valent Pneumococcal Conjugate Vaccine (study 6115A1-3005; ClinicalTrials.gov Identifier: NCT00546572). The plots represent the percent of sera with OPA positive titer (i.e., ≥1:8).

Figure 4 Reverse cumulative distribution curves (RCDC) of pre and post Immunization – pneumococcal serotype 9A (Pn9A).

Reverse cumulative distribution curves of OPA titers to serotype 9A from a matched pre- and post-vaccination serum panel (N = 66) vaccinated with a 13 valent Pneumococcal Conjugate Vaccine (study 6115A1-3005; ClinicalTrials.gov Identifier: NCT00546572). The plots represent the percent of sera with OPA positive titer (i.e., ≥1:8).

Petitioner Merck Ex-1015, 1273

Figure 5 Reverse cumulative distribution curves (RCDC) of pre and post Immunization – pneumococcal serotype 9L (Pn9L).

Reverse cumulative distribution curves of OPA titers to serotype 9L from a matched pre- and post-vaccination serum panel (N = 66) vaccinated with with a 13 valent Pneumococcal Conjugate Vaccine (study 6115A1-3005; ClinicalTrials.gov Identifier: NCT00546572). The plots represent the percent of sera with OPA positive titer (i.e., ≥1:8).

Figure 6 Reverse cumulative distribution curves (RCDC) of pre and post Immunization – pneumococcal serotype 9N (Pn9N).

Reverse cumulative distribution curves of OPA titers to serotype 9N from a matched pre- and post-vaccination serum panel (N = 66) vaccinated with with a 13 valent Pneumococcal Conjugate Vaccine (study 6115A1-3005; ClinicalTrials.gov Identifier: NCT00546572). The plots represent the percent of sera with OPA positive titer (i.e., ≥1:8).

Figure 7 Serogroup 9 Cross-Functional OPA responses from matched Pre/Post-vaccination sera from Study B1851088 (ClinicalTrials.gov Identifier: NCT01646398) following 13vPnC immunization.

A subset of 91 matched pre- and post-vaccinated serum panel from adults vaccinated with 13vPnC (study B1851088) were assessed in OPAs for the presence of functional antibodies against serotypes 9V, 9A, 9L, and 9N. The percent of samples with OPA positive titer (i.e., ≥1:8) is indicated above each group.

Figure 8 Serogroup 9 Cross-Functional OPA responses from matched Pre/Post-vaccination sera from Study B1851088 Following 23vPS immunization.

A subset of 83 matched pre- and post-vaccinated serum panel from adults vaccinated with 23vPS (study B1851088) were assessed in OPAs for the presence of functional antibodies against serotypes 9V, 9A, 9L, and 9N. The percent of samples with OPA positive titer (i.e., ≥1:8) is indicated above each group.

Figure 9 Serogroup 9 Cross-Functional OPA titers from matched Pre/Post-vaccination sera from Study B1851088 following 13vPnC (13v) or 23vPS (23v) immunization.

Petitioner Merck Ex-1015, 1274

Figure 10 Pn9V & Pn9N 13vPnC reverse cumulative distribution curves (RCDC) from Study B1851088.
Reverse cumulative distribution curves of OPA titers to serotype 9V and 9N from a matched pre- and post-vaccination serum panel (N = 91) vaccinated with 13vPnC (Japan Study B1851088).

Figure 11 Pn9V & Pn9N 23vPS reverse cumulative distribution curves (RCDC) from Study B1851088.
Reverse cumulative distribution curves of OPA titers to serotype 9V and 9N from a matched pre- and post-vaccination serum panel (N = 83) vaccinated with 23vPS (Japan Study B1851088).

Figure 12 Pn9V & Pn9A 13vPnC reverse cumulative distribution curves (RCDC) from Study B1851088.
Reverse cumulative distribution curves of OPA titers to serotype 9V and 9A from a matched pre- and post-vaccination serum panel (N = 91) vaccinated with 13vPnC (Japan Study B1851088).

Figure 13 Pn9V & Pn9A 23vPS reverse cumulative distribution curves (RCDC) from Study B1851088.
Reverse cumulative distribution curves of OPA titers to serotype 9V and 9A from a matched pre- and post-vaccination serum panel (N = 83) vaccinated with 23vPS (Japan Study B1851088).

Figure 14 Pn9V & Pn9L 13vPnC reverse cumulative distribution curves (RCDC) from Study B1851088.
Reverse cumulative distribution curves of OPA titers to serotype 9V and 9L from a matched pre- and post-vaccination serum panel (N = 91) vaccinated with 13vPnC (Japan Study B1851088).

Figure 15 Pn9V & Pn9L 23vPS reverse cumulative distribution curves (RCDC) from Study B1851088.

Petitioner Merck Ex-1015, 1275

Reverse cumulative distribution curves of OPA titers to serotype 9V and 9L from a matched pre- and post-vaccination serum panel (N = 83) vaccinated with 23vPS (Japan Study B1851088).

1 Immunogenic compositions of the invention

Immunogenic compositions of the present invention will typically comprise conjugated capsular saccharide antigens (also named glycoconjugates), wherein the saccharides are derived from serotypes of *S. pneumoniae*.

Preferably, the number of *S. pneumoniae* capsular saccharides can range from 1 serotype (or "v", valences) to 24 different serotypes (24v). In one embodiment there is one serotype. In one embodiment there are 2 different serotypes. In one embodiment there are 3 different serotypes. In one embodiment there are 4 different serotypes. In one embodiment there are 5 different serotypes. In one embodiment there are 6 different serotypes. In one embodiment there are 7 different serotypes. In one embodiment there are 8 different serotypes. In one embodiment there are 9 different serotypes. In one embodiment there are 10 different serotypes. In one embodiment there are 11 different serotypes. In one embodiment there are 12 different serotypes. In one embodiment there are 13 different serotypes. In one embodiment there are 14 different serotypes. In one embodiment there are 15 different serotypes. In one embodiment there are 16 different serotypes. In an embodiment there are 17 different serotypes. In an embodiment there are 18 different serotypes. In an embodiment there are 19 different serotypes. In an embodiment there are 20 different serotypes. In an embodiment there are 21 different serotypes. In an embodiment there are 22 different serotypes. In an embodiment there are 23 different serotypes. In an embodiment there are 24 different serotypes. The capsular saccharides are conjugated to a carrier protein to form glycoconjugates as described here below.

If the protein carrier is the same for 2 or more saccharides in the composition, the saccharides could be conjugated to the same molecule of the protein carrier (carrier molecules having 2 or more different saccharides conjugated to it) [see for instance WO 2004/083251].

In a preferred embodiment though, the saccharides are each individually conjugated to different molecules of the protein carrier (each molecule of protein carrier only having one type of saccharide conjugated to it). In said embodiment, the capsular saccharides are said to be individually conjugated to the carrier protein.

Petitioner Merck Ex-1015, 1276

For the purposes of the invention the term 'glycoconjugate' indicates a capsular saccharide linked covalently to a carrier protein. In one embodiment a capsular saccharide is linked directly to a carrier protein. In a second embodiment a bacterial saccharide is linked to a protein through a spacer/linker.

1.1 Carrier protein of the invention

A component of the glycoconjugate of the invention is a carrier protein to which the saccharide is conjugated. The terms "protein carrier" or "carrier protein" or "carrier" may be used interchangeably herein. Carrier proteins should be amenable to standard conjugation procedures.

In a preferred embodiment, the carrier protein of the glycoconjugates is selected in the group consisting of: DT (Diphtheria toxin), TT (tetanus toxid) or fragment C of TT, CRM_{197} (a nontoxic but antigenically identical variant of diphtheria toxin), the A chain of diphtheria toxin mutant CRM_{197} (CN103495161), other DT mutants (such as CRM176, CRM228, CRM45 (Uchida et al. (1973) J. Biol. Chem. 218:3838-3844), CRM9, CRM102, CRM103 or CRM107; and other mutations described by Nicholls and Youle in Genetically Engineered Toxins, Ed: Frankel, Maecel Dekker Inc. (1992); deletion or mutation of Glu-148 to Asp, Gln or Ser and/or Ala 158 to Gly and other mutations disclosed in U.S. Patent Nos. 4,709,017 and 4,950,740; mutation of at least one or more residues Lys 516, Lys 526, Phe 530 and/or Lys 534 and other mutations disclosed in U.S. Patent Nos. 5,917,017 and 6,455,673; or fragment disclosed in U.S. Patent No. 5,843,711, pneumococcal pneumolysin (ply) (Kuo et al. (1995) Infect Immun 63:2706-2713) including ply detoxified in some fashion, for example dPLY-GMBS (WO 2004/081515 and WO 2006/032499) or dPLY-formol, PhtX, including PhtA, PhtB, PhtD, PhtE (sequences of PhtA, PhtB, PhtD or PhtE are disclosed in WO 00/37105 and WO 00/39299) and fusions of Pht proteins for example PhtDE fusions, PhtBE fusions, Pht A-E (WO 01/98334, WO 03/054007, WO 2009/000826), OMPC (meningococcal outer membrane protein - usually extracted from *Neisseria meningitidis* serogroup B (EP0372501), PorB (from *N. meningitidis*), PD (*Haemophilus influenzae* protein D; see, e.g., EP0594610 B), or immunologically functional equivalents thereof, synthetic peptides (EP0378881, EP0427347), heat shock proteins (WO 93/17712, WO 94/03208), pertussis proteins (WO 98/58668, EP0471177), cytokines, lymphokines, growth factors or hormones (WO 91/01146), artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens (Falugi et al.

Petitioner Merck Ex-1015, 1277

(2001) Eur J Immunol 31:3816-3824) such as N19 protein (Baraldoi et al. (2004) Infect Immun 72:4884-4887) pneumococcal surface protein PspA (WO 02/091998), iron uptake proteins (WO 01/72337), toxin A or B of *Clostridium difficile* (WO 00/61761), transferrin binding proteins, pneumococcal adhesion protein (PsaA), recombinant *Pseudomonas aeruginosa* exotoxin A (in particular non-toxic mutants thereof (such as exotoxin A bearing a substution at glutamic acid 553 (Douglas et al. (1987) J. Bacteriol. 169(11):4967-4971)). Other proteins, such as ovalbumin, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or purified protein derivative of tuberculin (PPD) also can be used as carrier proteins. Other suitable carrier proteins include inactivated bacterial toxins such as cholera toxoid (e.g., as described in WO 2004/083251), *Escherichia coli* LT, *E. coli* ST, and exotoxin A from *P. aeruginosa*.

In a preferred embodiment, the carrier protein of the glycoconjugates is independently selected from the group consisting of TT, DT, DT mutants (such as CRM_{197}), *H. influenzae* protein D, PhtX, PhtD, PhtDE fusions (particularly those described in WO 01/98334 and WO 03/054007), detoxified pneumolysin, PorB, N19 protein, PspA, OMPC, toxin A or B of *C. difficile* and PsaA.

In an embodiment, the carrier protein of the glycoconjugates of the invention is DT (Diphtheria toxoid). In another embodiment, the carrier protein of the glycoconjugates of the invention is TT (tetanus toxid).

In another embodiment, the carrier protein of the glycoconjugates of the invention is PD (*H. influenzae* protein D; see, e.g., EP0594610 B).

The CRM_{197} protein is a nontoxic form of diphtheria toxin but is immunologically indistinguishable from the diphtheria toxin. CRM_{197} is produced by *Corynebacterium diphtheriae* infected by the nontoxigenic phage $\beta197^{tox-}$ created by nitrosoguanidine mutagenesis of the toxigenic corynephage beta (Uchida et al. (1971) Nature New Biology 233:8-11). The CRM_{197} protein has the same molecular weight as the diphtheria toxin but differs therefrom by a single base change (guanine to adenine) in the structural gene. This single base change causes an amino acid substitution (glutamic acid for glycine) in the mature protein and eliminates the toxic properties of diphtheria toxin. The CRM_{197} protein is a safe and effective T-cell dependent carrier for saccharides. Further details about CRM_{197} and production thereof can be found, e.g., in U.S. Patent No. 5,614,382. In an embodiment, the capsular saccharides of the invention are conjugated to CRM_{197} protein or the A chain of CRM_{197} (see CN103495161). In an embodiment, the capsular saccharides of the invention are conjugated the A chain of CRM_{197} obtained

Petitioner Merck Ex-1015, 1278

via expression by genetically recombinant *E. coli* (see CN103495161). In an embodiment, the capsular saccharides of the invention are all conjugated to CRM_{197}. In an embodiment, the capsular saccharides of the invention are all conjugated to the A chain of CRM_{197}.

Accordingly, in frequent embodiments, the glycoconjugates of the invention comprise CRM_{197} as the carrier protein, wherein the capsular polysaccharide is covalently linked to CRM_{197}.

1.2 Capsular saccharide of the invention

The term "saccharide" throughout this specification may indicate polysaccharide or oligosaccharide and includes both. In frequent embodiments, the saccharide is a polysaccharide, in particular a *S. pneumoniae* capsular polysaccharide.

Capsular polysaccharides are prepared by standard techniques known to those of ordinary skill in the art.

In the present invention, capsular polysaccharides may be prepared, e.g., from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F of *S. pneumoniae*. Typically capsular polysaccharides are produced by growing each *S. pneumoniae* serotype in a medium (e.g., in a soy-based medium), the polysaccharides are then prepared from the bacteria culture. Bacterial strains of *S. pneumoniae* used to make the respective polysaccharides that are used in the glycoconjugates of the invention may be obtained from established culture collections or clinical specimens.

The population of the organism (each *S. pneumoniae* serotype) is often scaled up from a seed vial to seed bottles and passaged through one or more seed fermentors of increasing volume until production scale fermentation volumes are reached. At the end of the growth cycle the cells are lysed and the lysate broth is then harvested for downstream (purification) processing (see for example WO 2006/110381, WO 2008/118752, and U.S. Patent App. Pub. Nos. 2006/0228380, 2006/0228381, 2008/0102498 and 2008/0286838).

The individual polysaccharides are typically purified through centrifugation, precipitation, ultra-filtration, and/or column chromatography (see for example WO 2006/110352 and WO 2008/118752).

Purified polysaccharides may be activated (e.g., chemically activated) to make them capable of reacting (e.g., either directly to the carrier protein of via a linker such as an

Petitioner Merck Ex-1015, 1279

eTEC spacer) and then incorporated into glycoconjugates of the invention, as further described herein.

S. pneumoniae capsular polysaccharides comprise repeating oligosaccharide units which may contain up to 8 sugar residues.

In an embodiment, capsular saccharide of the invention may be one oligosaccharide unit, or a shorter than native length saccharide chain of repeating oligosaccharide units. In an embodiment, capsular saccharide of the invention is one repeating oligosaccharide unit of the relevant serotype.

In an embodiment, capsular saccharide of the invention may be oligosaccharides. Oligosaccharides have a low number of repeat units (typically 5-15 repeat units) and are typically derived synthetically or by hydrolysis of polysaccharides.

Preferably though, all of the capsular saccharides of the present invention and in the immunogenic compositions of the present invention are polysaccharides. High molecular weight capsular polysaccharides are able to induce certain antibody immune responses due to the epitopes present on the antigenic surface. The isolation and purification of high molecular weight capsular polysaccharides is preferably contemplated for use in the conjugates, compositions and methods of the present invention.

In some embodiments, the purified polysaccharides before conjugation have a molecular weight of between 5 kDa and 4,000 kDa. In other such embodiments, the polysaccharide has a molecular weight of between 10 kDa and 4,000 kDa; between 50 kDa and 4,000 kDa; between 50 kDa and 3,000 kDa; between 50 kDa and 2,000 kDa; between 50 kDa and 1,500 kDa; between 50 kDa and 1,000 kDa; between 50 kDa and 750 kDa; between 50 kDa and 500 kDa; between 100 kDa and 4,000 kDa; between 100 kDa and 3,000 kDa; 100 kDa and 2,000 kDa; between 100 kDa and 1,500 kDa; between 100 kDa and 1,000 kDa; between 100 kDa and 750 kDa; between 100 kDa and 500 kDa; between 100 and 400 kDa; between 200 kDa and 4,000 kDa; between 200 kDa and 3,000 kDa; between 200 kDa and 2,000 kDa; between 200 kDa and 1,500 kDa; between 200 kDa and 1,000 kDa; or between 200 kDa and 500 kDa.

In further embodiments, the capsular polysaccharide has a molecular weight of between 70 kDa to 150 kDa; 80 kDa to 160 kDa; 90 kDa to 250 kDa; 100 kDa to 1,000; 100 kDa to 500 kDa; 100 kDa to 400 kDa; 100 kDa to 160 kDa; 150 kDa to 600 kDa; 200 kDa to 1,000 kDa; 200 kDa to 600 kDa; 200 kDa to 400 kDa; 300 kDa to 1,000 KDa; 300 kDa

Petitioner Merck Ex-1015, 1280

to 600 kDa; 300 kDa to 500 kDa or 500 kDa to 600 kDa. Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

A polysaccharide can become slightly reduced in size during normal purification procedures. Additionally, as described herein, polysaccharide can be subjected to sizing techniques before conjugation. Mechanical or chemical sizing maybe employed. Chemical hydrolysis maybe conducted using acetic acid. Mechanical sizing maybe conducted using High Pressure Homogenization Shearing. The molecular weight ranges mentioned above refer to purified polysaccharides before conjugation (e.g., before activation).

In a preferred embodiment the purified polysaccharides, are capsular polysaccharide from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F or 33F of *S. pneumoniae*, wherein the capsular polysaccharide has a molecular weight falling within one of the molecular weight ranges as described here above.

As used herein, the term "molecular weight" of polysaccharide or of carrier protein-polysaccharide conjugate refers to molecular weight calculated by size exclusion chromatography (SEC) combined with multiangle laser light scattering detector (MALLS).

In some embodiments, the pneumococcal saccharides from serotypes 9V, 18C, 11A, 15B, 22F and/or 33F of the invention are O-acetylated. In some embodiments, the pneumococcal saccharides from serotypes 9V, 11A, 15B, 22F and/or 33F of the invention are O-acetylated.

The degree of O-acetylation of the polysaccharide can be determined by any method known in the art, for example, by proton NMR (see for example Lemercinier et al. (1996) Carbohydrate Research 296:83-96, Jones et al. (2002) J. Pharmaceutical and Biomedical Analysis 30:1233-1247, WO 2005/033148 and WO 00/56357). Another commonly used method is described in Hestrin (1949) J. Biol. Chem. 180:249-261. Preferably, the presence of O-acetyl groups is determined by ion-HPLC analysis.

The purified polysaccharides described herein are chemically activated to make the saccharides capable of reacting with the carrier protein. These pneumococcal conjugates are prepared by separate processes and formulated into a single dosage formulation as described below.

Petitioner Merck Ex-1015, 1281

1.3 Glycoconjugates of the invention

The purified saccharides are chemically activated to make the saccharides (i.e., activated saccharides) capable of reacting with the carrier protein, either directly or via a linker. Once activated, each capsular saccharide is separately conjugated to a carrier protein to form a glycoconjugate. In one embodiment, each capsular saccharide is conjugated to the same carrier protein. The chemical activation of the saccharides and subsequent conjugation to the carrier protein can be achieved by the activation and conjugation methods disclosed herein.

Capsular polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and/or 33F of *S. pneumoniae* are prepared as disclosed above.

In an embodiment, the polysaccharides are activated with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated polysaccharide is then coupled directly or via a spacer (linker) group to an amino group on the carrier protein (preferably CRM_{197}). For example, the spacer could be cystamine or cysteamine to give a thiolated polysaccharide which could be coupled to the carrier via a thioether linkage obtained after reaction with a maleimide-activated carrier protein (for example using N-[γ-maleimidobutyrloxy]succinimide ester (GMBS)) or a haloacetylated carrier protein (for example using iodoacetimide, N-succinimidyl bromoacetate (SBA; SIB), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-SIAB), N-succinimidyl iodoacetate (SIA), or succinimidyl 3-[bromoacetamido]proprionate (SBAP)). Preferably, the cyanate ester (optionally made by CDAP chemistry) is coupled with hexane diamine or adipic acid dihydrazide (ADH) and the amino-derivatised saccharide is conjugated to the carrier protein (e.g., CRM_{197}) using carbodiimide (e.g., EDAC or EDC) chemistry via a carboxyl group on the protein carrier. Such conjugates are described for example in WO 93/15760, WO 95/08348 and WO 96/129094.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and/or 33F are prepared using CDAP chemistry. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, and 23F are prepared using CDAP chemistry. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6A, 6B, 7F, 8, 9V, 14, 18C, 19F, and 23F are prepared using CDAP

Petitioner Merck Ex-1015, 1282

chemistry. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19A, 19F, and 23F are prepared using CDAP chemistry. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6A, 6B, 7F, 8, 9V, 14, 18C, 19A, 19F, and 23F are prepared using CDAP chemistry. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 14, 18C, 19A, 19F, and 23F are prepared using CDAP chemistry.

Other suitable techniques for conjugation use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S--NHS, EDC, TSTU. Many are described in International Patent Application Publication No. WO 98/42721. Conjugation may involve a carbonyl linker which may be formed by reaction of a free hydroxyl group of the saccharide with CDI (see Bethell et al. (1979) 1. Biol. Chern. 254:2572-2574; Hearn et al. (1981) J. Chromatogr. 218:509-518) followed by reaction with a protein to form a carbamate linkage. This may involve reduction of the anomeric terminus to a primary hydroxyl group, optional protection/deprotection of the primary hydroxyl group, reaction of the primary hydroxyl group with CDI to form a CDI carbamate intermediate and coupling the CDI carbamate intermediate with an amino group on a protein.

In an preferred embodiment, at least one of capsular polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F of *S. pneumoniae* is conjugated to the carrier protein by reductive amination (such as described in U.S. Patent Appl. Pub. Nos. 2006/0228380, 2007/184072, 2007/0231340 and 2007/0184071, WO 2006/110381, WO 2008/079653, and WO 2008/143709).

In an embodiment of the present invention, the glycoconjugate from *S. pneumoniae* serotype 6A is prepared by reductive amination. In an embodiment of the present invention, the glycoconjugate from *S. pneumoniae* serotype 19A is prepared by reductive amination. In an embodiment of the present invention, the glycoconjugate from *S. pneumoniae* serotype 3 is prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 6A and 19A are prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 3, 6A and 19A are prepared by reductive amination.

In a preferred embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F are prepared by reductive

Petitioner Merck Ex-1015, 1283

amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 6B, 9V, 14, 18C, 19F and 23F are prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F are prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F are prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F and 23F are prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F are prepared by reductive amination. In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F are all prepared by reductive amination.

In another preferred embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 23F are all prepared by reductive amination.

In another preferred embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F, 22F and 23F are all prepared by reductive amination.

In another preferred embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F and 23F are all prepared by reductive amination.

In another preferred embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F are all prepared by reductive amination.

In another preferred embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 12F, 14, 15C, 18C, 19A, 19F, 22F, 23F and 33F are all prepared by reductive amination.

In another preferred embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F are all prepared by reductive amination.

Reductive amination involves two steps, (1) oxidation of the polysaccharide, (2) reduction of the activated polysaccharide and a carrier protein to form a conjugate.

Petitioner Merck Ex-1015, 1284

Before oxidation, the polysaccharide is optionally hydrolyzed. Mechanical or chemical hydrolysis maybe employed. Chemical hydrolysis maybe conducted using acetic acid.

The oxidation step may involve reaction with periodate. For the purpose of the present invention, the term "periodate" includes both periodate and periodic acid; the term also includes both metaperiodate (IO_4^-) and orthoperiodate (IO_6^{5-}) and includes the various salts of periodate (e.g., sodium periodate and potassium periodate). In an embodiment the capsular polysaccharide is oxidized in the presence of metaperiodate, preferably in the presence of sodium periodate ($NaIO_4$). In another embodiment the capsular polysaccharide is oxydized in the presence of orthoperiodate, preferably in the presence of periodic acid.

In an embodiment, the oxidizing agent is a stable nitroxyl or nitroxide radical compound, such as piperidine-N-oxy or pyrrolidine-N-oxy compounds, in the presence of an oxidant to selectively oxidize primary hydroxyls (as described in WO 2014/097099). In said reaction, the actual oxidant is the N-oxoammonium salt, in a catalytic cycle. In an aspect, said stable nitroxyl or nitroxide radical compound are piperidine-N-oxy or pyrrolidine-N-oxy compounds. In an aspect, said stable nitroxyl or nitroxide radical compound bears a TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) or a PROXYL (2,2,5,5-tetramethyl-1-pyrrolidinyloxy) moiety. In an aspect, said stable nitroxyl radical compound is TEMPO or a derivative thereof. In an aspect, said oxidant is a molecule bearing a N-halo moiety. In an aspect, said oxidant is selected from the group consisting of N-ChloroSuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid and 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. Preferably said oxidant is N-Chlorosuccinimide.

In a preferred embodiment, capsular polysaccharides from serotypes 12F *S. pneumoniae* are conjugated to the carrier protein by reductive amination, wherein the oxidizing agent is 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO) free radical and N-Chlorosuccinimide (NCS) as the cooxidant (as described inWO 2014/097099). Therefore in one aspect, the glycoconjugates from *S. pneumoniae* serotype 12F are obtainable by a method comprising the steps of: a) reacting a 12F saccharide with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and N-chlorosuccinimide (NCS) in an aqueous solvent to produce an activated saccharide; and b) reacting the activated

Petitioner Merck Ex-1015, 1285

saccharide with a carrier protein comprising one or more amine groups (said method is designated "TEMPO/NCS-reductive amination" thereafter).

Optionally the oxidation reaction is quenched by addition of a quenching agent. The quenching agent maybe selected from vicinal diols, 1,2-aminoalcohols, amino acids, glutathione, sulfite, bisulfate, dithionite, metabisulfite, thiosulfate, phosphites, hypophosphites or phosphorous acid (such as glycerol, ethylene glycol, propan-1,2-diol, butan-1,2-diol or butan-2,3-diol, ascorbic acid).

Following the oxidation step of the polysaccharide, the polysaccharide is said to be activated and is referred to an "activated polysaccharide" here below. The activated polysaccharide and the carrier protein may be lyophilised (freeze-dried), either independently (discrete lyophilization) or together (co-lyophilized). In one embodiment the activated polysaccharide and the carrier protein are co-lyophilized. In another embodiment the activated polysaccharide and the carrier protein are lyophilized independently.

In one embodiment the lyophilization takes place in the presence of a non-reducing sugar, possible non-reducing sugars include sucrose, trehalose, raffinose, stachyose, melezitose, dextran, mannitol, lactitol and palatinit.

The second step of the conjugation process is the reduction of the activated polysaccharide and a carrier protein to form a conjugate (so-called reductive amination), using a reducing agent. Reducing agents which are suitable include the cyanoborohydrides (such as sodium cyanoborohydride, sodium triacetoxyborohydride or sodium or zinc borohydride in the presence of Bronsted or Lewis acids), amine boranes such as pyridine borane, 2-Picoline Borane, 2,6-diborane-methanol, dimethylamine-borane, t-BuMe^{i}PrN-BH_3, benzylamine-BH_3 or 5-ethyl-2-methylpyridine borane (PEMB) or borohydride exchange resin. In one embodiment the reducing agent is sodium cyanoborohydride.

In an embodiment, the reduction reaction is carried out in aqueous solvent (e.g., selected from PBS, MES, HEPES, Bis-tris, ADA, PIPES, MOPSO, BES, MOPS, DIPSO, MOBS, HEPPSO, POPSO, TEA, EPPS, Bicine or HEPB, at a pH between 6.0 and 8.5, 7.0 and 8.0, or 7.0 and 7.5), in another embodiment the reaction is carried out in aprotic solvent. In an embodiment, the reduction reaction is carried out in DMSO (dimethylsulfoxide) or in DMF (dimethylformamide) solvent. The DMSO or DMF solvent may be used to reconstitute the activated polysaccharide and carrier protein which has been lyophilized.

Petitioner Merck Ex-1015, 1286

At the end of the reduction reaction, there may be unreacted aldehyde groups remaining in the conjugates, these may be capped using a suitable capping agent. In one embodiment this capping agent

is sodium borohydride ($NaBH_4$). Following the conjugation (the reduction reaction and optionally the capping), the glycoconjugates may be purified (enriched with respect to the amount of polysaccharide-protein conjugate) by a variety of techniques known to the skilled person. These techniques include dialysis, concentration/diafiltration operations, tangential flow filtration precipitation/elution, column chromatography (DEAE or hydrophobic interaction chromatography), and depth filtration. In an embodiment, the glycoconjugates are purified by diafilitration or ion exchange chromatography or size exclusion chromatography.

In one embodiment the glycoconjugates are sterile filtered.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, and 23F are prepared using CDAP chemistry and the glycoconjugate from *S. pneumoniae* serotype 6A is prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, and 23F are prepared using CDAP chemistry and the glycoconjugate from *S. pneumoniae* serotype 19A is prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, and 23F are prepared using CDAP chemistry and the glycoconjugates from *S. pneumoniae* serotype 6A and 19A are prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, and 23F are prepared using CDAP chemistry and the glycoconjugates from *S. pneumoniae* serotype 3, 6A and 19A are prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, 22F and 23F are prepared using CDAP chemistry and the glycoconjugate from *S. pneumoniae* serotype 6A is prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, 22F, and 23F are prepared using CDAP

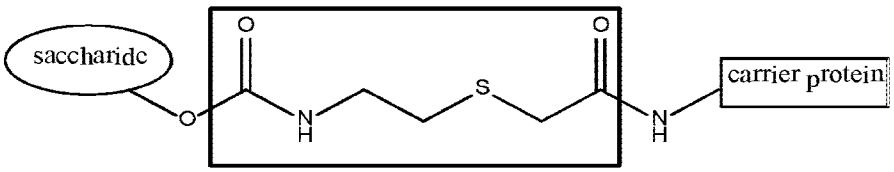
Petitioner Merck Ex-1015, 1287

chemistry and the glycoconjugate from *S. pneumoniae* serotype 19A is prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, 22F, and 23F are prepared using CDAP chemistry and the glycoconjugates from *S. pneumoniae* serotype 6A and 19A are prepared by reductive amination.

In an embodiment of the present invention, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 8, 9V, 14, 18C, 19F, 22F and 23F are prepared using CDAP chemistry and the glycoconjugates from *S. pneumoniae* serotype 3, 6A and 19A are prepared by reductive amination.

In an embodiment, the glycoconjugates of the invention are prepared using the eTEC conjugation, such as described in WO 2014/027302. Said glycoconjugates comprise a saccharide covalently conjugated to a carrier protein through one or more eTEC spacers, wherein the saccharide is covalently conjugated to the eTEC spacer through a carbamate linkage, and wherein the carrier protein is covalently conjugated to the eTEC spacer through an amide linkage. The eTEC linked glycoconjugates of the invention may be represented by the general formula (I):



(I),

where the atoms that comprise the eTEC spacer are contained in the central box.

The eTEC spacer includes seven linear atoms (i.e., $-C(O)NH(CH_2)_2SCH_2C(O)-$) and provides stable thioether and amide bonds between the saccharide and carrier protein. Synthesis of the eTEC linked glycoconjugate involves reaction of an activated hydroxyl group of the saccharide with the amino group of a thioalkylamine reagent, e.g., cystamine or cysteinamine or a salt thereof, forming a carbamate linkage to the saccharide to provide a thiolated saccharide. Generation of one or more free sulfhydryl groups is accomplished by reaction with a reducing agent to provide an activated thiolated saccharide. Reaction of the free sulfhydryl groups of the activated thiolated saccharide with an activated carrier protein having one or more α-haloacetamide groups on amine containing residues generates a thioether bond to form the conjugate, wherein the carrier protein is attached to the eTEC spacer through an amide bond.

In said glycoconjugates of the invention, the saccharide may be a polysaccharide or an oligosaccharide. The carrier protein may be selected from any suitable carrier as

Petitioner Merck Ex-1015, 1288

described herein or known to those of skill in the art. In frequent embodiments, the saccharide is a polysaccharide. In some such embodiments, the carrier protein is CRM_{197}. In some such embodiments, the eTEC linked glycoconjugate comprises a *S. pneumoniae* serotype 33F capsular polysaccharide.

In particularly preferred embodiments, the eTEC linked glycoconjugate comprises a pneumococcal serotype 33F (Pn33F) capsular polysaccharide, which is covalently conjugated to CRM_{197} through an eTEC spacer (serotype 33F eTEC linked glycoconjugates).

In some embodiments, the glycoconjugate from *S. pneumoniae* serotypes 1, 7F, 9V and/or 18C of the invention are O-acetylated. In some embodiments, the glycoconjugate from *S. pneumoniae* serotypes 1, 7F and 9V is O-acetylated and the glycoconjugate from *S. pneumoniae* serotype 18C is de-O-acetylated.

In some embodiments, the glycoconjugate from *S. pneumoniae* serotype 1 comprise a saccharide which has a degree of O-acetylation of between 10 and 100%, between 20 and 100%, between 30 and 100%, between 40 and 100%, between 50 and 100%, between 60 and 100%, between 70 and 100%, between 75 and 100%, 80 and 100%, 90 and 100%, 50 and 90%, 60 and 90%, 70 and 90% or 80 and 90%. In other embodiments, the degree of O-acetylation is ≥ 10%, ≥ 20%, ≥ 30%, ≥ 40%, ≥ 50%, ≥ 60%, ≥ 70%, ≥ 80%, ≥ 90%, or about 100%.

In some embodiments, the glycoconjugate from *S. pneumoniae* serotype 7F comprise a saccharide which has a degree of O-acetylation of between 10 and 100%, between 20 and 100%, between 30 and 100%, between 40 and 100%, between 50 and 100%, between 60 and 100%, between 70 and 100%, between 75 and 100%, 80 and 100%, 90 and 100%, 50 and 90%, 60 and 90%, 70 and 90% or 80 and 90%. In other embodiments, the degree of O-acetylation is ≥ 10%, ≥ 20%, ≥ 30%, ≥ 40%, ≥ 50%, ≥ 60%, ≥ 70%, ≥ 80%, ≥ 90%, or about 100%.

In some embodiments, the glycoconjugate from *S. pneumoniae* serotype 9V comprise a saccharide which has a degree of O-acetylation of between 10 and 100%, between 20 and 100%, between 30 and 100%, between 40 and 100%, between 50 and 100%, between 60 and 100%, between 70 and 100%, between 75 and 100%, 80 and 100%, 90 and 100%, 50 and 90%, 60 and 90%, 70 and 90% or 80 and 90%. In other embodiments, the degree of O-acetylation is ≥ 10%, ≥ 20%, ≥ 30%, ≥ 40%, ≥ 50%, ≥ 60%, ≥ 70%, ≥ 80%, ≥ 90%, or about 100%.

Petitioner Merck Ex-1015, 1289

In some embodiments, the glycoconjugate from *S. pneumoniae* serotype 18C comprise a saccharide which has a degree of O-acetylation of between 10 and 100%, between 20 and 100%, between 30 and 100%, between 40 and 100%, between 50 and 100%, between 60 and 100%, between 70 and 100%, between 75 and 100%, 80 and 100%, 90 and 100%, 50 and 90%, 60 and 90%, 70 and 90% or 80 and 90%. In other embodiments, the degree of O-acetylation is ≥ 10%, ≥ 20%, ≥ 30%, ≥ 40%, ≥ 50%, ≥ 60%, ≥ 70%, ≥ 80%, ≥ 90%, or about 100%. Preferably though, the glycoconjugate from *S. pneumoniae* serotype 18C is de-O-acetylated. In some said embodiments, the glycoconjugate from *S. pneumoniae* serotype 18C comprise a saccharide which has a degree of O-acetylation of between 0 and 50%, between 0 and 40%, between 0 and 30%, between 0 and 20%, between 0 and 10%, between 0 and 5%, or between 0 and 2%. In other embodiments, the degree of O-acetylation is ≤ 50%, ≤ 40%, ≤ 30%, ≤ 20%, ≤ 10%, ≤ 5%, ≤ 2%, or ≤ 1%.

By % of O-acetylation it is meant the percentage of a given saccharide relative to 100% (where each repeat unit is fully acetylated relative to its acetylated structure).

In some embodiments, the glycoconjugates of the present invention comprise a saccharide having a molecular weight of between 10 kDa and 2,000 kDa. In other such embodiments, the saccharide has a molecular weight of between 50 kDa and 1,000 kDa. In other such embodiments, the saccharide has a molecular weight of between 70 kDa and 900 kDa. In other such embodiments, the saccharide has a molecular weight of between 100 kDa and 800 kDa. In other such embodiments, the saccharide has a molecular weight of between 200 kDa and 600 kDa. In further such embodiments, the saccharide has a molecular weight of 100 kDa to 1000 kDa; 100 kDa to 900 kDa; 100 kDa to 800 kDa; 100 kDa to 700 kDa; 100 kDa to 600 kDa; 100 kDa to 500 kDa; 100 kDa to 400 kDa; 100 kDa to 300 kDa; 150 kDa to 1,000 kDa; 150 kDa to 900 kDa; 150 kDa to 800 kDa; 150 kDa to 700 kDa; 150 kDa to 600 kDa; 150 kDa to 500 kDa; 150 kDa to 400 kDa; 150 kDa to 300 kDa; 200 kDa to 1,000 kDa; 200 kDa to 900 kDa; 200 kDa to 800 kDa; 200 kDa to 700 kDa; 200 kDa to 600 kDa; 200 kDa to 500 kDa; 200 kDa to 400 kDa; 200 kDa to 300; 250 kDa to 1,000 kDa; 250 kDa to 900 kDa; 250 kDa to 800 kDa; 250 kDa to 700 kDa; 250 kDa to 600 kDa; 250 kDa to 500 kDa; 250 kDa to 400 kDa; 250 kDa to 350 kDa; 300 kDa to 1,000 kDa; 300 kDa to 900 kDa; 300 kDa to 800 kDa; 300 kDa to 700 kDa; 300 kDa to 600 kDa; 300 kDa to 500 kDa; 300 kDa to 400 kDa; 400 kDa to 1,000 kDa; 400 kDa to 900 kDa; 400 kDa to 800 kDa; 400 kDa to 700 kDa; 400 kDa to 600 kDa; 500 kDa to 600 kDa. Any whole number integer within

Petitioner Merck Ex-1015, 1290

any of the above ranges is contemplated as an embodiment of the disclosure. In some such embodiments, the glycoconjugate is prepared using reductive amination.

In some embodiments, the glycoconjugate of the invention has a molecular weight of between 400 kDa and 15,000 kDa; between 500 kDa and 10,000 kDa; between 2,000 kDa and 10,000 kDa; between 3,000 kDa and 8,000 kDa kDa; or between 3,000 kDa and 5,000 kDa. In other embodiments, the glycoconjugate has a molecular weight of between 500 kDa and 10,000 kDa. In other embodiments, glycoconjugate has a molecular weight of between 1,000 kDa and 8,000 kDa. In still other embodiments, the glycoconjugate has a molecular weight of between 2,000 kDa and 8,000 kDa or between 3,000 kDa and 7,000 kDa. In further embodiments, the glycoconjugate of the invention has a molecular weight of between 200 kDa and 20,000 kDa; between 200 kDa and 15,000 kDa; between 200 kDa and 10,000 kDa; between 200 kDa and 7,500 kDa; between 200 kDa and 5,000 kDa; between 200 kDa and 3,000 kDa; between 200 kDa and 1,000 kDa; between 500 kDa and 20,000 kDa; between 500 kDa and 15,000 kDa; between 500 kDa and 12,500 kDa; between 500 kDa and 10,000 kDa; between 500 kDa and 7,500 kDa; between 500 kDa and 6,000 kDa; between 500 kDa and 5,000 kDa; between 500 kDa and 4,000 kDa; between 500 kDa and 3,000 kDa; between 500 kDa and 2,000 kDa; between 500 kDa and 1,500 kDa; between 500 kDa and 1,000 kDa; between 750 kDa and 20,000 kDa; between 750 kDa and 15,000 kDa; between 750kDa and 12,500 kDa; between 750kDa and 10,000 kDa; between 750kDa and 7,500 kDa; between 750 kDa and 6,000 kDa; between 750 kDa and 5,000 kDa; between 750 kDa and 4,000 kDa; between 750 kDa and 3,000 kDa; between 750 kDa and 2,000 kDa; between 750 kDa and 1,500 kDa; between 1,000 kDa and 15,000 kDa; between 1,000 kDa and 12,500 kDa; between 1,000 kDa and 10,000 kDa; between 1,000 kDa and 7,500 kDa; between 1,000 kDa and 6,000 kDa; between 1,000 kDa and 5,000 kDa; between 1,000 kDa and 4,000 kDa; between 1,000 kDa and 2,500 kDa; between 2,000 kDa and 15,000 kDa; between 2,000 kDa and 12,500 kDa; between 2,000 kDa and 10,000 kDa; between 2,000 kDa and 7,500 kDa; between 2,000 kDa and 6,000 kDa; between 2,000 kDa and 5,000 kDa; between 2,000 kDa and 4,000 kDa; or between 2,000 kDa and 3,000 kDa.

In further embodiments, the glycoconjugate of the invention has a molecular weight of between 3,000 kDa and 20,000 kDa; between 3,000 kDa and 15,000 kDa; between 3,000 kDa and 10,000 kDa; between 3,000 kDa and 7,500 kDa; between 3,000 kDa and 5,000 kDa; between 4,000 kDa and 20,000 kDa; between 4,000 kDa and 15,000 kDa;

Petitioner Merck Ex-1015, 1291

between 4,000 kDa and 12,500 kDa; between 4,000 kDa and 10,000 kDa; between 4,000 kDa and 7,500 kDa; between 4,000 kDa and 6,000 kDa; or between 4,000 kDa and 5,000 kDa.

In further embodiments, the glycoconjugate of the invention has a molecular weight of between 5,000 kDa and 20,000 kDa; between 5,000 kDa and 15,000 kDa; between 5,000 kDa and 10,000 kDa; between 5,000 kDa and 7,500 kDa; between 6,000 kDa and 20,000 kDa; between 6,000 kDa and 15,000 kDa; between 6,000 kDa and 12,500 kDa; between 6,000 kDa and 10,000 kDa or between 6,000 kDa and 7,500 kDa.

The molecular weight of the glycoconjugate is measured by SEC-MALLS. Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

In a preferred embodiment, the serotype 22F glycoconjugate of the invention comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 or 0.7 or about 0.8 mM acetate per mM serotype 22F polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.5, 0.6 or 0.7 mM acetate per mM serotype 22F polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.6 mM acetate per mM serotype 22F polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.7 mM acetate per mM serotype 22F polysaccharide.

In a preferred embodiment, the serotype 33F glycoconjugate of the invention comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 or 0.8 mM acetate per mM serotype 33F capsular polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.5, 0.6 or 0.7 mM acetate per mM serotype 33F capsular polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.6 mM acetate per mM serotype 33F capsular polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.7 mM acetate per mM serotype 33F capsular polysaccharide. In a preferred embodiment, the presence of O-acetyl groups is determined by ion-HPLC analysis.

In a preferred embodiment, the serotype 15B glycoconjugate of the invention comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 or 0.8 mM acetate per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.5, 0.6 or 0.7 mM acetate per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.6 mM acetate per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the glycoconjugate comprises at least 0.7 mM acetate per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the presence of O-acetyl groups is determined by ion-HPLC analysis.

Petitioner Merck Ex-1015, 1292

In a preferred embodiment, the serotype 15B glycoconjugate of the invention comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 or 0.8 mM glycerol per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the serotype 15B glycoconjugate of the invention comprises at least 0.5, 0.6 or 0.7 mM glycerol per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the serotype 15B glycoconjugate of the invention comprises at least 0.6 mM glycerol per mM serotype 15B capsular polysaccharide. In a preferred embodiment, the serotype 15B glycoconjugate of the invention comprises at least 0.7 mM glycerol per mM serotype 15B capsular polysaccharide.

In a preferred embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.3, 0.5, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, 3.8, 4.2, 4.6 or about 5.0 mM acetate per mM serotype 11A polysaccharide. In a preferred embodiment, the serotype 11A glycoconjugate comprises at least 1.8, 2.2 or 2.6 mM acetate per mM serotype 11A polysaccharide. In an embodiment, the glycoconjugate comprises at least 0.6 mM acetate per mM serotype 11A polysaccharide. In a preferred embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, 3.8, 4.2 or about 4.6 mM acetate per mM serotype 11A polysaccharide and less than about 5.0 mM acetate per mM serotype 11A polysaccharide. In an embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, or about 3.0 mM acetate per mM serotype 11A polysaccharide and less than about 3.4 mM acetate per mM serotype 11A polysaccharide. In an embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, or about 3.0 mM acetate per mM serotype 11A polysaccharide and less than about 3.3 mM acetate per mM serotype 11A polysaccharide. Any of the above number is contemplated as an embodiment of the disclosure.

In a preferred embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or about 1.0 mM glycerol per mM serotype 11A polysaccharide. In a preferred embodiment, the serotype 11A glycoconjugate comprises at least 0.2, 0.3 or 0.4 mM glycerol per mM serotype 11A polysaccharide. In a preferred embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 or about 0.9 mM glycerol per mM serotype 11A polysaccharide and less than about 1.0 mM glycerol per mM serotype 11A polysaccharide. In a preferred embodiment, the serotype 11A glycoconjugate of the invention comprises at least 0.3, 0.4, 0.5, 0.6, or about 0.7 mM

Petitioner Merck Ex-1015, 1293

glycerol per mM serotype 11A polysaccharide and less than about 0.8 mM glycerol per mM serotype 11A polysaccharide. Any of the above number is contemplated as an embodiment of the disclosure.

Another way to characterize the glycoconjugates of the invention is by the number of lysine residues in the carrier protein (e.g., CRM_{197}) that become conjugated to the saccharide which can be characterized as a range of conjugated lysines (degree of conjugation). The evidence for lysine modification of the carrier protein, due to covalent linkages to the polysaccharides, can be obtained by amino acid analysis using routine methods known to those of skill in the art. Conjugation results in a reduction in the number of lysine residues recovered, compared to the carrier protein starting material used to generate the conjugate materials. In a preferred embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 15, between 2 and 13, between 2 and 10, between 2 and 8, between 2 and 6, between 2 and 5, between 2 and 4, between 3 and 15, between 3 and 13, between 3 and 10, between 3 and 8, between 3 and 6, between 3 and 5, between 3 and 4, between 5 and 15, between 5 and 10, between 8 and 15, between 8 and 12, between 10 and 15 or between 10 and 12. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14 or about 15. In a preferred embodiment, the degree of conjugation of the glycoconjugate of the invention is between 4 and 7. In some such embodiments, the carrier protein is CRM_{197}.

The glycoconjugates of the invention may also be characterized by the ratio (weight/weight) of saccharide to carrier protein. In some embodiments, the ratio of polysaccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3 (e.g., about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, or about 3.0). In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 2.0, between 0.5 and 1.5, between 0.8 and 1.2, between 0.5 and 1.0, between 1.0 and 1.5 or between 1.0 and 2.0. In further embodiments, the saccharide to carrier protein ratio (w/w) is between 0.8 and 1.2. In a preferred embodiment, the ratio of capsular polysaccharide to carrier protein in the conjugate is between 0.9 and 1.1. In some such embodiments, the carrier protein is CRM_{197}.

Petitioner Merck Ex-1015, 1294

The glycoconjugates and immunogenic compositions of the invention may contain free saccharide that is not covalently conjugated to the carrier protein, but is nevertheless present in the glycoconjugate composition. The free saccharide may be non-covalently associated with (i.e., non-covalently bound to, adsorbed to, or entrapped in or with) the glycoconjugate.

In a preferred embodiment, the glycoconjugate comprises less than about 50%, 45%, 40%, 35%, 30%, 25%, 20% or 15% of free polysaccharide compared to the total amount of polysaccharide. In a preferred embodiment the glycoconjugate comprises less than about 25% of free polysaccharide compared to the total amount of polysaccharide. In a preferred embodiment the glycoconjugate comprises less than about 20% of free polysaccharide compared to the total amount of polysaccharide. In a preferred embodiment the glycoconjugate comprises less than about 15% of free polysaccharide compared to the total amount of polysaccharide.

The glycoconjugates may also be characterized by their molecular size distribution (K_d). Size exclusion chromatography media (CL-4B) can be used to determine the relative molecular size distribution of the conjugate. Size Exclusion Chromatography (SEC) is used in gravity fed columns to profile the molecular size distribution of conjugates. Large molecules excluded from the pores in the media elute more quickly than small molecules. Fraction collectors are used to collect the column eluate. The fractions are tested colorimetrically by saccharide assay. For the determination of K_d, columns are calibrated to establish the fraction at which molecules are fully excluded (V_0), (K_d=0), and the fraction representing the maximum retention (V_i), (K_d=1). The fraction at which a specified sample attribute is reached (V_e), is related to K_d by the expression, $K_d = (V_e - V_0)/ (V_i - V_0)$.

In a preferred embodiment, at least 30% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 40% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 60% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, between 50% and 80% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, between 65% and 80% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

Petitioner Merck Ex-1015, 1295

The frequency of attachment of the saccharide chain to a lysine on the carrier protein is another parameter for characterizing the glycoconjugates of the invention. For example, in some embodiments, at least one covalent linkage between the carrier protein and the polysaccharide occurs for every 4 saccharide repeat units of the polysaccharide. In another embodiment, the covalent linkage between the carrier protein and the polysaccharide occurs at least once in every 10 saccharide repeat units of the polysaccharide. In another embodiment, the covalent linkage between the carrier protein and the polysaccharide occurs at least once in every 15 saccharide repeat units of the polysaccharide. In a further embodiment, the covalent linkage between the carrier protein and the polysaccharide occurs at least once in every 25 saccharide repeat units of the polysaccharide.

In frequent embodiments, the carrier protein is CRM_{197} and the covalent linkage via an eTEC spacer between the CRM_{197} and the polysaccharide occurs at least once in every 4, 10, 15 or 25 saccharide repeat units of the polysaccharide.

In other embodiments, the conjugate comprises at least one covalent linkage between the carrier protein and saccharide for every 5 to 10 saccharide repeat units; every 2 to 7 saccharide repeat units; every 3 to 8 saccharide repeat units; every 4 to 9 saccharide repeat units; every 6 to 11 saccharide repeat units; every 7 to 12 saccharide repeat units; every 8 to 13 saccharide repeat units; every 9 to 14 saccharide repeat units; every 10 to 15 saccharide repeat units; every 2 to 6 saccharide repeat units, every 3 to 7 saccharide repeat units; every 4 to 8 saccharide repeat units; every 6 to 10 saccharide repeat units; every 7 to 11 saccharide repeat units; every 8 to 12 saccharide repeat units; every 9 to 13 saccharide repeat units; every 10 to 14 saccharide repeat units; every 10 to 20 saccharide repeat units; every 4 to 25 saccharide repeat units or every 2 to 25 saccharide repeat units. In frequent embodiments, the carrier protein is CRM_{197}.

In another embodiment, at least one linkage between carrier protein and saccharide occurs for every 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 saccharide repeat units of the polysaccharide. In an embodiment, the carrier protein is CRM_{197}. Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

Petitioner Merck Ex-1015, 1296

1.4 Combination of glycoconjugates of the invention

In an embodiment the immunogenic composition of the invention comprises any of the glycoconjugates disclosed herein.

1.4.1 Combinations of glycoconjugates

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate from *S. pneumoniae* serotype 9V.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the two following *S. pneumoniae* serotypes: 9V and 4, 9V and 6B, 9V and 14, 9V and 18C, 9V and 19F or 9V and 23F.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the seven following *S. pneumoniae* serotypes: 9V, 4, 6B, 14, 18C, 19F and 23F.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the eight following *S. pneumoniae* serotypes: 9V, 1, 4, 6B, 14, 18C, 19F, and 23F; 9V, 4, 5, 6B, 14, 18C, 19F, and 23F; 9V, 4, 6B 7F, 14, 18C, 19F, and 23F.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the ten following *S. pneumoniae* serotypes: 9V, 1, 5, 4, 6B, 7F, 14, 18C, 19F and 23F.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the eleven following *S. pneumoniae* serotypes: 9V, 1, 4, 5, 6A, 6B, 7F, 14, 18C, 19F, and 23F; 9V, 1, 4, 5, 6B, 7F, 14, 18C, 19A, 19F, and 23F.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the twelve following *S. pneumoniae* serotypes: 9V, 1, 4, 5, 6A, 6B, 7F, 14, 18C, 19A, 19F, and 23F.

In an embodiment the immunogenic composition of the invention comprises at least one glycoconjugate of each of the thirteen following *S. pneumoniae* serotypes: 9V, 1, 3, 4, 5, 6A, 6B, 7F, 14, 18C, 19A, 19F, and 23F.

1.4.2 Additional combinations of glycoconjugates

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 15B.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 22F.

Petitioner Merck Ex-1015, 1297

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 33F.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 8.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 10A.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 11A.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of *S. pneumoniae* serotype 12F.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of each of the two following *S. pneumoniae* serotypes:

15B and 22F,
15B and 33F,
15B and 12F,
15B and 10A,
15B and 11A,
15B and 8,
22F and 33F,
22F and 12F,
22F and 10A,
22F and 11A,
22F and 8,
33F and 12F,
33F and 10A,
33F and 11A,
33F and 8,
12F and 10A,
12F and 11A,
12F and 8,
10A and 11A,
10A and 8, or
11A and 8.

Petitioner Merck Ex-1015, 1298

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of each of the three following *S. pneumoniae* serotypes:

15B and 22F and 33F,
15B and 22F and 12F,
15B and 22F and 10A,
15B and 22F and 11A,
15B and 22F and 8,
15B and 33F and 12F,
15B and 33F and 10A,
15B and 33F and 11A,
15B and 33F and 8,
15B and 12F and 10A,
15B and 12F and 11A,
15B and 12F and 8,
15B and 10A and 11A,
15B and 10A and 8,
15B and 11A and 8,
22F and 33F and 12F,
22F and 33F and 10A,
22F and 33F and 11A,
22F and 33F and 8,
22F and 12F and 10A,
22F and 12F and 11A,
22F and 12F and 8,
22F and 10A and 11A,
22F and 10A and 8,
22F and 11A and 8,
33F and 12F and 10A,
33F and 12F and 11A,
33F and 12F and 8,
33F and 10A and 11A,
33F and 10A and 8,
33F and 11A and 8,

Petitioner Merck Ex-1015, 1299

12F and 10A and 11A,
12F and 10A and 8,
12F and 11A and 8, or
10A and 11A and 8.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of each of the four following *S. pneumoniae* serotypes:

15B and 22F and 33F and 12F,
15B and 22F and 33F and 10A,
15B and 22F and 33F and 11A,
15B and 22F and 33F and 8,
15B and 22F and 12F and 10A,
15B and 22F and 12F and 11A,
15B and 22F and 12F and 8,
15B and 22F and 10A and 11A,
15B and 22F and 10A and 8,
15B and 22F and 11A and 8,
15B and 33F and 12F and 10A,
15B and 33F and 12F and 11A,
15B and 33F and 12F and 8,
15B and 33F and 10A and 11A,
15B and 33F and 10A and 8,
15B and 33F and 11A and 8,
15B and 12F and 10A and 11A,
15B and 12F and 10A and 8,
15B and 12F and 11A and 8,
15B and 10A and 11A and 8,
22F and 33F and 12F and 10A,
22F and 33F and 12F and 11A,
22F and 33F and 12F and 8,
22F and 33F and 10A and 11A,
22F and 33F and 10A and 8,
22F and 33F and 11A and 8,
22F and 12F and 10A and 11A,

Petitioner Merck Ex-1015, 1300

22F and 12F and 10A and 8,
22F and 12F and 11A and 8,
22F and 10A and 11A and 8,
33F and 12F and 10A and 11A,
33F and 12F and 10A and 8,
33F and 12F and 11A and 8,
33F and 10A and 11A and 8 or
12F and 10A and 11A and 8.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of each of the five following *S. pneumoniae* serotypes:

15B and 22F and 33F and 12F and 10A,
15B and 22F and 33F and 12F and 11A,
15B and 22F and 33F and 12F and 8,
15B and 22F and 33F and 10A and 11A,
15B and 22F and 33F and 10A and 8,
15B and 22F and 33F and 11A and 8,
15B and 22F and 12F and 10A and 11A,
15B and 22F and 12F and 10A and 8,
15B and 22F and 12F and 11A and 8,
15B and 22F and 10A and 11A and 8,
15B and 33F and 12F and 10A and 11A,
15B and 33F and 12F and 10A and 8,
15B and 33F and 12F and 11A and 8,
15B and 33F and 10A and 11A and 8,
15B and 12F and 10A and 11A and 8,
22F and 33F and 12F and 10A and 11A,
22F and 33F and 12F and 10A and 8,
22F and 33F and 12F and 11A and 8,
22F and 33F and 10A and 11A and 8,
22F and 12F and 10A and 11A and 8 or
33F and 12F and 10A and 11A and 8.

Petitioner Merck Ex-1015, 1301

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of each of the six following *S. pneumoniae* serotypes:

15B and 22F and 33F and 12F and 10A and 11A,

15B and 22F and 33F and 12F and 10A and 8,

15B and 22F and 33F and 12F and 11A and 8,

15B and 22F and 33F and 10A and 11A and 8,

15B and 22F and 12F and 10A and 11A and 8,

15B and 33F and 12F and 10A and 11A and 8 or

22F and 33F and 12F and 10A and 11A and 8.

In an embodiment any of the immunogenic composition defined at 1.4.1 above comprises in addition at least one glycoconjugate of each of the seven following *S. pneumoniae* serotypes: 15B and 22F and 33F and 12F and 10A and 11A and 8.

In an embodiment any of the immunogenic composition above comprises in addition glycoconjugates from *S. pneumoniae* serotype 2.

In an embodiment any of the immunogenic composition above comprises in addition glycoconjugates from *S. pneumoniae* serotype 17F.

In an embodiment any of the immunogenic composition above comprises in addition glycoconjugates from *S. pneumoniae* serotype 20.

In an embodiment any of the immunogenic composition above comprises in addition glycoconjugates from *S. pneumoniae* serotype 15C.

Preferably, all the glycoconjugates of the above immunogenic composition are individually conjugated to the carrier protein.

In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 9V is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotype 22F is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 33F is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 15B is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 12F is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 10A is conjugated to CRM_{197}. In an embodiment of any of the

Petitioner Merck Ex-1015, 1302

above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 11A is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 8 is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 14, 18C, 19F and 23F are conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotypes 1, 5 and 7F are conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotypes 6A and 19A are conjugated to CRM_{197}. In an embodiment of any of the above immunogenic composition, the glycoconjugates from *S. pneumoniae* serotype 3 is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotype 2 is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotype 17F is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotype 20 is conjugated to CRM_{197}. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotype 15C is conjugated to CRM_{197}.

In an embodiment, the glycoconjugates of the above immunogenic compositions are all individually conjugated to CRM_{197}.

In an embodiment, the glycoconjugate from *S. pneumoniae* serotype 9V of any of the above immunogenic composition is individually conjugated to PD.

In an embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14 and/or 23F of any of the above immunogenic compositions are individually conjugated to PD.

In an embodiment, the glycoconjugate from *S. pneumoniae* serotype 18C of any of the above immunogenic compositions is conjugated to TT.

In an embodiment, the glycoconjugate from *S. pneumoniae* serotype 19F of any of the above immunogenic compositions is conjugated to DT.

In an embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14 and/or 23F of any of the above immunogenic compositions are individually conjugated to PD, the glycoconjugate from *S. pneumoniae* serotype 18C is conjugated to TT and the glycoconjugate from *S. pneumoniae* serotype 19F is conjugated to DT.

Petitioner Merck Ex-1015, 1303

In an embodiment the above immunogenic composition comprises from 7 to 24 different serotypes of *S. pneumoniae*. In one embodiment the above immunogenic composition comprises glycoconjugates from 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 different serotypes.

In an embodiment the above immunogenic composition comprises from 7 to 20 different serotypes of *S. pneumoniae*. In one embodiment the above immunogenic composition comprises glycoconjugates from 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 different serotypes. In one embodiment the above immunogenic composition comprises glycoconjugates from 16 or 20 different serotypes.

In an embodiment the above immunogenic composition is a 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20-valent pneumococcal conjugate composition. In an embodiment the above immunogenic composition is a 14, 15, 16, 17, 18 or 19 valent pneumococcal conjugate composition. In an embodiment the above immunogenic composition is a 16-valent pneumococcal conjugate composition. In an embodiment the above immunogenic composition is a 19-valent pneumococcal conjugate composition. In an embodiment the above immunogenic composition is a 20-valent pneumococcal conjugate composition.

In an embodiment, the immunogenic composition of the invention comprises glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F.

In an embodiment, the immunogenic composition of the invention comprises glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F.

In an embodiment, the immunogenic composition of the invention comprises conjugated *S. pneumoniae* saccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F.

In an embodiment, the immunogenic composition of the invention comprises conjugated *S. pneumoniae* saccharides from serotypes 1, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F.

In an embodiment, the glycoconjugates of the immunogenic composition of the invention consists of glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment, the glycoconjugates of the immunogenic composition of the invention consists of glycoconjugates from serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F, 22F,

Petitioner Merck Ex-1015, 1304

23F and 33F. In an embodiment, the glycoconjugates of the immunogenic composition of the invention consists of glycoconjugates from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment, the glycoconjugates of the immunogenic composition of the invention consists of glycoconjugates from 1, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F.

Preferably, all the glycoconjugates of the immunogenic composition of the invention are individually conjugated to the carrier protein. In an embodiment, the glycoconjugates of the immunogenic composition above are individually conjugated to CRM_{197}.

1.4.3 Further combinations of glycoconjugates

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotype 9N.

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotype 9A.

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotype 9L.

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotypes 9N and 9A.

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotypes 9N and 9L.

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotypes 9A and 9L.

In an embodiment any of the immunogenic compositions defined at 1.4.1 or 1.4.2 above do not comprise capsular saccharide from *S. pneumoniae* serotypes 9N, 9A and 9L.

After conjugation of the capsular polysaccharide to the carrier protein, the glycocopnjugates are purified (enriched with respect to the amount of polysaccharide-protein conjugate) by a variety of techniques. These techniques include concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration. See, e.g., U.S. Appl. Publication No. 2007/0184072 and WO 2008/079653. After the individual glycoconjugates are purified, they are compounded to formulate the immunogenic composition of the present invention.

Petitioner Merck Ex-1015, 1305

2 Dosage of the immunogenic compositions

2.1 Polysaccharide amount

The amount of glycoconjugate(s) in each dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented.

The amount of a particular glycoconjugate in an immunogenic composition can be calculated based on total polysaccharide for that conjugate (conjugated and non-conjugated). For example, a glycoconjugate with 20% free polysaccharide will have about 80 µg of conjugated polysaccharide and about 20 µg of non-conjugated polysaccharide in a 100 µg polysaccharide dose.The amount of glycoconjugate can vary depending upon the streptococcal serotype. The saccharide concentration can be determined by the uronic acid assay.

The "immunogenic amount" of the different polysaccharide components in the immunogenic composition, may diverge and each may comprise about 1.0 µg, about 2.0 µg, about 3.0 µg, about 4.0 µg, about 5.0 µg, about 6.0 µg, about 7.0 µg, about 8.0 µg, about 9.0 µg, about 10.0 µg, about 15.0 µg, about 20.0 µg, about 30.0 µg, about 40.0 µg, about 50.0 µg, about 60.0 µg, about 70.0 µg, about 80.0 µg, about 90.0 µg, or about 100.0 µg of any particular polysaccharide antigen.

Generally, each dose will comprise 0.1 µg to 100 µg of polysaccharide for a given serotype, particularly 0.5 µg to 20 µg, more particularly 1 µg to 10 µg, and even more particularly 2 µg to 5 µg. Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

In an embodiment, each dose will comprise 1 µg, 2 µg, 3 µg, 4 µg, 5 µg, 6 µg, 7 µg, 8 µg, 9 µg, 10 µg, 15 µg or 20 µg of polysaccharide for a given serotype.

2.2 Carrier amount

Generally, each dose will comprise 5 µg to 150 µg of carrier protein, particularly 10 µg to 100 µg of carrier protein, more particularly 15 µg to 100 µg of carrier protein, more particularly 25 to 75 µg of carrier protein, more particularly 30 µg to 70 µg of carrier protein, more particularly 30 to 60 µg of carrier protein, more particularly 30 µg to 50 µg of carrier protein and even more particularly 40 to 60 µg of carrier protein. In an embodiment, said carrier protein is CRM_{197}.

Petitioner Merck Ex-1015, 1306

In an embodiment, each dose will comprise about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg or about 75 µg of carrier protein. In an embodiment, said carrier protein is CRM_{197}.

3 Further antigens

Immunogenic compositions of the invention comprise conjugated *S. pneumoniae* saccharide antigens (glycoconjugates). They may also further include antigens from other pathogens, particularly from bacteria and/or viruses. Preferred further antigens are selected from: a diphtheria toxoid (D), a tetanus toxoid (T), a pertussis antigen (P), which is typically acellular (Pa), a hepatitis B virus (HBV) surface antigen (HBsAg), a hepatitis A virus (HAV) antigen, a conjugated *Haemophilus influenzae* type b capsular saccharide (Hib), inactivated poliovirus vaccine (IPV).

In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa. In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa-Hib, D-T-Pa-IPV or D-T-Pa-HBsAg. In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa-HBsAg-IPV or D-T-Pa-HBsAg-Hib. In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa-HBsAg-IPV-Hib.

Pertussis antigens: *Bordetella pertussis* causes whooping cough. Pertussis antigens in vaccines are either cellular (whole cell, in the form of inactivated B.pertussis cells) or acellular. Preparation of cellular pertussis antigens is well documented (e.g., it may be obtained by heat inactivation of phase I culture of *B. pertussis*). Preferably, however, the invention uses acellular antigens. Where acellular antigens are used, it is preferred to use one, two or (preferably) three of the following antigens: (1) detoxified pertussis toxin (pertussis toxoid, or PT); (2) filamentous hemagglutinin (FHA); (3) pertactin (also known as the 69 kiloDalton outer membrane protein). FHA and pertactin may be treated with formaldehyde prior to use according to the invention. PT is preferably detoxified by treatment with formaldehyde and/or glutaraldehyde. Acellular pertussis antigens are

Petitioner Merck Ex-1015, 1307

preferably adsorbed onto one or more aluminum salt adjuvants. As an alternative, they may be added in an unadsorbed state. Where pertactin is added then it is preferably already adsorbed onto an aluminum hydroxide adjuvant. PT and FHA may be adsorbed onto an aluminum hydroxide adjuvant or an aluminum phosphate. Adsorption of all of PT, FHA and pertactin to aluminum hydroxide is most preferred.

Inactivated poliovirus vaccine: Poliovirus causes poliomyelitis. Rather than use oral poliovirus vaccine, preferred embodiments of the invention use IPV. Prior to administration to patients, polioviruses must be inactivated, and this can be achieved by treatment with formaldehyde. Poliomyelitis can be caused by one of three types of poliovirus. The three types are similar and cause identical symptoms, but they are antigenically different and infection by one type does not protect against infection by others. It is therefore preferred to use three poliovirus antigens in the invention: poliovirus Type 1 (e.g., Mahoney strain), poliovirus Type 2 (e.g., MEF-1 strain), and poliovirus Type 3 (e.g., Saukett strain). The viruses are preferably grown, purified and inactivated individually, and are then combined to give a bulk trivalent mixture for use with the invention.

Diphtheria toxoid: *Corynebacterium diphtheriae* causes diphtheria. Diphtheria toxin can be treated (e.g., using formalin or formaldehyde) to remove toxicity while retaining the ability to induce specific anti-toxin antibodies after injection. These diphtheria toxoids are used in diphtheria vaccines. Preferred diphtheria toxoids are those prepared by formaldehyde treatment. The diphtheria toxoid can be obtained by growing *C.diphtheriae* in growth medium, followed by formaldehyde treatment, ultrafiltration and precipitation. The toxoided material may then be treated by a process comprising sterile filtration and/or dialysis. The diphtheria toxoid is preferably adsorbed onto an aluminum hydroxide adjuvant.

Tetanus toxoid: *Clostridium tetani* causes tetanus. Tetanus toxin can be treated to give a protective toxoid. The toxoids are used in tetanus vaccines. Preferred tetanus toxoids are those prepared by formaldehyde treatment. The tetanus toxoid can be obtained by growing *C. tetani* in growth medium, followed by formaldehyde treatment, ultrafiltration and precipitation. The material may then be treated by a process comprising sterile filtration and/or dialysis.

Hepatitis A virus antigens: Hepatitis A virus (HAV) is one of the known agents which causes viral hepatitis. A preferred HAV component is based on inactivated virus, and inactivation can be achieved by formalin treatment.

Petitioner Merck Ex-1015, 1308

Hepatitis B virus (HBV) is one of the known agents which causes viral hepatitis. The major component of the capsid is a protein known as HBV surface antigen or, more commonly, HBsAg, which is typically a 226-amino acid polypeptide with a molecular weight of ~24 kDa. All existing hepatitis B vaccines contain HBsAg, and when this antigen is administered to a normal vaccinee it stimulates the production of anti-HBsAg antibodies which protect against HBV infection.

For vaccine manufacture, HBsAg has been made in two ways: purification of the antigen in particulate form from the plasma of chronic hepatitis B carriers or expression of the protein by recombinant DNA methods (e.g., recombinant expression in yeast cells). Unlike native HBsAg (i.e., as in the plasma-purified product), yeast-expressed HBsAg is generally non-glycosylated, and this is the most preferred form of HBsAg for use with the invention.

Conjugated *Haemophilus influenzae* type b antigens: Haemophilus influenzae type b (Hib) causes bacterial meningitis. Hib vaccines are typically based on the capsular saccharide antigen, the preparation of which is well documented. The Hib saccharide can be conjugated to a carrier protein in order to enhance its immunogenicity, especially in children. Typical carrier proteins are tetanus toxoid, diphtheria toxoid, CRM197, *H.influenzae* protein D, and an outer membrane protein complex from serogroup B meningococcus. The saccharide moiety of the conjugate may comprise full-length polyribosylribitol phosphate (PRP) as prepared from Hib bacteria, and/or fragments of full-length PRP. Hib conjugates may or may not be adsorbed to an aluminum salt adjuvant.

In an embodiment the immunogenic compositions of the invention further include a conjugated N.meningitidis serogroup Y capsular saccharide (MenY), and/or a conjugated *N. meningitidis* serogroup C capsular saccharide (MenC).

In an embodiment the immunogenic compositions of the invention further include a conjugated *N. meningitidis* serogroup A capsular saccharide (MenA), a conjugated N. meningitidis serogroup W135 capsular saccharide (MenW135), a conjugated *N. meningitidis* serogroup Y capsular saccharide (MenY), and/or a conjugated *N. meningitidis* serogroup C capsular saccharide (MenC).

In an embodiment the immunogenic compositions of the invention further include a conjugated *N. meningitidis* serogroup W135 capsular saccharide (MenW135), a conjugated *N. meningitidis* serogroup Y capsular saccharide (MenY), and/or a conjugated *N. meningitidis* serogroup C capsular saccharide (MenC).

Petitioner Merck Ex-1015, 1309

4 Adjuvant(s)

In some embodiments, the immunogenic compositions disclosed herein may further comprise at least one adjuvant (e.g., one, two or three adjuvants). The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. Antigens may act primarily as a delivery system, primarily as an immune modulator or have strong features of both. Suitable adjuvants include those suitable for use in mammals, including humans.

Examples of known suitable delivery-system type adjuvants that can be used in humans include, but are not limited to, alum (e.g., aluminum phosphate, aluminum sulfate or aluminum hydroxide), calcium phosphate, liposomes, oil-in-water emulsions such as MF59 (4.3% w/v squalene, 0.5% w/v polysorbate 80 (Tween 80), 0.5% w/v sorbitan trioleate (Span 85)), water-in-oil emulsions such as Montanide, and poly(D,L-lactide-co-glycolide) (PLG) microparticles or nanoparticles.

In an embodiment, the immunogenic compositions disclosed herein comprise aluminum salts (alum) as adjuvant (e.g., aluminum phosphate, aluminum sulfate or aluminum hydroxide). In a preferred embodiment, the immunogenic compositions disclosed herein comprise aluminum phosphate or aluminum hydroxide as adjuvant. In an embodiment, the immunogenic compositions disclosed herein comprise from 0.1 mg/mL to 1 mg/mL or from 0.2 mg/mL to 0.3 mg/ml of elemental aluminum in the form of aluminum phosphate. In an embodiment, the immunogenic compositions disclosed herein comprise about 0.25 mg/mL of elemental aluminum in the form of aluminum phosphate.

Examples of known suitable immune modulatory type adjuvants that can be used in humans include, but are not limited to, saponin extracts from the bark of the Aquilla tree (QS21, Quil A), TLR4 agonists such as MPL (Monophosphoryl Lipid A), 3DMPL (3-O-deacylated MPL) or GLA-AQ, LT/CT mutants, cytokines such as the various interleukins (e.g., IL-2, IL-12) or GM-CSF, and the like.

Examples of known suitable immune modulatory type adjuvants with both delivery and immune modulatory features that can be used in humans include, but are not limited to ISCOMS (see, e.g., Sjölander et al. (1998) J. Leukocyte Biol. 64:713; WO 90/03184, WO 96/11711, WO 00/48630, WO 98/36772, WO 00/41720, WO 2006/134423 and WO 2007/026190) or GLA-EM which is a combination of a TLR4 agonist and an oil-in-water emulsion.

For veterinary applications including but not limited to animal experimentation, one can use Complete Freund's Adjuvant (CFA), Freund's Incomplete Adjuvant (IFA) ,

Petitioner Merck Ex-1015, 1310

Emulsigen, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

Further exemplary adjuvants to enhance effectiveness of the pneumococcal vaccines as disclosed herein include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (b) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOX™); (2) saponin adjuvants, such as QS21, STIMULON™ (Cambridge Bioscience, Worcester, MA), Abisco® (Isconova, Sweden), or Iscomatrix® (Commonwealth Serum Laboratories, Australia), may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent (e.g., WO 00/07621); (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO 99/44636)), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) (see, e.g., GB-2220221, EP0689454), optionally in the substantial absence of alum when used with pneumococcal saccharides (see, e.g., WO 00/56358); (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (see, e.g., EP0835318, EP0735898, EP0761231); (7) a polyoxyethylene ether or a polyoxyethylene ester (see, e.g., WO99/52549); (8) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO 01/21152); (9) a saponin and an immunostimulatory oligonucleotide (e.g., a CpG oligonucleotide) (WO 00/62800);

Petitioner Merck Ex-1015, 1311

(10) an immunostimulant and a particle of metal salt (see e.g., WO00/23105); (11) a saponin and an oil-in-water emulsion e.g., WO 99/11241; (12) a saponin (e.g., QS21) + 3dMPL + IM2 (optionally + a sterol) e.g., WO 98/57659; (13) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutarninyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), etc.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a CpG Oligonucleotide as adjuvant. A CpG oligonucleotide as used herein refers to an immunostimulatory CpG oligodeoxynucleotide (CpG ODN), and accordingly these terms are used interchangeably unless otherwise indicated. Immunostimulatory CpG oligodeoxynucleotides contain one or more immunostimulatory CpG motifs that are unmethylated cytosine-guanine dinucleotides, optionally within certain preferred base contexts. The methylation status of the CpG immunostimulatory motif generally refers to the cytosine residue in the dinucleotide. An immunostimulatory oligonucleotide containing at least one unmethylated CpG dinucleotide is an oligonucleotide which contains a 5' unmethylated cytosine linked by a phosphate bond to a 3' guanine, and which activates the immune system through binding to Toll-like receptor 9 (TLR-9). In another embodiment the immunostimulatory oligonucleotide may contain one or more methylated CpG dinucleotides, which will activate the immune system through TLR9 but not as strongly as if the CpG motif(s) was/were unmethylated. CpG immunostimulatory oligonucleotides may comprise one or more palindromes that in turn may encompass the CpG dinucleotide. CpG oligonucleotides have been described in a number of issued patents, published patent applications, and other publications, including U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; and 6,339,068.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise any of the CpG Oligonucleotide described at pages 3, lines 22, to page 12, line 36, of WO 2010/125480.

Different classes of CpG immunostimulatory oligonucleotides have been identified. These are referred to as A, B, C and P class, and are described in greater detail at pages 3, lines 22, to page 12, line 36, of WO 2010/125480. Methods of the invention embrace the use of these different classes of CpG immunostimulatory oligonucleotides.

Petitioner Merck Ex-1015, 1312

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise an A class CpG oligonucleotide. In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a B class CpG Oligonucleotide.

The B class CpG oligonucleotide sequences of the invention are those broadly described above as well as disclosed in publishedWO 96/02555, WO 98/18810, and in U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; and 6,339,068. Exemplary sequences include but are not limited to those disclosed in these latter applications and patents.

In an embodiment, the "B class" CpG oligonucleotide of the invention has the following nucleic acid sequence:

5' TCGTCGTTTTTCGGTGCTTTT 3' (SEQ ID NO: 1), or

5' TCGTCGTTTTTCGGTCGTTTT 3' (SEQ ID NO: 2), or

5' TCGTCGTTTTGTCGTTTTGTCGTT 3' (SEQ ID NO: 3), or

5' TCGTCGTTTCGTCGTTTTGTCGTT 3' (SEQ ID NO: 4), or

5' TCGTCGTTTTGTCGTTTTTTTCGA 3' (SEQ ID NO: 5).

In any of these sequences, all of the linkages may be all phosphorothioate bonds. In another embodiment, in any of these sequences, one or more of the linkages may be phosphodiester, preferably between the "C" and the "G" of the CpG motif making a semi-soft CpG oligonucleotide. In any of these sequences, an ethyl-uridine or a halogen may substitute for the 5' T; examples of halogen substitutions include but are not limited to bromo-uridine or iodo-uridine substitutions.

Some non-limiting examples of B-Class oligonucleotides include:

5' T*C*G*T*C*G*T*T*T*T*T*C*G*G*T*G*C*T*T*T*T 3' (SEQ ID NO: 6), or

5' T*C*G*T*C*G*T*T*T*T*T*C*G*G*T*C*G*T*T*T*T 3' (SEQ ID NO: 7), or

5' T*C*G*T*C*G*T*T*T*T*T*G*T*C*G*T*T*T*T*G*T*C*G*T*T 3' (SEQ ID NO: 8), or

5' T*C*G*T*C*G*T*T*T*T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T 3' (SEQ ID NO: 9), or

5' T*C*G*T*C*G*T*T*T*T*T*G*T*C*G*T*T*T*T*T*T*T*T*C*G*A 3' (SEQ ID NO: 10).

wherein "*" refers to a phosphorothioate bond.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a C class CpG oligonucleotide.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a P class CpG oligonucleotide.

Petitioner Merck Ex-1015, 1313

In one embodiment the oligonucleotide includes at least one phosphorothioate linkage. In another embodiment all internucleotide linkages of the oligonucleotide are phosphorothioate linkages. In another embodiment the oligonucleotide includes at least one phosphodiester-like linkage. In another embodiment the phosphodiester-like linkage is a phosphodiester linkage. In another embodiment a lipophilic group is conjugated to the oligonucleotide. In one embodiment the lipophilic group is cholesterol.

In an embodiment, all the internucleotide linkage of the CpG oligonucleotides disclosed herein are phosphodiester bonds ("soft" oligonucleotides, as described in WO 2007/026190). In another embodiment, CpG oligonucleotides of the invention are rendered resistant to degradation (e.g., are stabilized). A "stabilized oligonucleotide " refers to an oligonucleotide that is relatively resistant to *in vivo* degradation (e.g., via an exo- or endo-nuclease). Nucleic acid stabilization can be accomplished via backbone modifications. Oligonucleotides having phosphorothioate linkages provide maximal activity and protect the oligonucleotide from degradation by intracellular exo- and endo-nucleases.

The immunostimulatory oligonucleotides may have a chimeric backbone, which have combinations of phosphodiester and phosphorothioate linkages. For purposes of the instant invention, a chimeric backbone refers to a partially stabilized backbone, wherein at least one internucleotide linkage is phosphodiester or phosphodiester-like, and wherein at least one other internucleotide linkage is a stabilized internucleotide linkage, wherein the at least one phosphodiester or phosphodiester-like linkage and the at least one stabilized linkage are different. When the phosphodiester linkage is preferentially located within the CpG motif such molecules are called "semi-soft" as described in WO 2007/026190.

Other modified oligonucleotides include combinations of phosphodiester, phosphorothioate, methylphosphonate, methylphosphorothioate, phosphorodithioate, and/or p-ethoxy linkages.

Mixed backbone modified ODN may be synthesized as described in WO 2007/026190.

The size of the CpG oligonucleotide (i.e., the number of nucleotide residues along the length of the oligonucleotide) also may contribute to the stimulatory activity of the oligonucleotide. For facilitating uptake into cells, CpG oligonucleotide of the invention preferably have a minimum length of 6 nucleotide residues. Oligonucleotides of any size greater than 6 nucleotides (even many kb long) are capable of inducing an immune response if sufficient immunostimulatory motifs are present, because larger

Petitioner Merck Ex-1015, 1314

oligonucleotides are degraded inside cells. In certain embodiments, the CpG oligonucleotides are 6 to 100 nucleotides long, preferentially 8 to 30 nucleotides long. In important embodiments, nucleic acids and oligonucleotides of the invention are not plasmids or expression vectors.

In an embodiment, the CpG oligonucleotide disclosed herein comprise substitutions or modifications, such as in the bases and/or sugars as described at paragraphs 134 to 147 of WO 2007/026190.

In an embodiment, the CpG oligonucleotide of the present invention is chemically modified. Examples of chemical modifications are known to the skilled person and are described, for example in Uhlmann et al. (1990) Chem. Rev. 90:543; S. Agrawal, Ed., Humana Press, Totowa, USA 1993; Crooke. et al. (1996) Annu. Rev. Pharmacol. Toxicol. 36:107-129; and Hunziker et al., (1995) Mod. Synth. Methods 7:331-417. An oligonucleotide according to the invention may have one or more modifications, wherein each modification is located at a particular phosphodiester internucleoside bridge and/or at a particular β-D-ribose unit and/or at a particular natural nucleoside base position in comparison to an oligonucleotide of the same sequence which is composed of natural DNA or RNA.

In some embodiments of the invention, CpG-containing nucleic acids might be simply mixed with immunogenic carriers according to methods known to those skilled in the art (see, e.g., WO 03/024480).

In a particular embodiment of the present invention, any of the immunogenic composition disclosed herein comprises from 2 µg to 100 mg of CpG oligonucleotide, preferably from 0.1 mg to 50 mg CpG oligonucleotide, preferably from 0.2 mg to 10 mg CpG oligonucleotide, preferably from 0.3 mg to 5 mg CpG oligonucleotide, preferably from 0.3 mg to 5 mg CpG oligonucleotide, even preferably from 0.5 mg to 2 mg CpG oligonucleotide, even preferably from 0.75 mg to 1.5 mg CpG oligonucleotide. In a preferred embodiement, any of the immunogenic composition disclosed herein comprises about 1 mg CpG oligonucleotide.

5 Formulation

The immunogenic compositions of the invention may be formulated in liquid form (i.e., solutions or suspensions) or in a lyophilized form. Liquid formulations may advantageously be administered directly from their packaged form and are thus ideal for

Petitioner Merck Ex-1015, 1315

injection without the need for reconstitution in aqueous medium as otherwise required for lyophilized compositions of the invention.

Formulation of the immunogenic composition of the present invention can be accomplished using art-recognized methods. For instance, the individual pneumococcal conjugates can be formulated with a physiologically acceptable vehicle to prepare the composition. Examples of such vehicles include, but are not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions.

The present disclosure provides an immunogenic composition comprising any of combination of glycoconjugates disclosed herein and a pharmaceutically acceptable excipient, carrier, or diluent.

In an embodiment, the immunogenic composition of the invention is in liquid form, preferably in aqueous liquid form.

Immunogenic compositions of the disclosure may comprise one or more of a buffer, a salt, a divalent cation, a non-ionic detergent, a cryoprotectant such as a sugar, and an anti-oxidant such as a free radical scavenger or chelating agent, or any multiple combinations thereof.

In an embodiment, the immunogenic composition of the invention comprises a buffer. In an embodiment, said buffer has a pKa of about 3.5 to about 7.5. In some embodiments, the buffer is phosphate, succinate, histidine or citrate. In certain embodiments, the buffer is succinate at a final concentration of 1 mM to 10 mM. In one particular embodiment, the final concentration of the succinate buffer is about 5 mM.

In an embodiment, the immunogenic composition of the invention comprises a salt. In some embodiments, the salt is selected from the groups consisting of magnesium chloride, potassium chloride, sodium chloride and a combination thereof. In one particular embodiment, the salt is sodium chloride. In one particular embodiment, the immunogenic composition of the invention comprises sodium chloride at 150 mM.

In an embodiment, the immunogenic compositions of the invention comprise a surfactant. In an embodiment, the surfactant is selected from the group consisting of polysorbate 20 (TWEEN™20), polysorbate 40 (TWEEN™40), polysorbate 60 (TWEEN™60), polysorbate 65 (TWEEN™65), polysorbate 80 (TWEEN™80), polysorbate 85 (TWEEN™85), TRITON™ N-1 01, TRITON™ X-100, oxtoxynol 40, nonoxynol-9, triethanolamine, triethanolamine polypeptide oleate, polyoxyethylene-660 hydroxystearate (PEG-15, Solutol H 15), polyoxyethylene-35-ricinoleate

Petitioner Merck Ex-1015, 1316

(CREMOPHOR® EL), soy lecithin and a poloxamer. In one particular embodiment, the surfactant is polysorbate 80. In some said embodiment, the final concentration of polysorbate 80 in the formulation is at least 0.0001% to 10% polysorbate 80 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 80 in the formulation is at least 0.001% to 1% polysorbate 80 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 80 in the formulation is at least 0.01% to 1% polysorbate 80 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 80 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 1% polysorbate 80 (w/w).

In certain embodiments, the immunogenic composition of the invention has a pH of 5.5 to 7.5, more preferably a pH of 5.6 to 7.0, even more preferably a pH of 5.8 to 6.0.

In one embodiment, the present invention provides a container filled with any of the immunogenic compositions disclosed herein. In one embodiment, the container is selected from the group consisting of a vial, a syringe, a flask, a fermentor, a bioreactor, a bag, a jar, an ampoule, a cartridge and a disposable pen. In certain embodiments, the container is siliconized.

In an embodiment, the container of the present invention is made of glass, metals (e.g., steel, stainless steel, aluminum, etc.) and/or polymers (e.g., thermoplastics, elastomers, thermoplastic-elastomers). In an embodiment, the container of the present invention is made of glass.

In one embodiment, the present invention provides a syringe filled with any of the immunogenic compositions disclosed herein. In certain embodiments, the syringe is siliconized and/or is made of glass.

A typical dose of the immunogenic composition of the invention for injection has a volume of 0.1 mL to 2 mL, more preferabl 0.2 mL to 1 mL, even more preferably a volume of about 0.5 mL.

Therfore the container or syringe as defined above is filed with a volume of 0.1 mL to 2 mL, more preferably 0.2 mL to 1 mL, even more preferably a volume of about 0.5 mL of any of the immunogenic composition defined herein.

Petitioner Merck Ex-1015, 1317

6 Ability of the immunogenic compositions of the invention to elicit cross-reactive antibodies

In an embodiment, the immunogenic composition of the invention is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9N, 9A and/or 9L polysaccharide as determined by ELISA assay.

In the ELISA (Enzyme-linked Immunosorbent Assay) method, antibodies from the sera of vaccinated subjects are incubated with polysaccharides which have been adsorbed to a solid support.The bound antibodies are detected using enzyme-conjugated secondary detection antibodies.

In an embodiment said ELISA assay is the standardized ELISA assay as defined by the WHO in the "Training Manual For Enzyme Linked Immunosorbent Assay For The Quantitation Of Streptococcus Pneumoniae Serotype Specific IgG (Pn PS ELISA)." (available at http://www.vaccine.uab.edu/ELISA%20protocol.pdf, accessed on March 31, 2014).

The ELISA measures type specific IgG anti-*S. pneumoniae* capsular polysaccharide (PS) antibodies present in human serum. When dilutions of human sera are added to type-specific capsular PS-coated microtiter plates, antibodies specific for that capsular PS bind to the microtiter plates. The antibodies bound to the plates are detected using a goat anti-human IgG alkaline phosphatase-labeled antibody followed by a p-nitrophenyl phosphate substrate. The optical density of the colored end product is proportional to the amount of anticapsular PS antibody present in the serum.

In an embodiment, the immunogenic composition of the invention is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9N polysaccharide at a concentration of at least 0.05 µg/ml, 0.1 µg/ml, 0.2 µg/ml, 0.3 µg/ml, 0.35 µg/ml, 0.4 µg/ml or 0.5 µg/ml as determined by ELISA assay.

In an embodiment, the immunogenic composition of the invention is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9A polysaccharide at a concentration of at least 0.05 µg/ml, 0.1 µg/ml, 0.2 µg/ml, 0.3 µg/ml, 0.35 µg/ml, 0.4 µg/ml or 0.5 µg/ml as determined by ELISA assay.

In an embodiment, the immunogenic composition of the invention is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9L polysaccharide at a concentration of at least 0.05 µg/ml, 0.1 µg/ml, 0.2 µg/ml, 0.3 µg/ml, 0.35 µg/ml, 0.4 µg/ml or 0.5 µg/ml as determined by ELISA assay.

Petitioner Merck Ex-1015, 1318

In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in humans which are capable of killing *S. pneumoniae* serotype 9N, 9A and/or 9L as determined by *in vitro* opsonophagocytic assay (OPA) (see Example 1). In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in humans which are capable of killing *S. pneumoniae* serotype 9N as determined by *in vitro* opsonophagocytic assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in humans which are capable of killing *S. pneumoniae* serotype 9A as determined by *in vitro* opsonophagocytic assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in human which are capable of killing *S. pneumoniae* serotype 9L as determined by *in vitro* opsonophagocytic assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in humans which are capable of killing *S. pneumoniae* serotype 9N and 9A as determined by *in vitro* opsonophagocytic assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in humans which are capable of killing *S. pneumoniae* serotype 9N and 9L as determined by *in vitro* opsonophagocytic assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit functional antibodies in humans which are capable of killing *S. pneumoniae* serotype 9A and 9L as determined by *in vitro* opsonophagocytic assay (OPA).

The pneumococcal opsonophagocytic assay (OPA), which measures killing of *S. pneumoniae* cells by phagocytic effector cells in the presence of functional antibody and complement, is considered to be an important surrogate for evaluating the effectiveness of pneumococcal vaccines.

In vitro opsonophagocytic assay (OPA) can be conducted by incubating together a mixture of *Streptococcus pneumoniae* cells, a heat inactivated human serum to be tested, differentiated HL-60 cells (phagocytes) and an exogenous complement source (e.g., baby rabbit complement). Opsonophagocytosis proceeds during incubation and bacterial cells that are coated with antibody and complement are killed upon opsonophagocytosis. Colony forming units (cfu) of surviving bacteria that escape from opsonophagocytosis are determined by plating the assay mixture. The OPA titer is defined as the reciprocal dilution that results in a 50% reduction in bacterial count over control wells without test serum. The OPA titer is interpolated from the two dilutions that encompass this 50% killing cut-off.

Petitioner Merck Ex-1015, 1319

An endpoint titer of 1:8 or greater is considered a positive result in these killing type OPA.

In an embodiment, the immunogenic composition of the invention is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9N in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9N in at least 60%, 70%, 80%, 90%, or at least 93% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).

In an embodiment, the immunogenic composition of the invention is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9A in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9A in at least 60%, 70%, 80%, 90%, or at least 95% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).

In an embodiment, the immunogenic composition of the invention is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9L in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA). In an embodiment, the immunogenic composition of the invention is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9L in at least 60%, 70%, 80%, 90%, or at least 95%, of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).

In some embodiment, the subjects may have serotype specific OPA titers prior to pneumococcal vaccination due for example to natural exposures to *S. pneumoniae* (e.g., in case of adult subjects).

Therefore, comparaison of OPA activity of pre- and post-immunization serum with the immunogenic composition of the invention can be conducted and compared for their response to serotypes 9A, 9L, and 9N to assess the potential increase of responders (see Example 1).

In an embodiment the immunogenic composition of the invention significantly increases the proportion of responders (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

Therefore in an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9N (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

Petitioner Merck Ex-1015, 1320

In an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9A (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9L (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotypes 9N and 9A (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotypes 9N and 9L (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotypes 9A and 9L (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the proportion of responders against *S. pneumoniae* serotypes 9N, 9A and 9L (i.e., individual with a serum having a titer of at least 1:8 as determined by *in vitro* OPA) as compared to the pre-immunized population.

Comparaison of OPA activity of pre- and post-immunization serum with the immunogenic composition of the invention can also be done by comparing the potential increase in OPA titers.

Therefore, comparaison of OPA activity of pre- and post-immunization serum with the immunogenic composition of the invention can be conducted and compared for their response to serotypes 9A, 9L, and 9N to assess the potential for increase in OPA titers (see Example 1).

In an embodiment the immunogenic compositions of the invention are able to significantly increase the OPA titer of human subjects as compared to the pre-immunized population.

Petitioner Merck Ex-1015, 1321

Therefore in an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9N as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9N is at least 1.2, 1.5, 1.75, 2.0 or 2.1.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9A as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9A is at least 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0 or 7.5.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9L as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9L is at least 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 or 4.2.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotypes 9N and 9A as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9N is at least 1.2, 1.5, 1.75, 2.0 or 2.1 and the fold-rise in OPA titer against *S. pneumoniae* serotype 9A is at least 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0 or 7.5.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotypes 9N and 9L as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9N is at least 1.2, 1.5, 1.75, 2.0 or 2.1 and the fold-rise in OPA titer against *S. pneumoniae* serotype 9L is at least 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 or 4.2.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotypes 9A and 9L as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9A is at least 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0 or 7.5 and the fold-rise in OPA titer against *S. pneumoniae* serotype 9L is at least 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 or 4.2.

In an embodiment, the immunogenic composition of the invention is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotypes 9N, 9A and 9L as compared to the pre-immunized population. In an embodiment, the fold-rise in OPA titer against *S. pneumoniae* serotype 9N is at least 1.2, 1.5, 1.75, 2.0 or 2.1, the

Petitioner Merck Ex-1015, 1322

fold-rise in OPA titer against *S. pneumoniae* serotype 9A is at least 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0 or 7.5 and the fold-rise in OPA titer against *S. pneumoniae* serotype 9L is at least 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 or 4.2.

7 Uses of the immunogenic compositions of the invention

In an embodiment, the mmunogenic compositions disclosed herein are for use as a medicament.

The immunogenic compositions described herein may be used in various therapeutic or prophylactic methods for preventing, treating or ameliorating a bacterial infection, disease or condition in a subject. In particular, immunogenic compositions described herein may be used to prevent, treat or ameliorate a *S. pneumoniae* infection, disease or condition in a subject.

Thus in one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotypes 9N, 9A and 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 9N in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 9A in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 9N and 9A in a

Petitioner Merck Ex-1015, 1323

subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 9N and 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 9A and 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of inducing an immune response to *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of inducing an immune response to *S. pneumoniae* serotypes 9N in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of inducing an immune response to *S. pneumoniae* serotypes 9A in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the invention provides a method of inducing an immune response to *S. pneumoniae* serotypes 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention.

In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject.

In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotype 9N in a subject. In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotype 9A in a subject. In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotype 9L in a subject.

Petitioner Merck Ex-1015, 1324

In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N and 9A in a subject. In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotype 9N and 9L in a subject. In one aspect, the immunogenic compositions of the present invention are for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotype 9A and 9L in a subject.

In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L.

In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N. In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9A. In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9L.

In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N and 9A. In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N and 9L. In an embodiment, any of the immunogenic composition disclosed herein is for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9A and 9L.

In one aspect, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject.

In one aspect, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N and 9A in a subject. In one aspect, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition

Petitioner Merck Ex-1015, 1325

caused by *S. pneumoniae* serotype 9N and 9L in a subject. In one aspect, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotype 9A and 9L in a subject.

In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L.

In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9N. In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9A. In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9L.

In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9N and 9A. In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9N and 9L. In an embodiment, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9A and 9L.

In one aspect, the present invention provides a method for inducing an immune response to *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject. In one aspect, the present invention provides a method for inducing an immune response to *S. pneumoniae* serotype 9N in a subject. In one aspect, the present invention provides a method for inducing an immune response to *S. pneumoniae* serotype 9A in a subject. In one aspect, the present invention provides a method for inducing an immune response to *S. pneumoniae* serotype 9L in a subject. In an embodiment, the immunogenic compositions disclosed herein are for use as a vaccine. More particularly, the immunogenic compositions described herein may be used to prevent serotypes 9N, 9A

Petitioner Merck Ex-1015, 1326

and/or 9L *S. pneumoniae* infections in a subject. Thus in one aspect, the invention provides a method of preventing, an infection by serotypes 9N, 9A and/or 9L *S. pneumoniae* in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the invention. In some such embodiments, the infection is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteremia, sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess. In one aspect, the subject to be vaccinated is a mammal, such as a human, cat, sheep, pig, horse, bovine or dog.

In one aspect, the immunogenic compositions disclosed herein are for use in a method of preventing, treating or ameliorating an infection, disease or condition associated *S. pneumoniae* with serotypes 9N, 9A and/or 9L in a subject. In some such embodiments, the infection, disease or condition is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteremia, sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess.

In an aspect, the immunogenic composition disclosed herein are for use in a method of preventing, an infection by serotypes 9N, 9A and/or 9L of *S. pneumoniae* in a subject. In some such embodiments, the infection is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteremia, sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess. In one aspect, the subject to be vaccinated is a mammal, such as a human, cat, sheep, pig, horse, bovine or dog.

In one aspect, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition associated *S. pneumoniae* with serotypes 9N, 9A and/or 9L in a subject. In some such embodiments, the infection, disease or condition is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteremia, sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess.

Petitioner Merck Ex-1015, 1327

In an aspect, the present invention is directed toward the use of the immunogenic composition disclosed herein for the manufacture of a medicament for preventing, an infection by serotypes 9N, 9A and/or 9L of *S. pneumoniae* in a subject. In some such embodiments, the infection is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteremia, sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess. In one aspect, the subject to be vaccinated is a mammal, such as a human, cat, sheep, pig, horse, bovine or dog.

The immunogenic compositions of the present invention can be used to protect or treat a human susceptible to *S. pneumoniae* serotypes 9N, 9A and/or 9L infection, by means of administering the immunogenic compositions via a systemic or mucosal route. In an embodiment, the immunogenic compositions disclosed herein are administered by intramuscular, intraperitoneal, intradermal or subcutaneous routes. In an embodiment, the immunogenic compositions disclosed herein are administered by intramuscular, intraperitoneal, intradermal or subcutaneous injection. In an embodiment, the immunogenic compositions disclosed herein are administered by intramuscular or subcutaneous injection.

In an embodiment, the immunogenic compositions of the present disclosure comprise at least one glycoconjugate from *S. pneumoniae* 9V (such as the glycoconjugates of paragraph 1.3 above).

8 Subject to be treated with the immunogenic compositions of the invention

As disclosed herein, the immunogenic compositions described herein may be used in various therapeutic or prophylactic methods for preventing, treating or ameliorating a bacterial infection, disease or condition in a subject.

In a preferred embodiment, said subject is a human. In a most preferred embodiment, said subject is a newborn (i.e., under three months of age), an infant (i.e., from 3 months to one year of age) or a toddler (i.e., from one year to four years of age).

In an embodiment, the immunogenic compositions disclosed herein are for use as a vaccine.

In such embodiment, the subject to be vaccinated may be less than 1 year of age. For example, the subject to be vaccinated can be about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11 or about 12 months of age. In

Petitioner Merck Ex-1015, 1328

an embodiment, the subject to be vaccinated is about 2, 4 or 6 months of age. In another embodiment, the subject to be vaccinated is less than 2 years of age. For example the subject to be vaccinated can be about 12 to about 15 months of age. In some cases, as little as one dose of the immunogenic composition according to the invention is needed, but under some circumstances, a second, third or fourth dose may be given (see section 9 below).

In an embodiment of the present invention, the subject to be vaccinated is a human adult 50 years of age or older, more preferably a human adult 55 years of age or older. In an embodiment, the subject to be vaccinated is a human adult 65 years of age or older, 70 years of age or older, 75 years of age or older or 80 years of age or older.

In an embodiment the subject to be vaccinated is an immunocompromised individual, in particular a human. An immunocompromised individual is generally defined as a person who exhibits an attenuated or reduced ability to mount a normal humoral or cellular defense to challenge by infectious agents.

In an embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from a disease or condition that impairs the immune system and results in an antibody response that is insufficient to protect against or treat pneumococcal disease.

In an embodiment, said disease is a primary immunodeficiency disorder. Preferably, said primary immunodeficiency disorder is selected from the group consisting of: combined T- and B-cell immunodeficiencies, antibody deficiencies, well-defined syndromes, immune dysregulation diseases, phagocyte disorders, innate immunity deficiencies, autoinflammatory disorders, and complement deficiencies. In an embodiment, said primary immunodeficiency disorder is selected from the one disclosed on page 24, line 11, to page 25, line 19, of WO 2010/125480.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from a disease selected from the groups consisting of: HIV-infection, acquired immunodeficiency syndrome (AIDS), cancer, chronic heart or lung disorders, congestive heart failure, diabetes mellitus, chronic liver disease, alcoholism, cirrhosis, spinal fluid leaks, cardiomyopathy, chronic bronchitis, emphysema, chronic obstructive pulmonary disease (COPD), spleen dysfunction (such as sickle cell disease), lack of spleen function (asplenia), blood malignancy, leukemia, multiple myeloma, Hodgkin's disease, lymphoma, kidney failure, nephrotic syndrome and asthma.

Petitioner Merck Ex-1015, 1329

In an embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from malnutrition.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated is taking a drug or treatment that lowers the body's resistance to infection. In an embodiment, said drug is selected from the one disclosed on page 26, line 33, to page 26, line 4, of WO 2010/125480.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated is a smoker.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a white blood cell count (leukocyte count) below 5 x 10^9 cells per liter, or below 4 x 10^9 cells per liter, or below 3 x 10^9 cells per liter, or below 2 x 10^9 cells per liter, or below 1 x 10^9 cells per liter, or below 0.5 x 10^9 cells per liter, or below 0.3 x 10^9 cells per liter, or below 0.1 x 10^9 cells per liter.

White blood cell count (leukocyte count): The number of white blood cells (WBC) in the blood. The WBC is usually measured as part of the CBC (complete blood count). White blood cells are the infection-fighting cells in the blood and are distinct from the red (oxygen-carrying) blood cells known as erythrocytes. There are different types of white blood cells, including neutrophils (polymorphonuclear leukocytes; PMN), band cells (slightly immature neutrophils), T-type lymphocytes (T-cells), B-type lymphocytes (B-cells), monocytes, eosinophils, and basophils. All the types of white blood cells are reflected in the white blood cell count. The normal range for the white blood cell count is usually between 4,300 and 10,800 cells per cubic millimeter of blood. This can also be referred to as the leukocyte count and can be expressed in international units as 4.3 - 10.8 x 10^9 cells per liter.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from neutropenia. In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a neutrophil count below 2 x 10^9 cells per liter, or below 1 x 10^9 cells per liter, or below 0.5 x 10^9 cells per liter, or below 0.1 x 10^9 cells per liter, or below 0.05 x 10^9 cells per liter.

A low white blood cell count or "neutropenia" is a condition characterized by abnormally low levels of neutrophils in the circulating blood. Neutrophils are a specific kind of white blood cell that help prevent and fight infections. The most common reason that cancer patients experience neutropenia is as a side effect of chemotherapy. Chemotherapy-

Petitioner Merck Ex-1015, 1330

induced neutropenia increases a patient's risk of infection and disrupts cancer treatment.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a CD4+ cell count below 500/mm3, or CD4+ cell count below 300/mm3, or CD4+ cell count below 200/mm3, CD4+ cell count below 100/mm3, CD4+ cell count below 75/mm3, or CD4+ cell count below 50/mm3.

CD4 cell tests are normally reported as the number of cells in mm3. Normal CD4 counts are between 500 and 1600, and CD8 counts are between 375 and 1100. CD4 counts drop dramatically in people with HIV.

In an embodiment of the invention, any of the immunocompromised subject disclosed herein is a human male or a human female.

9 Regimen

In some cases, as little as one dose of the immunogenic composition according to the invention is needed, but under some circumstances, such as conditions of greater immune deficiency, a second, third or fourth dose may be given. Following an initial vaccination, subjects can receive one or several booster immunizations adequately spaced.

In an embodiment, the schedule of vaccination of the immunogenic composition according to the invention is a single dose. In a particular embodiment, said single dose schedule is for healthy persons being at least 2 years of age.

In an embodiment, the schedule of vaccination of the immunogenic composition according to the invention is a multiple dose schedule. In a particular embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 1 month to about 2 months. In a particular embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 1 month, or a series of 2 doses separated by an interval of about 2 months.

In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month to about 2 months. In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month, or a series of 3 doses separated by an interval of about 2 months.

In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month to about 2 months followed by a fourth dose about 10 months to about 13 months after the first dose. In another embodiment, said

Petitioner Merck Ex-1015, 1331

multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month followed by a fourth dose about 10 months to about 13 months after the first dose, or a series of 3 doses separated by an interval of about 2 months followed by a fourth dose about 10 months to about 13 months after the first dose.

In an embodiment, the multiple dose schedule consists of at least one dose (e.g., 1, 2 or 3 doses) in the first year of age followed by at least one toddler dose.

In an embodiment, the multiple dose schedule consists of a series of 2 or 3 doses separated by an interval of about 1 month to about 2 months (for example 28-56 days between doses), starting at 2 months of age, and followed by a toddler dose at 12-18 months of age. In an embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 to 2 months (for example 28-56 days between doses), starting at 2 months of age, and followed by a toddler dose at 12-15 months of age. In another embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 2 months, starting at 2 months of age, and followed by a toddler dose at 12-18 months of age.

In an embodiment, the multiple dose schedule consists of a 4-dose series of vaccine at 2, 4, 6, and 12-15 months of age.

In an embodiment, a prime dose is given at day 0 and one or more boosts are given at intervals that range from about 2 to about 24 weeks, preferably with a dosing interval of 4-8 weeks.

In an embodiment, a prime dose is given at day 0 and a boost is given about 3 months later.

10 Kit and process

In an embodiment, the invention is directed toward a kit comprising an immunogenic composition disclosed herein and an information leaflet.

In an embodiment said information leaflet mentions the ability of the composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N.

In an embodiment said information leaflet mentions the ability of the composition to elicit functional antibodies against *S. pneumoniae* serotype 9N.

In an embodiment said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N at a concentration ≥ 0.35 µg/mL in a human population.

Petitioner Merck Ex-1015, 1332

In an embodiment said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotype 9N at a concentration ≥ 0.35 µg/mL in a human population.

In an embodiment said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9A, 9L and/or 9N in a human population.

In an embodiment said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9N in a human population.

In an embodiment, the invention is directed toward a process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

- producing an immunogenic composition of the present disclosure and
- combining in the same kit said immunogenic composition and information leaflet, wherein said information leaflet mentions the ability of said composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N.

In an embodiment, the invention is directed toward a process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

- producing an immunogenic composition of the present disclosure and
- combining in the same kit said immunogenic composition and information leaflet, wherein said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N at a concentration ≥ 0.35 µg/mL in a human population.

In an embodiment, the invention is directed toward a process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

- producing an immunogenic composition of the present disclosure and
- combining in the same kit said immunogenic composition and information leaflet, wherein said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9A, 9L and/or 9N in a human population.

In an embodiment, the invention is directed toward a process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

- producing an immunogenic composition of the present disclosure;

Petitioner Merck Ex-1015, 1333

- printing an information leaflet wherein said information leaflet mentions the ability of said composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N;

- combining in the same kit said immunogenic composition and said information leaflet.

In an embodiment, the invention is directed toward a process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

- producing an immunogenic composition of the present disclosure;

- printing an information leaflet wherein said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N at a concentration ≥ 0.35 µg/mL in a human population;

- combining in the same kit said immunogenic composition and said information leaflet.

In an embodiment, the invention is directed toward a process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

- producing an immunogenic composition of the present disclosure;

- printing an information leaflet wherein said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9A, 9L and/or 9N in a human population;

- combining in the same kit said immunogenic composition and said information leaflet.

11 Methods

In an embodiment, the invention is directed toward a method comprising the step of:

- injecting to a subject an immunologically effective amount of any of the immunogenic compositions defined in the present document;

- collecting a serum sample from said subject;

- testing said serum sample for opsonophagocytic killing activity against *S. pneumoniae* serotype 9N by *in vitro* opsonophagocytic killing assay (OPA).

In an embodiment, the invention is directed toward a method comprising the step of:

- injecting to a subject an immunologically effective amount of any of the immunogenic compositions defined in the present document;

Petitioner Merck Ex-1015, 1334

- collecting a serum sample from said subject;

- testing said serum sample for opsonophagocytic killing activity against *S. pneumoniae* serotype 9A by *in vitro* opsonophagocytic killing assay (OPA).

In an embodiment, the invention is directed toward a method comprising the step of:

- injecting to a subject an immunologically effective amount of any of the immunogenic compositions defined in the present document;

- collecting a serum sample from said subject;

- testing said serum sample for opsonophagocytic killing activity against *S. pneumoniae* serotype 9L by *in vitro* opsonophagocytic killing assay (OPA).

In an embodiment, the invention is directed toward a method comprising the step of:

- injecting to a subject an immunologically effective amount of any of the immunogenic compositions defined in the present document;

- collecting a serum sample from said subject;

- testing said serum sample for opsonophagocytic killing activity against *S. pneumoniae* serotypes 9N, 9A and/or 9L by *in vitro* opsonophagocytic killing assay (OPA).

Particular embodiments of the disclosure are set forth in the following numbered paragraphs:

1. An immunogenic composition comprising at least one glycoconjugate from *S. pneumoniae* serotype 9V for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L.

2. The immunogenic composition of paragraph 1, wherein said composition does not comprise capsular saccharide from *S. pneumoniae* serotype 9N.

3. The immunogenic composition of any one of paragraphs 1-2, wherein said composition does not comprise capsular saccharide from *S. pneumoniae* serotype 9A.

4. The immunogenic composition composition of any one of paragraphs 1-3, wherein said composition does not comprise capsular saccharide from *S. pneumoniae* serotype 9L.

5. The immunogenic composition of paragraph 1, wherein said composition does not comprise capsular saccharide from *S. pneumoniae* serotypes 9N, 9A and 9L

Petitioner Merck Ex-1015, 1335

6. The immunogenic composition composition of any one of paragraphs 1-5, further comprising at least one glycoconjugate from *S. pneumoniae* serotype 4.

7. The immunogenic composition composition of any one of paragraphs 1-6 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 6B.

8. The immunogenic composition composition of any one of paragraphs 1-7 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 14.

9. The immunogenic composition composition of any one of paragraphs 1-8 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 18C.

10. The immunogenic composition composition of any one of paragraphs 1-9 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 19F.

11. The immunogenic composition composition of any one of paragraphs 1-10 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 23F.

12. The immunogenic composition composition of any one of paragraphs 1-5 further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 14, 18C, 19F and 23F.

13. The immunogenic composition of any one of paragraphs 1-12 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 1.

14. The immunogenic composition of any one of paragraphs 1-13 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 5.

15. The immunogenic composition of any one of paragraphs 1-14 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 7F.

16. The immunogenic composition of any one of paragraphs 1-15 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 5 and 7F.

17. The immunogenic composition of any one of paragraphs 1-16 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 6A.

18. The immunogenic composition of any one of paragraphs 1-17 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 19A.

19. The immunogenic composition of any one of paragraphs 1-15 further comprising glycoconjugates from *S. pneumoniae* serotypes 6A and 19A.

Petitioner Merck Ex-1015, 1336

20. The immunogenic composition of any one of paragraphs 1-19 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 3.

21. The immunogenic composition of any one of paragraphs 1-20 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 15B.

22. The immunogenic composition of any one of paragraphs 1-21 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 22F.

23. The immunogenic composition of any one of paragraphs 1-22 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 33F.

24. The immunogenic composition of any one of paragraphs 1-23 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 12F.

25. The immunogenic composition of any one of paragraphs 1-24 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 10A.

26. The immunogenic composition of any one of paragraphs 1-25 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 11A.

27. The immunogenic composition of any one of paragraphs 1-26 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 8.

28. The immunogenic composition of any one of paragraphs 1-20 further comprising glycoconjugates from *S. pneumoniae* serotypes 22F and 33F.

29. The immunogenic composition of any one of paragraphs 1-20 further comprising glycoconjugates from *S. pneumoniae* serotypes 15B, 22F and 33F.

30. The immunogenic composition of any one of paragraphs 1-23 further comprising glycoconjugates from *S. pneumoniae* serotypes 12F, 10A, 11A and 8.

31. The immunogenic composition of any one of paragraphs 1-30 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 2.

32. The immunogenic composition of any one of paragraphs 1-31 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 17F.

33. The immunogenic composition of any one of paragraphs 1-32 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 20.

34. The immunogenic composition of any one of paragraphs 1-30 further comprising glycoconjugates from *S. pneumoniae* serotypes 2, 17F and 20.

Petitioner Merck Ex-1015, 1337

35. The immunogenic composition of any one of paragraphs 1-34 further comprising at least one glycoconjugate from *S. pneumoniae* serotype 15C.

36. The immunogenic composition of any one of paragraphs 1-35 which is a 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24-valent pneumococcal conjugate composition.

37. The immunogenic composition of any one of paragraphs 1-35 which is a 14, 15, 16, 17, 18, 19 or 20 valent pneumococcal conjugate composition.

38. The immunogenic composition of any one of paragraphs 1-35 which is a 16-valent pneumococcal conjugate composition.

39. The immunogenic composition of any one of paragraphs 1-35 which is a 20-valent pneumococcal conjugate composition.

40. The immunogenic composition of any one of paragraphs 1-39 wherein said glycoconjugates are individually conjugated to CRM_{197}.

41. The immunogenic composition of any one of paragraphs 1-39 wherein all glycoconjugates are individually conjugated to CRM_{197}.

42. The immunogenic composition of any one of paragraphs 1-39 wherein, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14 and/or 23F are individually conjugated to PD.

43. The immunogenic composition of any one of paragraphs 9-39 or 42 wherein the glycoconjugate from *S. pneumoniae* serotype 18C is conjugated to TT.

44. The immunogenic composition of any one of paragraphs 10-39 or 42-43 wherein the glycoconjugate from *S. pneumoniae* serotype 19F is conjugated to DT.

45. The immunogenic composition of any one of paragraphs 1-44 wherein said glycoconjugates are prepared using CDAP chemistry.

46. The immunogenic composition of any one of paragraphs 1-44 wherein said glycoconjugates are prepared by reductive amination.

47. The immunogenic composition of any one of paragraphs 1-44 wherein said glycoconjugate from *S. pneumoniae* serotype 6A is prepared by reductive amination.

48. The immunogenic composition of any one of paragraphs 1-44 or 47 wherein said glycoconjugate from *S. pneumoniae* serotype 19A is prepared by reductive amination.

Petitioner Merck Ex-1015, 1338

49. The immunogenic composition of any one of paragraphs 1-44 or 47-48 wherein said glycoconjugate from *S. pneumoniae* serotype 3 is prepared by reductive amination.

50. The immunogenic composition of any one of paragraphs 1-49 wherein said immunogenic composition further comprise antigens from other pathogens.

51. The immunogenic composition of any one of paragraphs 1-49 wherein said immunogenic composition further comprise antigens selected from: a diphtheria toxoid (D), a tetanus toxoid (T), a pertussis antigen (P), which is typically acellular (Pa), a hepatitis B virus (HBV) surface antigen (HBsAg), a hepatitis A virus (HAV) antigen, a conjugated Haemophilus influenzae type b capsular saccharide (Hib), inactivated poliovirus vaccine (IPV).

52. The immunogenic composition of any one of paragraphs 1-51 wherein said immunogenic composition further comprise at least one adjuvant, most preferably any of the adjuvant disclosed herein.

53. The immunogenic composition of any one of paragraphs 1-51 wherein said immunogenic composition further comprise at least one adjuvant selected from the group consisting of aluminum phosphate, aluminum sulfate and aluminum hydroxide.

54. The immunogenic composition of any one of paragraphs 1-51 wherein said immunogenic composition comprise from 0.1 mg/mL to 1 mg/mL of elemental aluminum in the form of aluminum phosphate as adjuvant.

55. The immunogenic composition of any one of paragraphs 1-54 which is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9N polysaccharide at a concentration of at least 0.35 µg/ml as determined by ELISA assay.

56. The immunogenic composition of any one of paragraphs 1-55 which is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9A polysaccharide at a concentration of at least 0.35 µg/ml as determined by ELISA assay.

57. The immunogenic composition of any one of paragraphs 1-56 which is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9L polysaccharide at a concentration of at least 0.35 µg/ml as determined by ELISA assay.

Petitioner Merck Ex-1015, 1339

58. The immunogenic composition of any one of paragraphs 1-57 which is able to elicit functional antibodies in human which are capable of killing *S. pneumoniae* serotype 9N, 9A and/or 9L as determined by in vitro opsonophagocytic assay (OPA).

59. The immunogenic composition of any one of paragraphs 1-58 which is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9N in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).

60. The immunogenic composition of any one of paragraphs 1-59 which is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9A in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).

61. The immunogenic composition of any one of paragraphs 1-60 which is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9L in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).

62. The immunogenic composition of any one of paragraphs 1-61 which is able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9N as compared to the pre-immunized population.

63. The immunogenic composition of any one of paragraphs 1-62 which is able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9A as compared to the pre-immunized population.

64. The immunogenic composition of any one of paragraphs 1-63 which is able to significantly increase the proportion of responders against *S. pneumoniae* serotype 9L as compared to the pre-immunized population

65. The immunogenic composition of any one of paragraphs 1-64 which is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9N as compared to the pre-immunized population.

66. The immunogenic composition of any one of paragraphs 1-65 which is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9A as compared to the pre-immunized population.

67. The immunogenic composition of any one of paragraphs 1-66 which is able to significantly increase the OPA titers of human subjects against *S. pneumoniae* serotype 9L as compared to the pre-immunized population.

68. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N.

69. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9A.

Petitioner Merck Ex-1015, 1340

70. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9L.

71. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N and 9A.

72. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N and 9L.

73. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9A and 9L.

74. The immunogenic composition of any one of paragraphs 1-67, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and 9L.

75. The immunogenic composition of any one of paragraphs 1-67 for use in a method for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject.

76. The immunogenic composition of any one of paragraphs 1-67 for use to prevent serotypes 9N, 9A and/or 9L *S. pneumoniae* infection in a subject.

77. The immunogenic composition of any one of paragraphs 1-67 for use in a method to protect or treat a human susceptible to *S. pneumoniae* serotypes 9N, 9A and/or 9L infection, by means of administering said immunogenic compositions via a systemic or mucosal route.

78. A method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of any one of paragraphs 1-67.

79. A method of preventing an infection by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of any one of paragraphs 1-67.

Petitioner Merck Ex-1015, 1341

80. The immunogenic composition of any one of paragraphs 1-67, wherein said subject is a human being less than 1 year of age.

81. The immunogenic composition of any one of paragraphs 1-67, wherein said subject is a human is a human being less than 2 year of age.

82. The immunogenic composition of any one of paragraphs 1-67, wherein said subject is a human adult 50 years of age or older.

83. The immunogenic composition of any one of paragraphs 1-82 for use in a multiple dose vaccination schedule.

84. A kit comprising an immunogenic composition disclosed herein and an information leaflet.

85. A kit comprising an immunogenic composition of any one of paragraphs 1-67 and an information leaflet.

86. The kit of paragraph 85 or 86 wherein said information leaflet mentions the ability of the composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N.

87. The kit of paragraph 85 or 86 wherein said information leaflet mentions the ability of the composition to elicit functional antibodies against *S. pneumoniae* serotype 9N.

88. The kit of paragraph 85 or 86 wherein said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N at a concentration ≥ 0.35 µg/mL in a human population.

89. The kit of paragraph 85 or 86 wherein said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotype 9N at a concentration ≥ 0.35 µg/mL in a human population.

90. The kit of any one of paragraphs 85-89 wherein said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9A, 9L and/or 9N in a human population.

91. The kit of any one of paragraphs 85-89 wherein said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9N in a human population.

92. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

-producing an immunogenic composition of of any one of paragraphs 1-67 and

Petitioner Merck Ex-1015, 1342

-combining in the same kit said immunogenic composition and information leaflet, wherein said information leaflet mentions the ability of said composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N.

93. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

-producing an immunogenic composition of any one of paragraphs 1-67 and

- combining in the same kit said immunogenic composition and information leaflet, wherein said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N at a concentration ≥ 0.35 µg/mL in a human population.

94. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

-producing an immunogenic composition of any one of paragraphs 1-67 and

- combining in the same kit said immunogenic composition and information leaflet, wherein said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9A, 9L and/or 9N in a human population.

95. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

-producing an immunogenic composition of any one of paragraphs 1-67;

-printing an information leaflet wherein said information leaflet mentions the ability of said composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N;

-combining in the same kit said immunogenic composition and said information leaflet.

96. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

-producing an immunogenic composition of of any one of paragraphs 1-67;

-printing an information leaflet wherein said information leaflet mentions the ability of the composition to elicit anti-capsular antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N at a concentration ≥ 0.35 µg/mL in a human population;

-combining in the same kit said immunogenic composition and said information leaflet.

97. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:

-producing an immunogenic composition of of any one of paragraphs 1-67;

Petitioner Merck Ex-1015, 1343

- printing an information leaflet wherein said information leaflet mentions the ability of the composition to elicit OPA titers against *S. pneumoniae* serotypes 9A, 9L and/or 9N in a human population;

-combining in the same kit said immunogenic composition and said information leaflet.

98. A method comprising the step of:

- injecting to a subject an immunologically effective amount of the immunogenic composition defined at any one of paragraphs 1-67;

- collecting a serum sample from said subject;

- testing said serum sample for opsonophagocytic killing activity against *S. pneumoniae* serotype 9N, 9A and/or 9L by *in vitro* opsonophagocytic killing assay (OPA).

99. A method of inducing an immune response to *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of any one of paragraphs 1-67.

100. Use of an immunogenic composition of any one of paragraphs 1-67 for the manufacture of a medicament for immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L.

101. Use of an immunogenic composition of any one of paragraphs 1-67 for the manufacture of a medicament for preventing, treating or ameliorating an infection, disease or condition caused by *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject.

102. Use of an immunogenic composition of any one of paragraphs 1-67 for the manufacture of a medicament for preventing infection by serotypes 9N, 9A and/or 9L *S. pneumoniae* in a subject.

As used herein, the term "about" means within a statistically meaningful range of a value, such as a stated concentration range, time frame, molecular weight, temperature or pH. Such a range can be within an order of magnitude, typically within 20%, more typically within 10%, and even more typically within 5% or within 1% of a given value or range. Sometimes, such a range can be within the experimental error typical of standard methods used for the measurement and/or determination of a given value or range. The allowable variation encompassed by the term "about" will depend upon the particular system under study, and can be readily appreciated by one of ordinary skill in the art. Whenever a range is recited within this application, every whole number integer within the range is also contemplated as an embodiment of the disclosure.

Petitioner Merck Ex-1015, 1344

The terms "comprising", "comprise" and "comprises" herein are intended by the inventors to be optionally substitutable with the terms "consisting of", "consist of" and "consists of", respectively, in every instance.

All references or patent applications cited within this patent specification are incorporated
by reference herein.

The invention is illustrated in the accompanying examples. The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

EXAMPLE

Example 1. Evaluation of Cross-Reactive Opsonophagocytic Immune Responses within Serogroup 9 of *Streptococcus pneumoniae*

The pneumococcal opsonophagocytic assay (OPA), which measures killing of *S. pneumoniae* cells by phagocytic effector cells in the presence of functional antibody and complement, is considered to be an important surrogate for evaluating the effectiveness of pneumococcal vaccines.

Materials and Methods

Two randomly selected subsets of immune sera from adults vaccinated with a 13-valent pneumococcal conjugate vaccine (13vPnC) were tested in OPA assays for the serotypes 9V, 9A, 9L and 9N. The sera were collected from U.S. clinical trials 6115A1-004 (N=59, post-vaccinated) and 6115A1-3005 (N=66, matched pre-and post-vaccination), respectively.

Study 6115A1-3005 (ClinicalTrials.gov Identifier: NCT00546572) was a phase 3, randomized, active-controlled, modified double-blind trial evaluating the safety, tolerability, and immunogenicity of PREVNAR 13® compared with a 23-valent pneumococcal polysaccharide vaccine (23vPS) in ambulatory elderly individuals aged 70 years and older who received 1 dose of 23vPS at least 5 years before study

Petitioner Merck Ex-1015, 1345

enrollment (see http://clinicaltrials.gov/ct2/show/NCT00546572, accessed on March 31, 2014).

Study 6115A1-004 (ClinicalTrials.gov Identifier: NCT00427895) was a phase 3, randomized, active-controlled, modified double-blind trial evaluating the safety, tolerability, and immunogenicity of a 13-valent pneumococcal conjugate vaccine (13vPnC) compared to a 23-valent pneumococcal polysaccharide vaccine (23vPS) in adults 60 to 64 years old who are naive to 23vPS and the safety, tolerability, and immunogenicity of 13vPnC in adults 18 to 59 years old ho are naïve to 23vPS (see: http://clinicaltrials.gov/show/NCT00427895, accessed on March 31' 2014).

The 13-valent pneumococcal conjugate vaccine (13vPnC) tested in these studies contained conjugates from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, individually conjugated to diphtheria cross-reacting material 197 (CRM_{197}) carrier protein.

OPAs are used to measure functional antibodies in human sera agaisnt *S. pneumoniae* serotypes 9V, 9N, 9A and/or 9L. Test serum is set up in assay reactions that measure the ability of capsular polysaccharide specific immunoglobulin to opsonize bacteria, trigger complement deposition, thereby facilitating phagocytosis and killing of bacteria by phagocytes. The OPA titer is defined as the reciprocal dilution that results in a 50% reduction in bacterial count over control wells without test serum. The OPA titer is interpolated from the two dilutions that encompass this 50% killing cut-off.
OPA procedures were based on methods described in Hu et al., (2005) Clin Diagn Lab Immunol12:287–295. Test heat-inactivated serum was serially diluted 2.5-fold and was added together with the target bacteria in assay plates and incubated for 30 minutes with shaking. Differentiated HL-60 cells (phagocytes) and baby rabbit serum (3- to 4-week old, PEL-FREEZ®, Arkansas, 12.5% final concentration) were then added to the wells, at an approximate effector to target ratio of 200:1, and incubated at 37°C with shaking. To terminate the reaction, 80 µL of 0.9% NaCl was added to all wells, mixed, and a 10 µL aliquot was transferred to the wells of MULTISCREEN® HTS HV filter plates (MILLIPORE®) containing 200 µL of water. Liquid was filtered through the plates under vacuum, and 150 µL of HYSOY® medium was added to each well and filtered through. The filter plates were then incubated at 37°C, 5% CO_2 overnight and were then fixed with Destain Solution (Bio-Rad Laboratories, Inc., Hercules, CA). The plates were

Petitioner Merck Ex-1015, 1346

then stained with Coomassie Blue and destained once. Colonies were imaged and enumerated on a Cellular Technology Limited (CTL) (Shaker Heights, OH) IMMUNOSPOT® Analyzer. The OPA antibody titer was determined as the reciprocal of the lowest serum dilution resulting in 50% reduction in the number of bacterial colonies when compared to the bacteria-effector cell-complement control wells that did not contain serum.

Statistical Analysis: Pearson two-tailed correlations were calculated.

Results - OPA Responses in 9V, 9A, 9L and 9N

The cross-functional response from immune sera of adults immunized with 13vPnC against serotypes 9A, 9L, and 9N, was evaluated in the respective microcolony Opsonophagocytic Assays (mcOPAs), along with the homologous functional response to serotype 9V. Two randomly selected subsets of immune sera from adults vaccinated with 13vPnC were tested. The sera were collected from U.S. clinical trials 6115A1-004 (N=59, post-vaccinated) and 6115A1-3005 (N=66, matched pre-and post-vaccination), respectively.

Subjects in study 6115A1-004 were previously naïve to any pneumococcal vaccination and received a single dose of 13vPnC as part of the study protocol. The immune sera from study 6115A1-004 shows a similar percentage of responders for all the serogroups with values of 98.3%, 98.3%, 100% and 93.2% for 9V, 9A, 9L and 9N respectively (Figure 1), supporting the results from 6115A1-3005 (Figure 2). A relative good OPA titer correlations were observed between serotypes 9V and 9A (Pearson correlation ρ = 0.5456, $p < 0.0001$) or 9L (ρ = 0.7353, $p < 0.0001$), but not with 9N (ρ = 0.1217, $p <$ 0.3627).

Subjects in study 6115A1-3005 had previously received 1 dose of 23vPS at least 5 years before study enrollment and received a single dose of 13vPnC as part of the study protocol. Matched pre- and post-vaccination serum panel (N = 66) from adults immunized with 13vPnC (study 6115A1-3005) was evaluated on OPA for the homologous response to serotype 9V and for cross-reactivity of anti-9V antibodies to serotypes 9A, 9L, and 9N. As shown in Figure 2, a relatively high immunity (percentage responders) to 9A (84%), 9A (66%), 9L (82%) and 9N (86%) was detected in the OPA assay likely due to their previous immunization with 23vPS, which includes unconjugated polysaccharides from serotypes 9V and 9N. However, the percentage

Petitioner Merck Ex-1015, 1347

responders increased to 95% or more for all four serotypes after vaccination with 13vPnC, which only contains serotype 9V conjugate from serogroup 9. The fold-rise in titer values are shown in Table 1 and are similar between the serotypes also suggesting cross-reactivity.

Table 1. OPA Titer Fold-Rise Matched Pre- and Post-Vaccination, 13vPnC

	OPA Titers							
	9V		9A		9L		9N	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
GMT	221	1323	41	308	165	706	322	693
Fold-rise	5.9		7.5		4.2		2.1	

A more comprehensive analysis of the OPA titer distribution is shown in the reverse cumulative distribution curves (RCDC) in Figures 3-6. The RCDCs show an increase in serotype-specific immune response post vaccination for serotypes 9V, 9A, 9L and to a lesser extent 9N. The correlation of the fold-rise of titer of individual matched / samples between 9V 9A, 9V/9L, and 9V/9N were also analyzed using Pearson's correlation. Relatively good correlations of fold-rises of titers were observed between serotypes 9V and 9A (Pearson correlation $\rho = 0.8720$, $p < 0.0001$) or 9N ($\rho = 0.5801$, $p < 0.0001$), but to a lesser extent with 9L ($\rho = 0.1804$, $p < 0.1640$).

Conclusion

Based on these data, the 13vPnC vaccine is likely to provide broader serotype coverage by providing additional protection against serotypes 9A, 9L, and 9N.

Example 2. Further evaluation of Cross-Reactive Opsonophagocytic Immune Responses within Serogroup 9 of *Streptococcus pneumoniae*

The ability of 13vPnC-induced serotype 9V-specific antibodies to functionally cross-react with bacteria from serotypes 9A, 9L and 9N (i.e. kill bacteria in serotypes 9A, 9L and 9N OPAs) was further assessed in serum from a third clinical study.

Petitioner Merck Ex-1015, 1348

Materials and Methods

Randomly selected subsets of immune sera from adults vaccinated with a 13-valent pneumococcal conjugate vaccine (13vPnC) were tested in OPA assays for the serotypes 9V, 9A, 9L and 9N. The sera were collected from pre- and post-vaccination serum from the Japanese adult clinical study B1851088 (ClinicalTrials.gov Identifier: NCT01646398)

Study B1851088 was a phase 3, randomized, modified double-blind, active-controlled trial evaluating the safety, tolerability and immunogenicity of a 13-valent pneumococcal conjugate vaccine In Japanese elderly adults aged 65 years old and older who are naive to pneumococcal vaccine (see https://clinicaltrials.gov/ct2/show/NCT01646398 , accessed on November 30, 2015).

The 13-valent pneumococcal conjugate vaccine (13vPnC) tested in these studies contained conjugates from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, individually conjugated to diphtheria cross-reacting material 197 (CRM_{197}) carrier protein.

OPAs and statistical analysis were conducted as described at Example 1.

Results - OPA Responses in 9V, 9A, 9L and 9N

Subjects in study B1851088 were not previously vaccinated with pneumococcal vaccines and received a single dose of either 13vPnC or 23vPS (a 23-valent pneumococcal polysaccharide vaccine consisting of a mixture of purified capsular polysaccharides from types 1, 2, 3, 4, 5, 6B, 7F,8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F) as part of the study protocol. The cross-functional response to serotypes 9A, 9L, and 9N in immune sera from Japanese adults immunized with 13vPnC or 23vPS was evaluated in the respective serogroup 9 OPAs.

A subset of pre-and post-immune sera from adults vaccinated with 13vPnC or 23vPS were tested in OPA assays for serotypes 9A, 9L, and 9N. 9V OPA titers were obtained from reported clinical data. The sera were selected from Japan clinical trial B1851088 13vPnC arm (N = 91, matched pre-and post-vaccination) and 23vPS arm (N = 83, matched pre-and post-vaccination). The sera were chosen from the subjects who's pre-

Petitioner Merck Ex-1015, 1349

vaccination OPA titers were low or negative against 9V from reported clinical data. All subjects from B1851088 were not pre-exposed to 23vPS vaccine.

Relatively low baseline serogroup 9 OPA titer was observed in subjects prior to vaccination. Following vaccination with either 13vPnC or 23vPS, serogroup 9 OPA responses increased to high responder rates: 100%, 98%, 92%, and 96% for 9V, 9A, 9L, and 9N respectively from 13vPnC subjects and 100%, 88%, 94%, and 100% for 9V, 9A, 9L, and 9N respectively from 23vPS subjects (Figure 7 and Figure 8).

While 13vPnC only contains serotype 9V polysaccharide from serogroup 9, 23vPS contains polysaccharides from both 9V and 9N. Therefore, serotype 9N responses are homologous following 23vPS vaccination and heterologous (cross-reaction from serptype 9V) following 13vPnC vaccination.

Serogroup 9 OPA GMTs following vaccination are high for all serogroup 9 serotypes regardless of the vaccine received (Figure 9). Figure 10 through Figure 15 display reverse cumulative distribution curves for the serogroup 9 OPA responses to either 13vPnC or 23vPS vaccination.

Functional cross-reactive serogroup 9 OPA responses were evaluated in serum specimens from three pneumococcal vaccine studies (6115A1-004, 6155A1-3005 (example 1) and B1851088 (example 2)). Surprisingly, the 13vPnC induced immune response to serotype 9V cross-reacted with all heterologous serotypes within serogroup 9 (9A, 9L, and 9N) to varying degrees. Based on these data, the 13vPnC vaccine should provide additional protection against serotypes 9A, 9L, and 9N in addition to the expected protection against serotype 9V.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are hereby incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, certain changes and modifications may be practiced within the scope of the appended claims.

Petitioner Merck Ex-1015, 1350

Claims

1. An immunogenic composition comprising at least one glycoconjugate from *S. pneumoniae* serotype 9V for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and/or 9L.
2. The immunogenic composition of claim 1, wherein said composition does not comprise capsular saccharide from *S. pneumoniae* serotypes 9N, 9A and 9L
3. The immunogenic composition composition of any one of claims 1-2 further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 14, 18C, 19F and 23F.
4. The immunogenic composition of any one of claims 1-3 further comprising at least one glycoconjugate from *S. pneumoniae* serotypes 1, 3, 5, 6A, 7F, and 19A, .
5. The immunogenic composition of any one of claims 1-4 which is able to elicit IgG antibodies in human which are capable of binding *S. pneumoniae* serotypes 9N polysaccharide at a concentration of at least 0.35 μg/ml as determined by ELISA assay.
6. The immunogenic composition of any one of claims 1-5 which is able to elicit functional antibodies in human which are capable of killing *S. pneumoniae* serotype 9N, 9A and/or 9L as determined by in vitro opsonophagocytic assay (OPA).
7. The immunogenic composition of any one of claims 1-6 which is able to elicit a titer of at least 1:8 against *S. pneumoniae* serotype 9N in at least 50% of the subjects as determined by *in vitro* opsonophagocytic killing assay (OPA).
8. The immunogenic composition of any one of claims 1-7, for use in a method of immunizing a subject against infection by *S. pneumoniae* serotype 9N, 9A and 9L.
9. The immunogenic composition of any one of claims 1-7 for use to prevent serotypes 9N, 9A and/or 9L *S. pneumoniae* infection in a subject.
10. A method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of any one of claims 1-7.

Petitioner Merck Ex-1015, 1351

11. A kit comprising an immunogenic composition of any one of claims 1-7 and an information leaflet.

12. The kit of claim 11 wherein said information leaflet mentions the ability of the composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N.

13. A process for producing a kit comprising an immunogenic composition and an information leaflet, said process comprising the step of:
-producing an immunogenic composition of any one of claims 1-7;
-printing an information leaflet wherein said information leaflet mentions the ability of said composition to elicit functional antibodies against *S. pneumoniae* serotypes 9A, 9L and/or 9N;
-combining in the same kit said immunogenic composition and said information leaflet.

14. A method of inducing an immune response to *S. pneumoniae* serotypes 9N, 9A and/or 9L in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of any one of claims 1-7.

15. Use of an immunogenic composition of any one of claims 1-7 for the manufacture of a medicament for preventing infection by serotypes 9N, 9A and/or 9L *S. pneumoniae* in a subject.

Petitioner Merck Ex-1015, 1352

FIG. 1

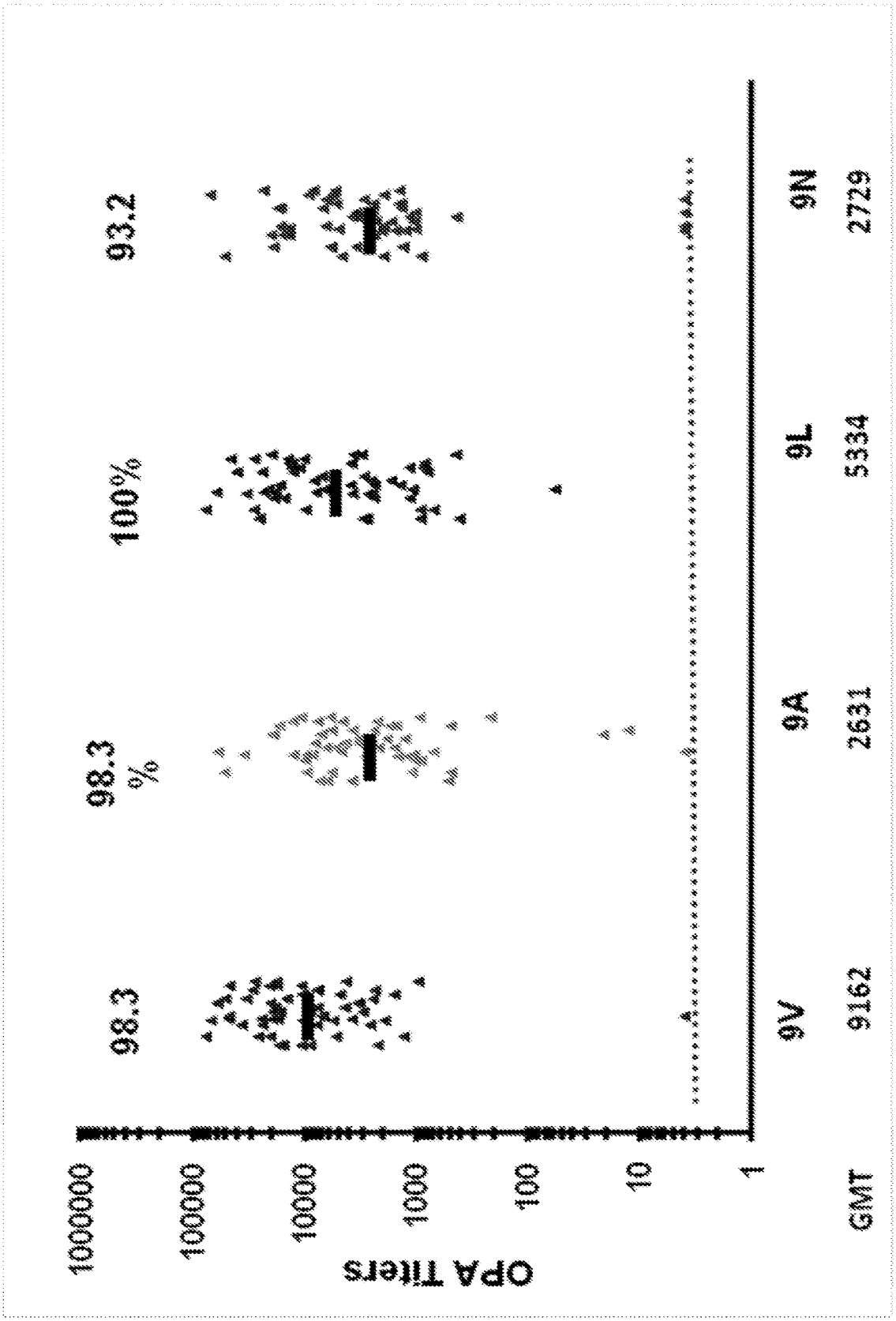

OPA Titers
1000000
100000
10000
1000
100
10
1
98.3
%
100%
93.2
98.3
9V
9A
9L
9N
GMT
9162
2631
5334
2729

FIG. 2

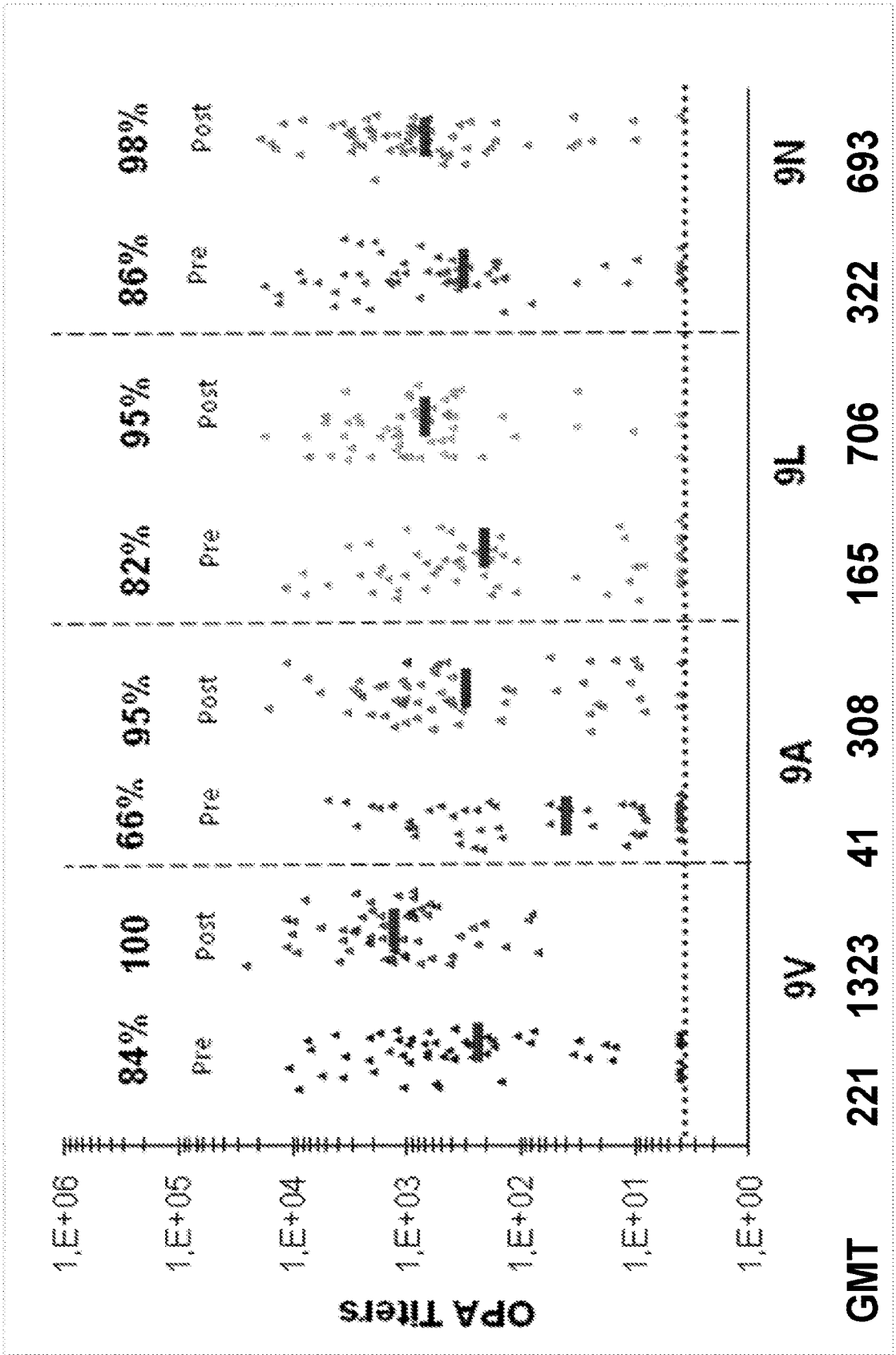

OPA Titers
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
Pre
Post
84%
100
66%
95%
82%
95%
86%
98%
GMT
221
1323
41
308
165
706
322
693
9V
9A
9L
9N

FIG. 3

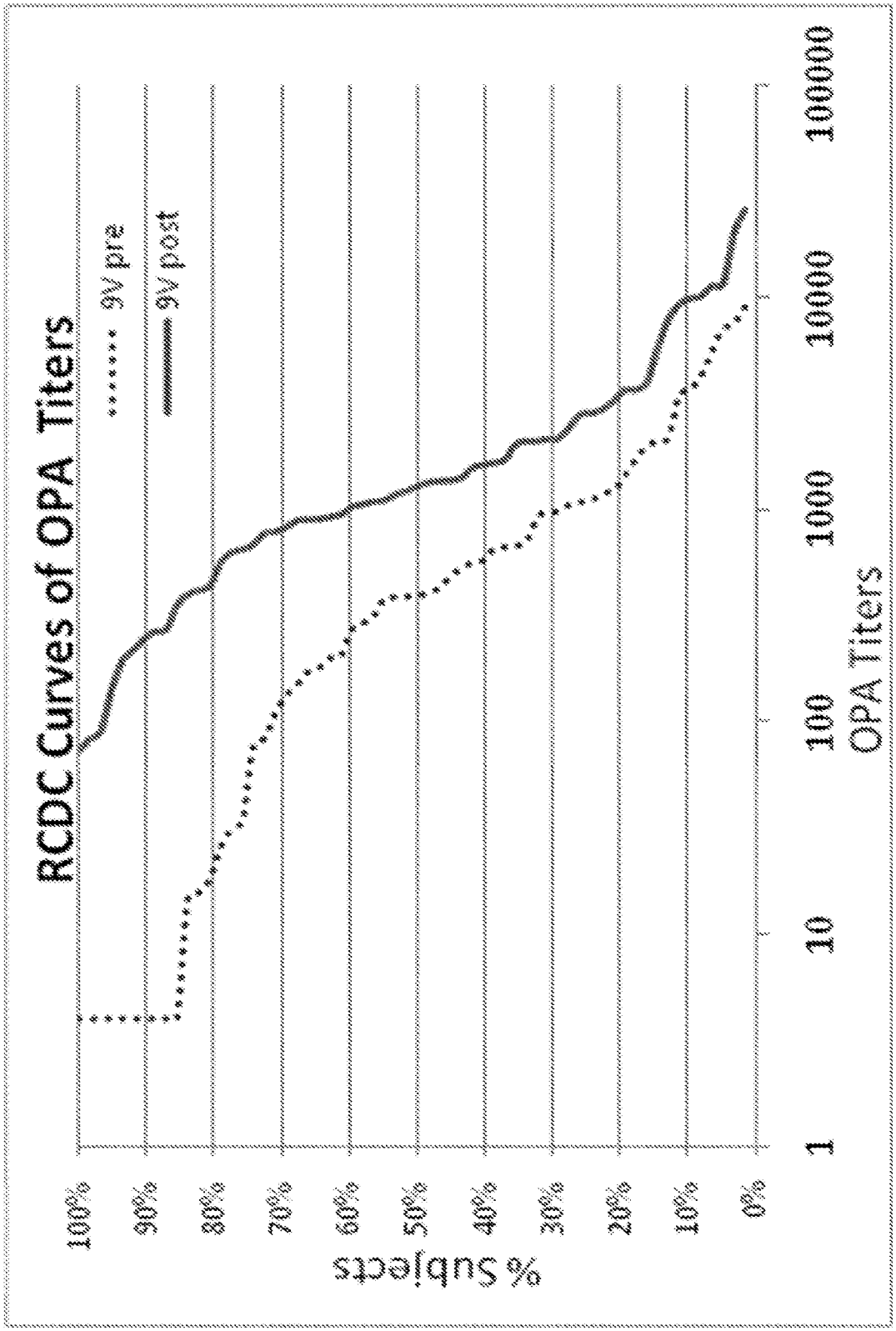

RCDC Curves of OPA Titers
9V pre
9V post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 4

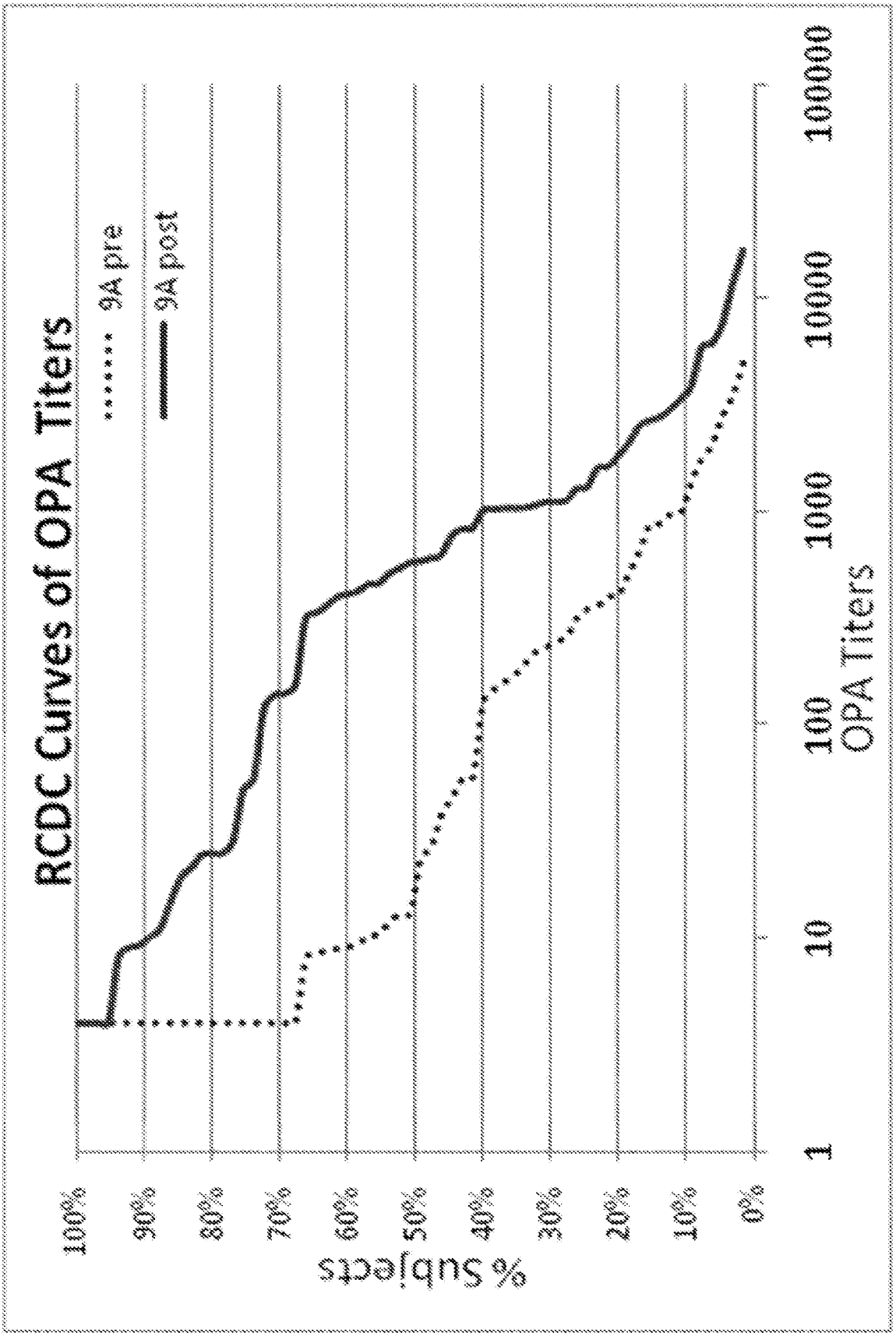

RCDC Curves of OPA Titers
9A pre
9A post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 5

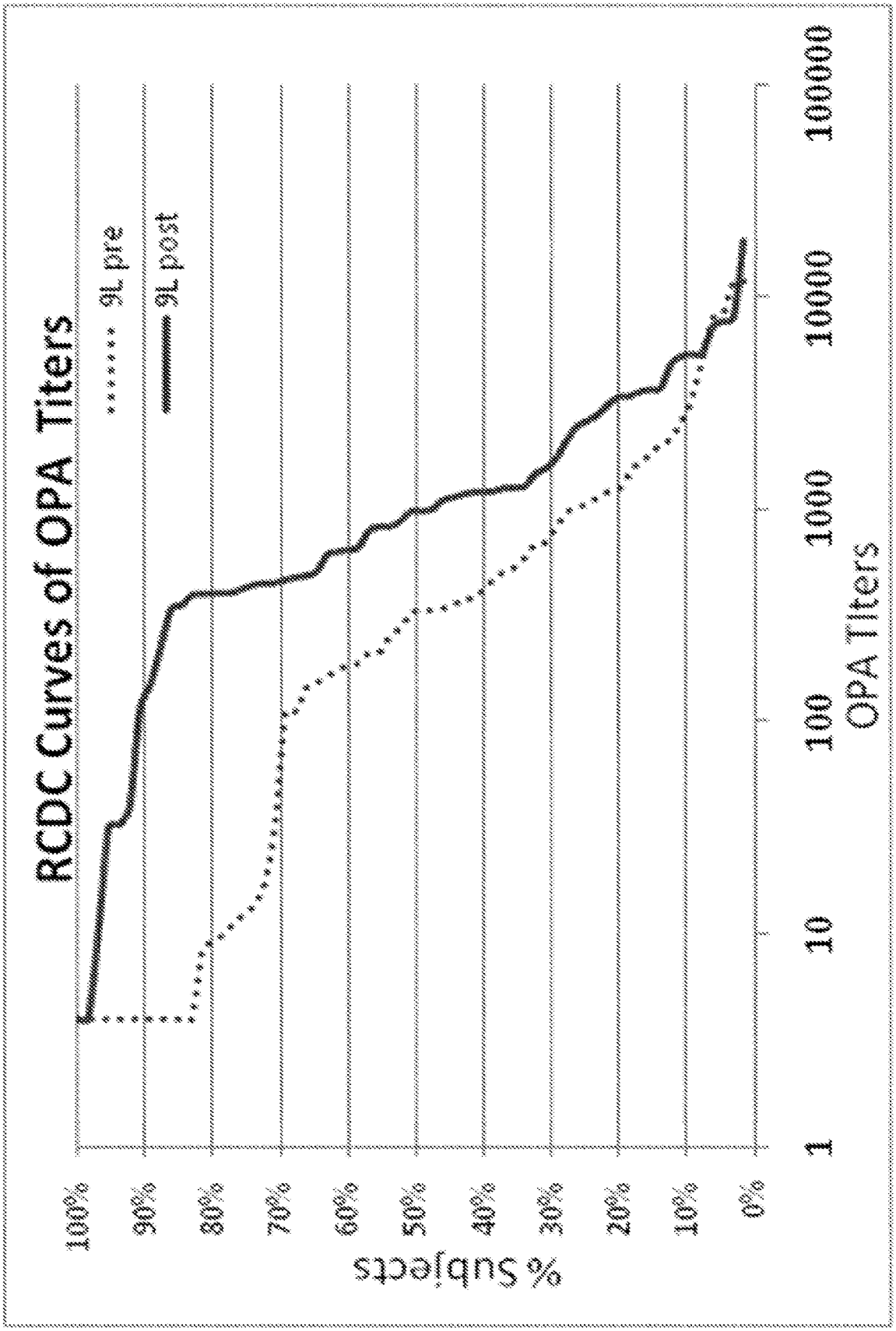

RCDC Curves of OPA Titers
9L pre
9L post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 6

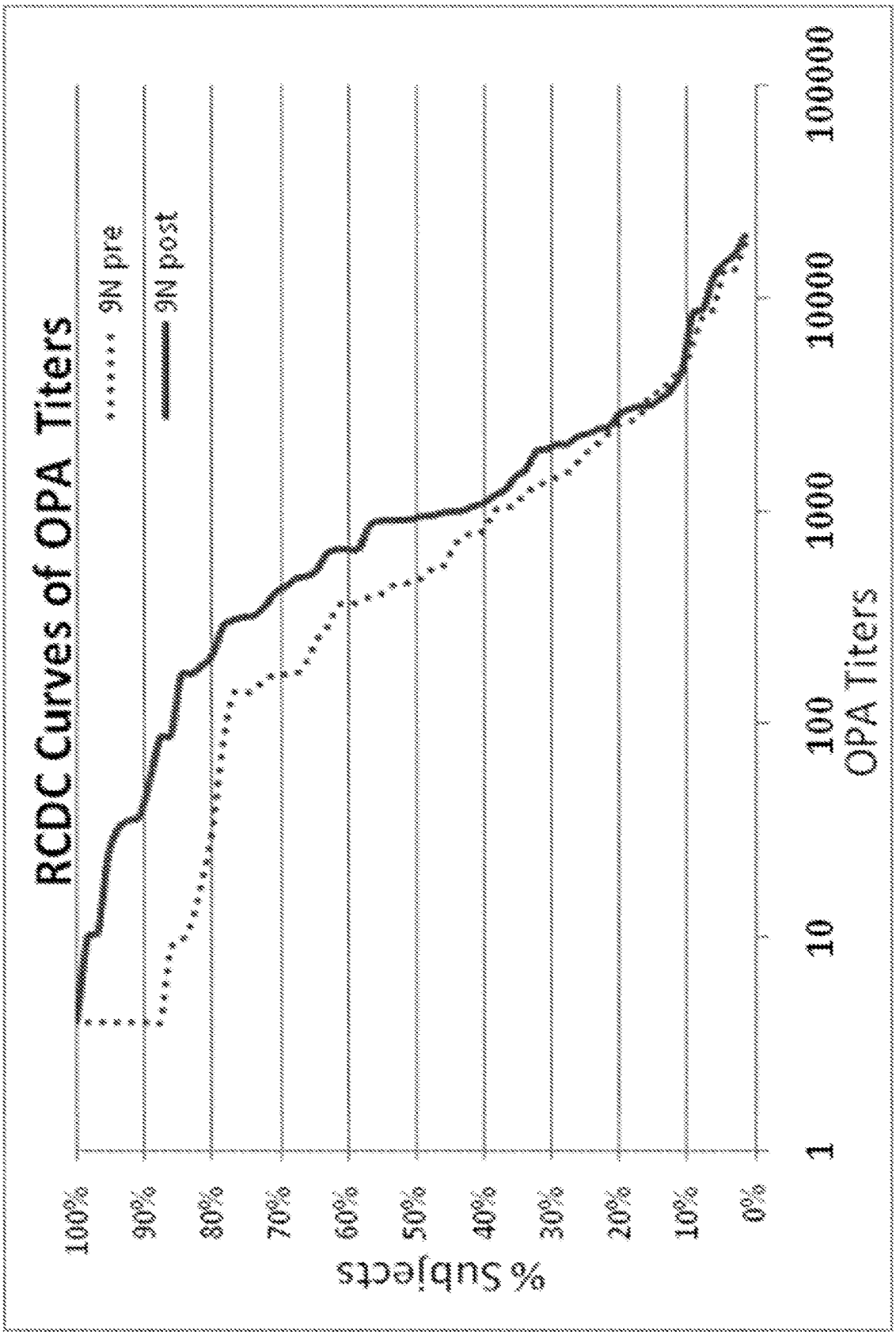

RCDC Curves of OPA Titers
9N pre
9N post
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 7

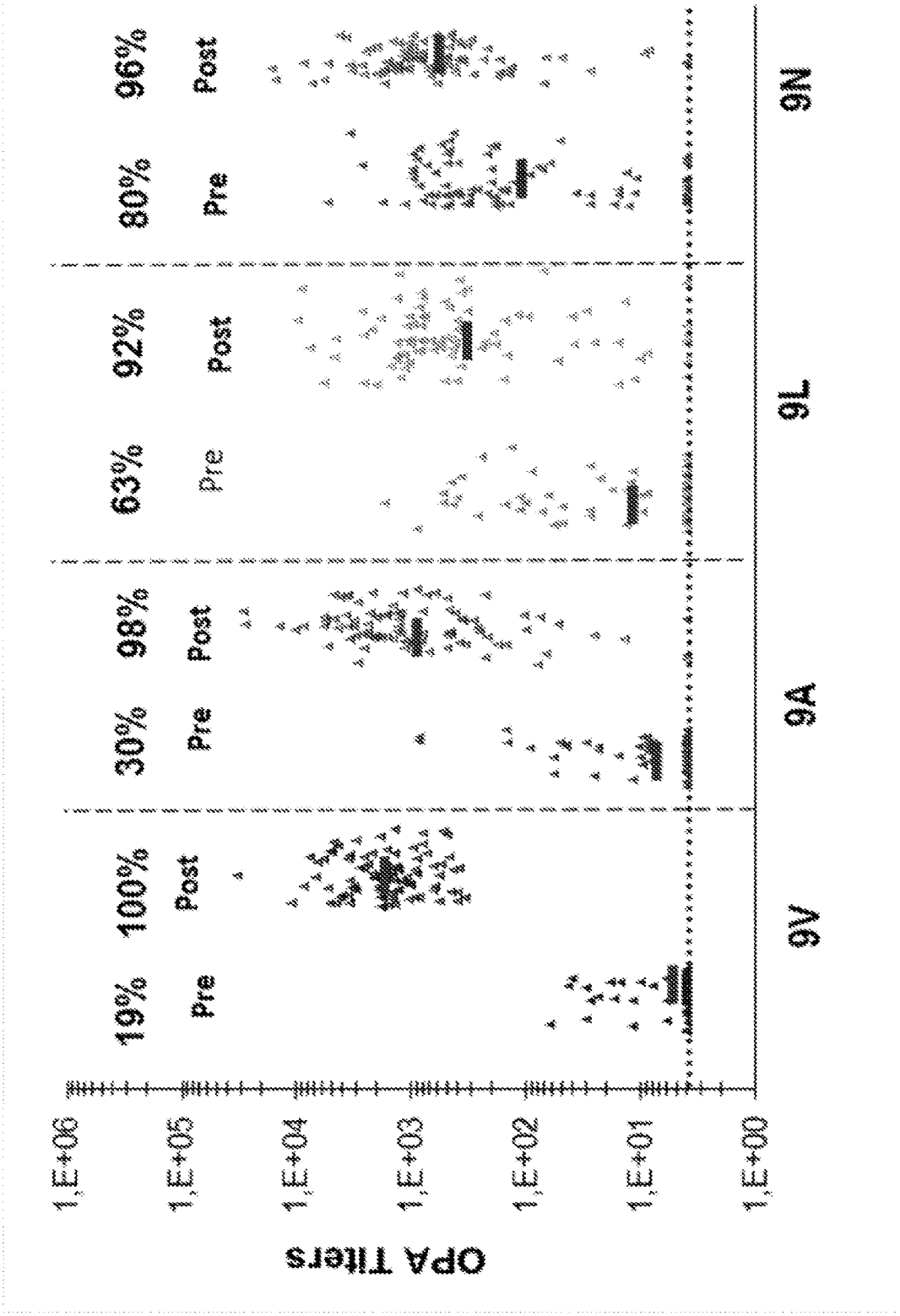

OPA Titers
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1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
9V
9A
9L
9N
19%
Pre
100%
Post
30%
Pre
98%
Post
63%
Pre
92%
Post
80%
Pre
96%
Post

FIG. 8

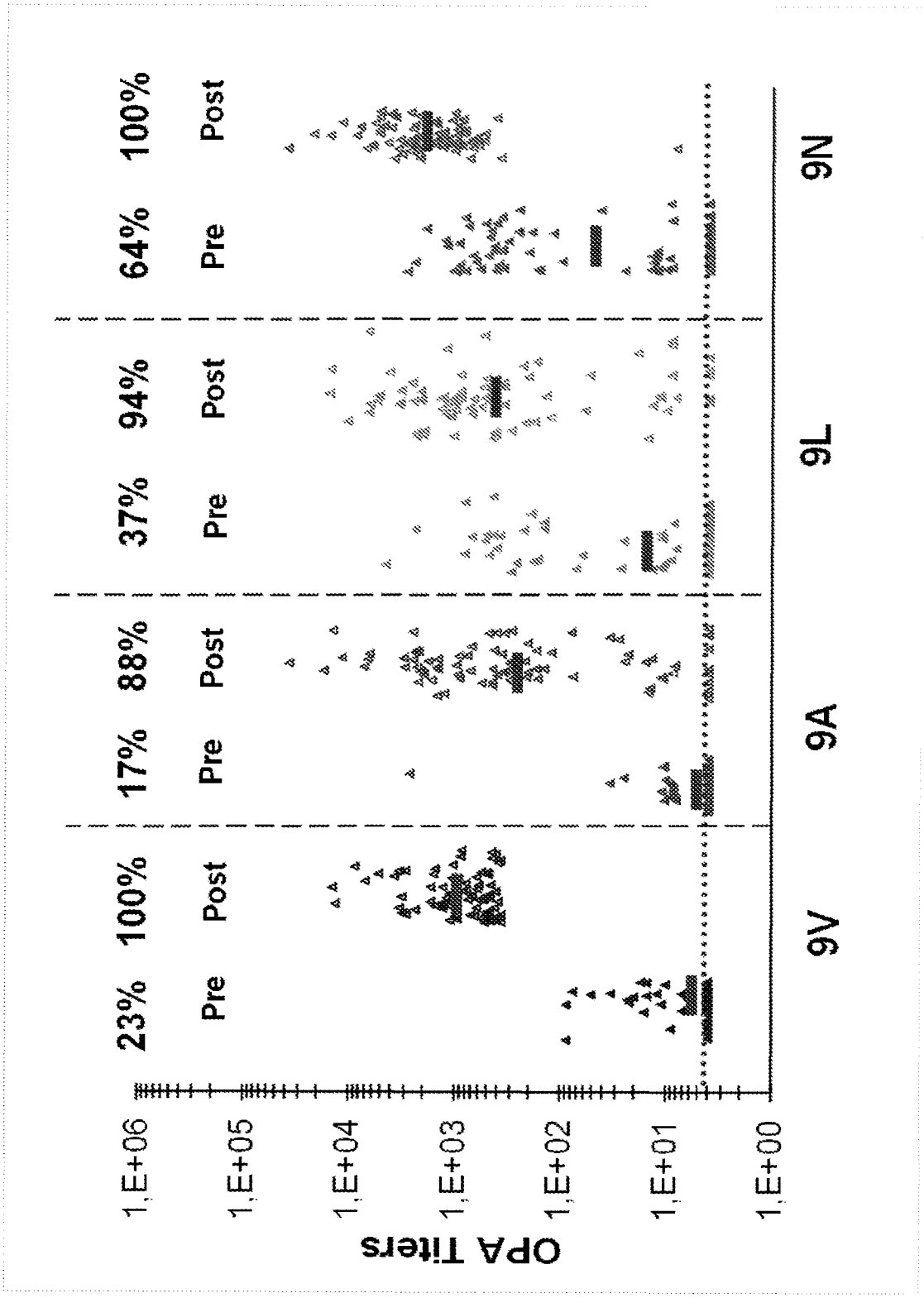

OPA Titers
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
9V
9A
9L
9N
23%
100%
Pre
Post
17%
88%
Pre
Post
37%
94%
Pre
Post
64%
100%
Pre
Post

FIG. 9

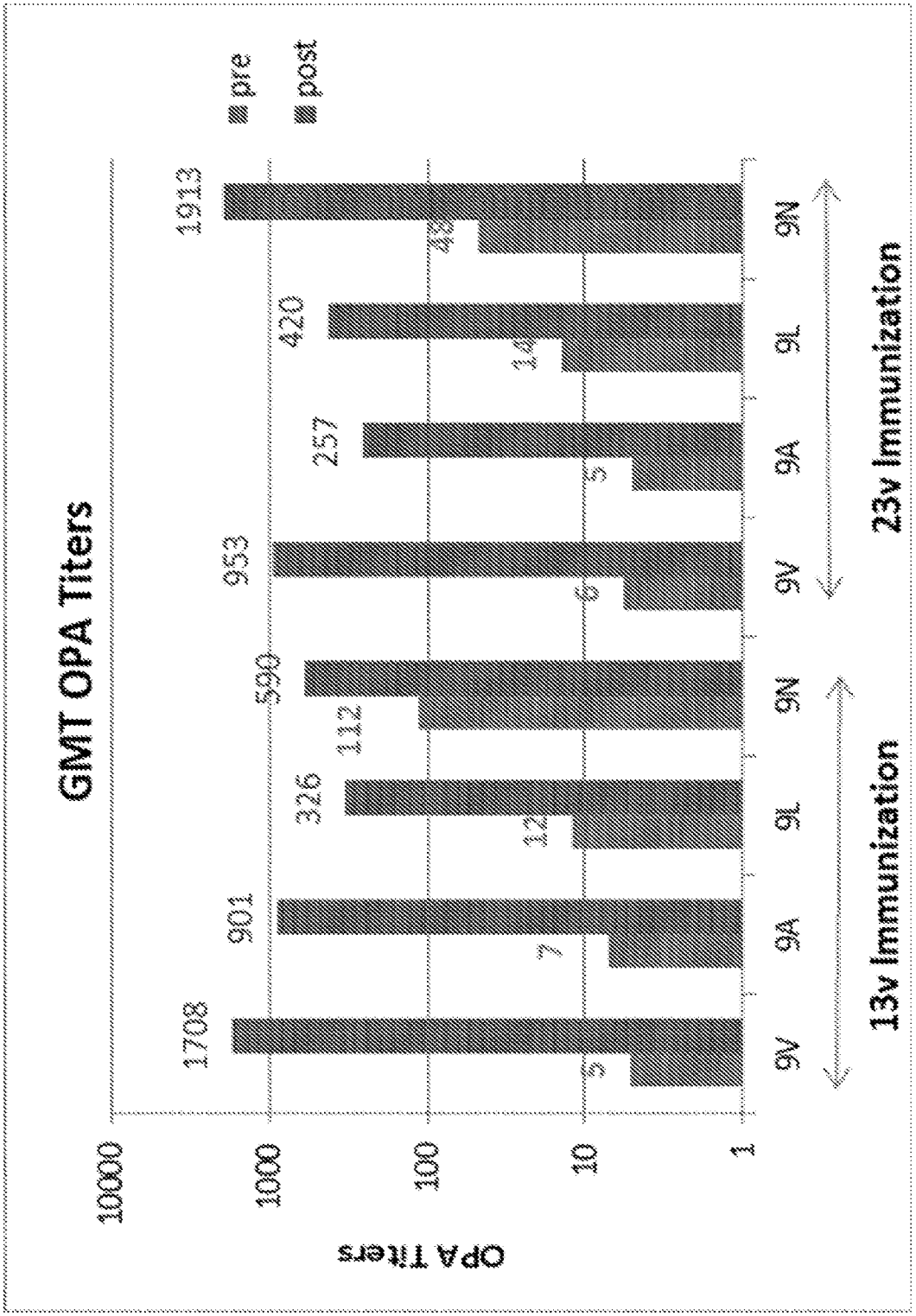

GMT OPA Titers
pre
post
OPA Titers
10000
1000
100
10
1
1708
5
901
7
326
12
590
112
953
6
420
14
257
5
1913
43
9V
9A
9L
9N
9V
9A
9L
9N
13v Immunization
23v Immunization

FIG. 10

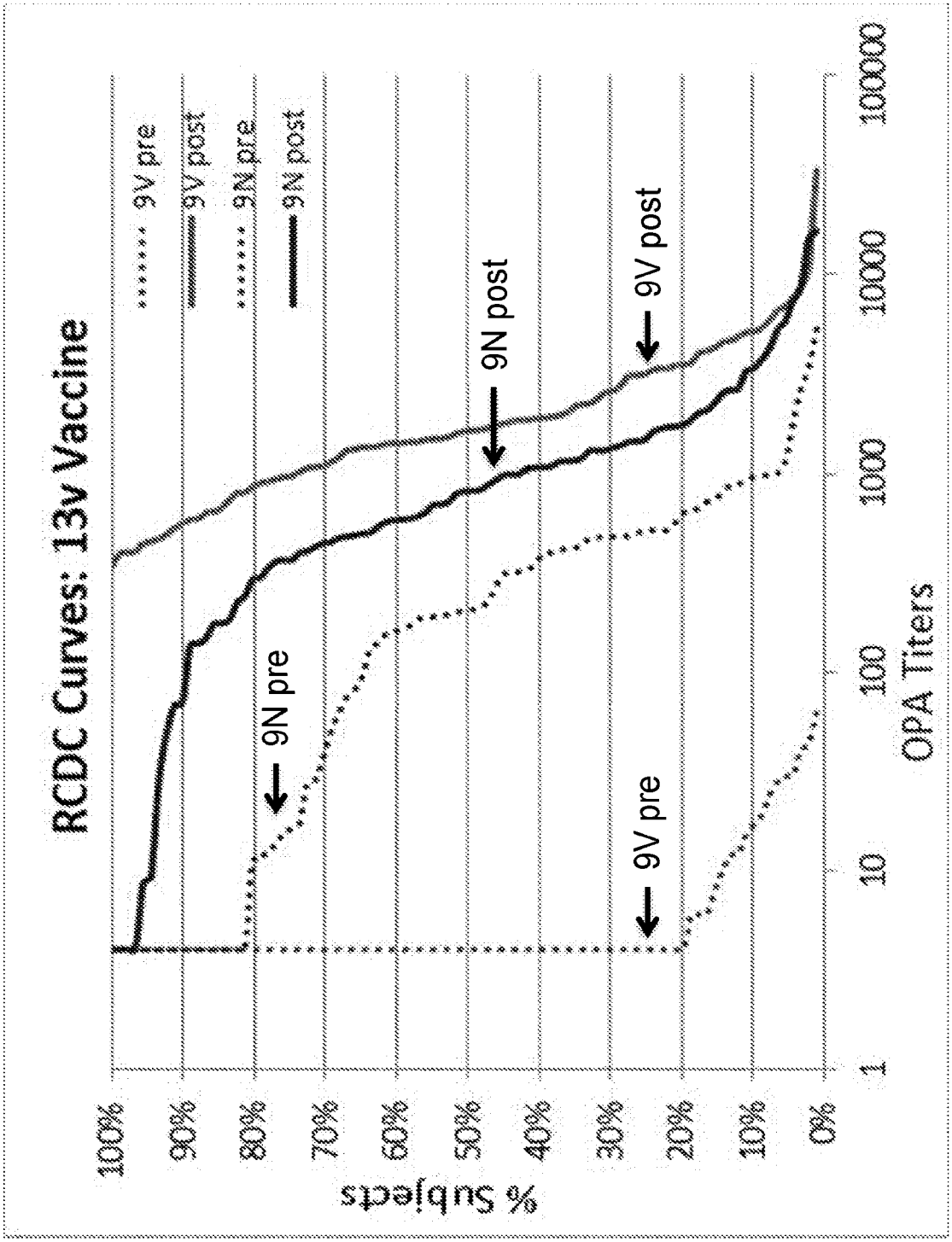

RCDC Curves: 13v Vaccine
9V pre
9V post
9N pre
9N post
9N pre
9N post
9V post
9V pre
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 11

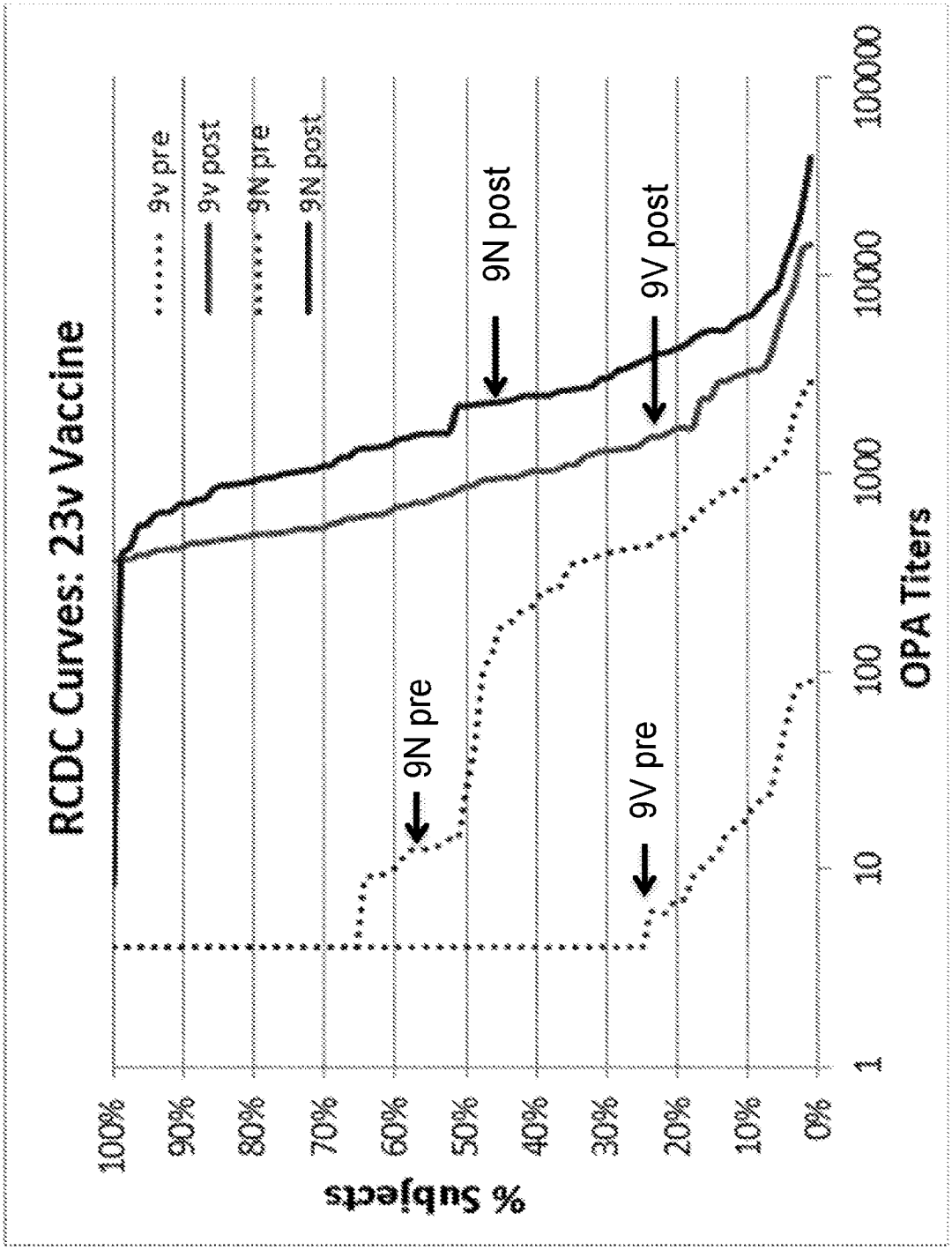

RCDC Curves: 23v Vaccine
9v pre
9v post
9N pre
9N post
9N post
9V post
9N pre
9V pre
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 12

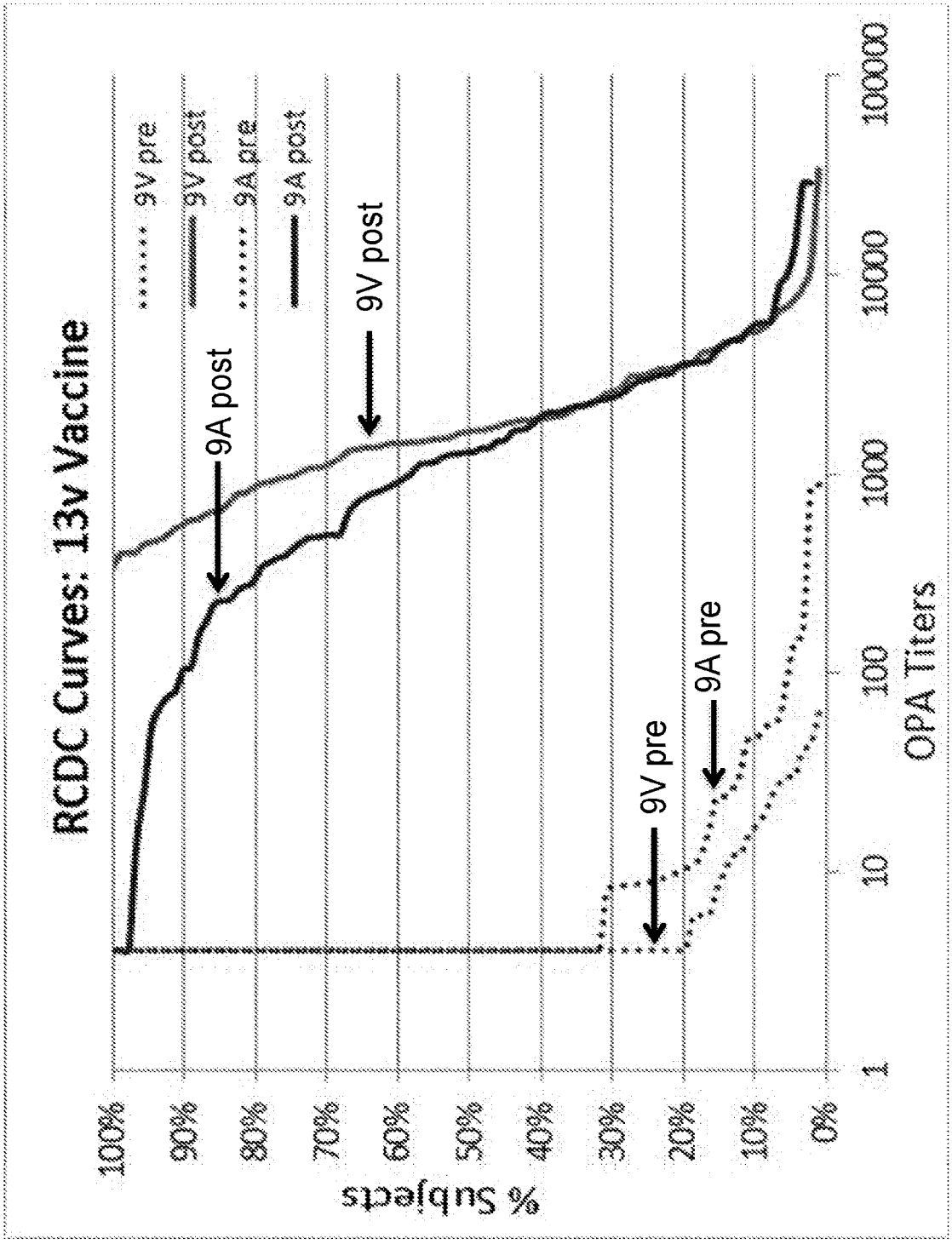

RCDC Curves: 13v Vaccine
9V pre
9V post
9A pre
9A post
9A post
9V post
9V pre
9A pre
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 13

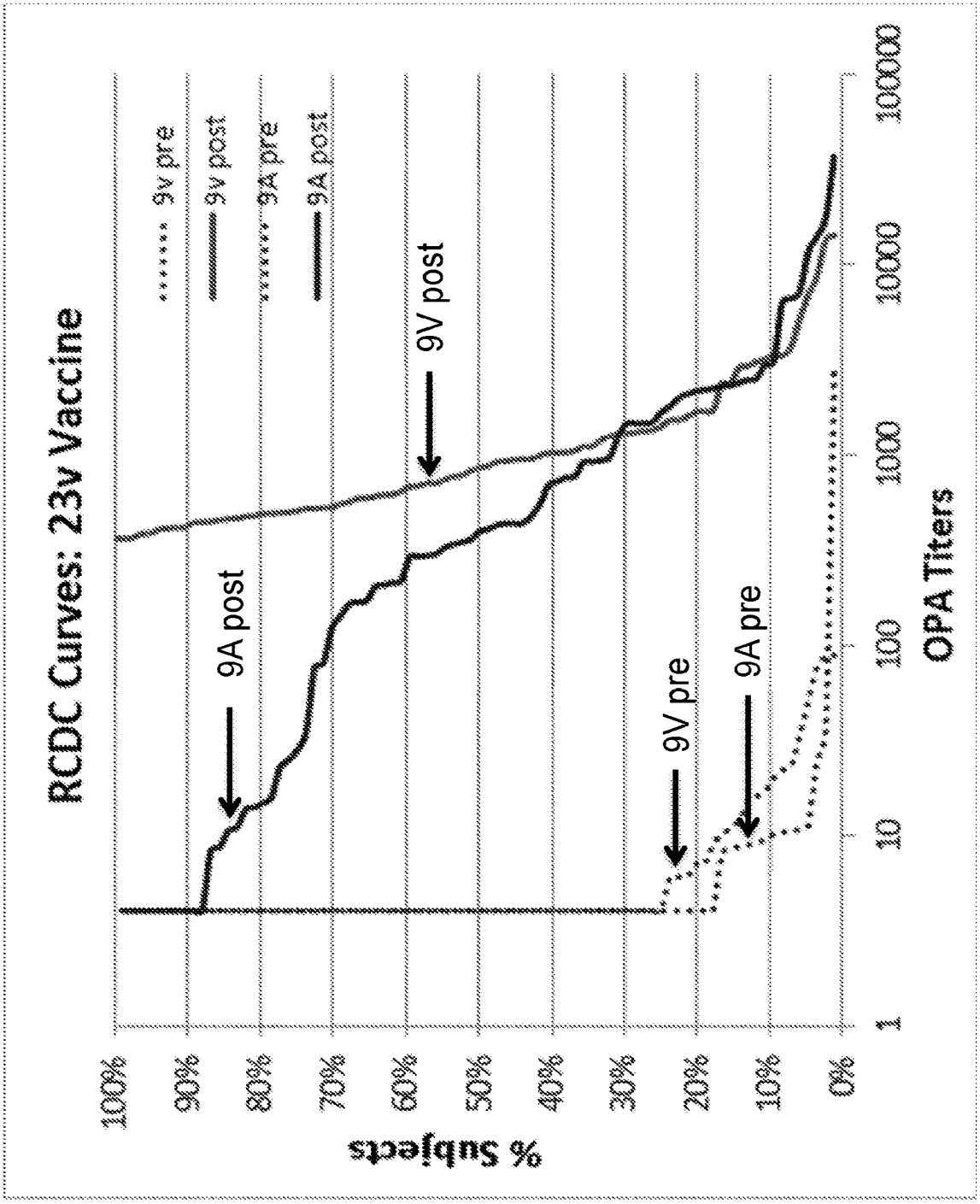

RCDC Curves: 23v Vaccine
9v pre
9v post
9A pre
9A post
9A post
9V post
9V pre
9A pre
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 14

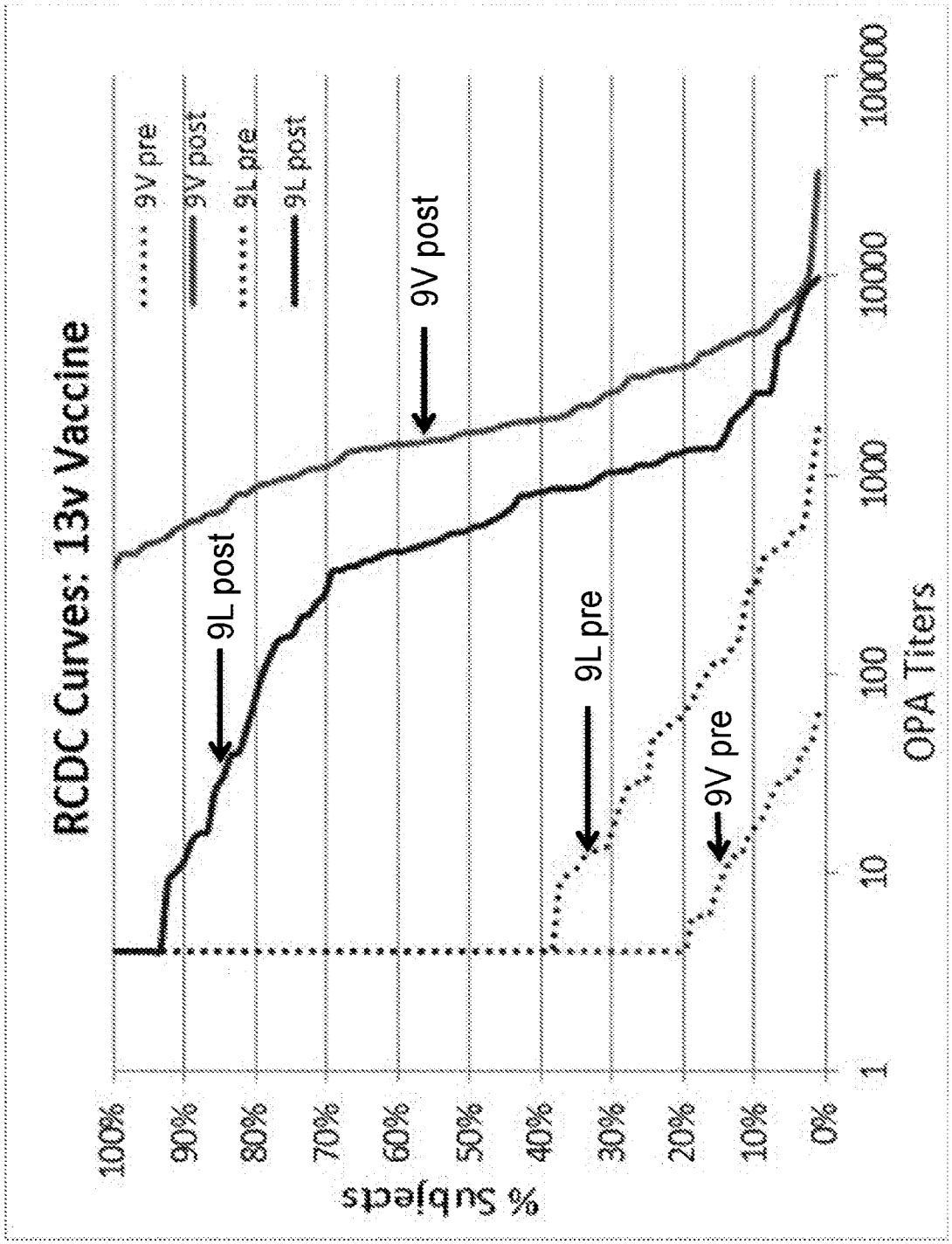
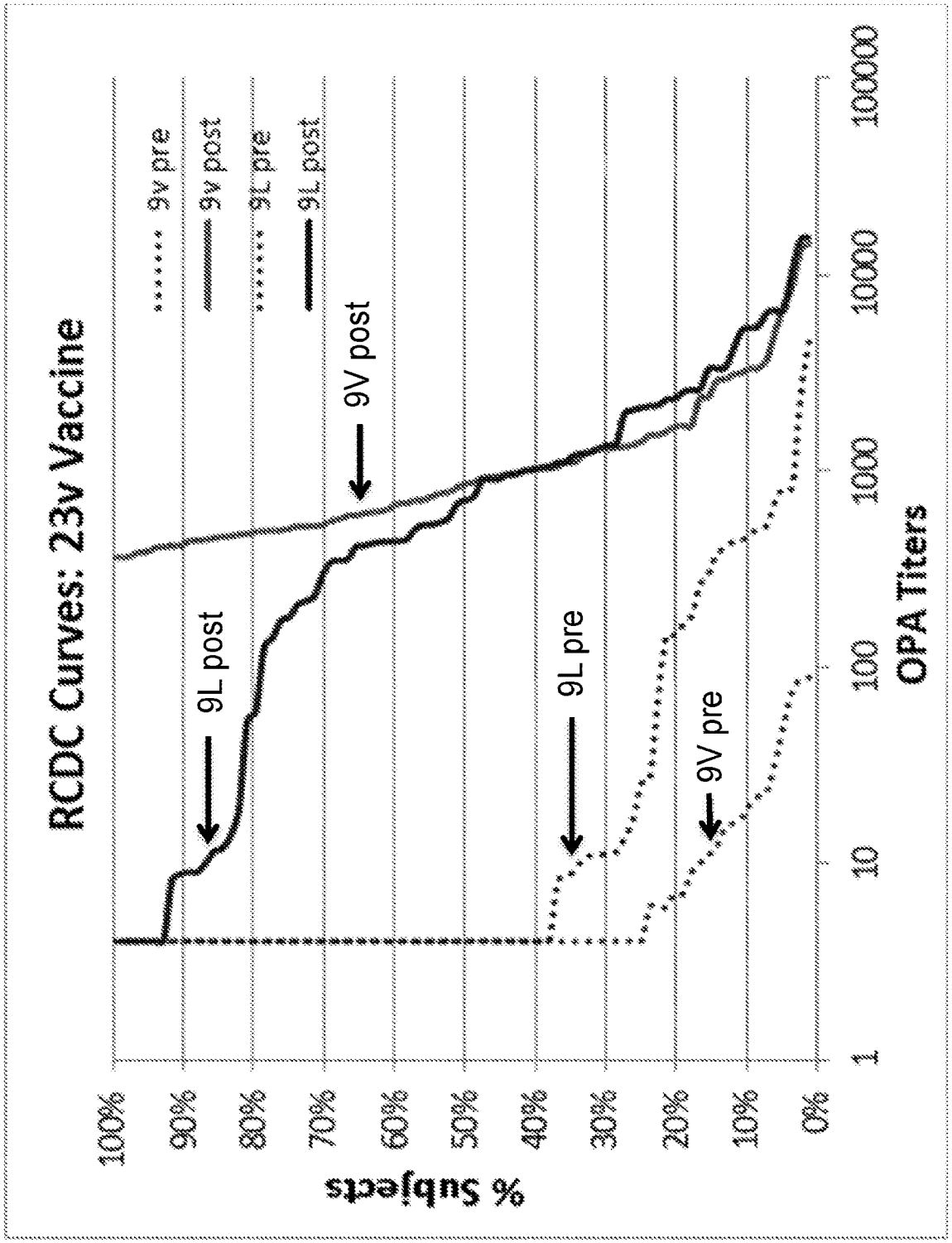

RCDC Curves: 13v Vaccine
9V pre
9V post
9L pre
9L post
9V post
9L post
9L pre
9V pre
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

FIG. 15


RCDC Curves: 23v Vaccine
9v pre
9v post
9L pre
9L post
9L post
9V post
9L pre
9V pre
% Subjects
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
10
100
1000
10000
100000
OPA Titers

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/050036

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/09 A61K39/385 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/092377 A1 (SK CHEMICALS CO LTD [KR]) 19 June 2014 (2014-06-19) cited in the application	1,3-7,9, 10,14,15
Y	abstract -& EP 2 932 979 A1 (SK CHEMICALS CO LTD [KR]) 21 October 2015 (2015-10-21) the whole document	11-13

X	WO 2013/191459 A1 (SK CHEMICALS CO LTD [KR]) 27 December 2013 (2013-12-27) cited in the application	1,3-7,9, 10,14,15
Y	abstract -& EP 2 865 392 A1 (SK CHEMICALS CO LTD [KR]) 29 April 2015 (2015-04-29) the whole documents, claims 1-8 and Figure 15	11-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2016	29/06/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Renggli-Zulliger, N

Form PCT/ISA/210 (second sheet) (April 2005)

Petitioner Merck Ex-1015, 1368

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 101 590 224 A (GUANGZHOU JINGDA MEDICAL SCIEN [CN]) 2 December 2009 (2009-12-02) cited in the application	1,3-7,9, 10,14,15
Y	abstract	11-13
Y	US 2007/184071 A1 (HAUSDORFF WILLIAM P [BE] ET AL) 9 August 2007 (2007-08-09) cited in the application the whole document	1-4,6, 9-15
Y	CYNTHIA G WHITNEY ET AL: "Decline in Invasive Pneumococcal Disease after the Introduction of Protein-Polysaccharide Conjugate Vaccine", N ENGL J MED, vol. 348, no. 18, 1 May 2003 (2003-05-01), pages 1737-1746, XP055281236, the whole document	1-4,6, 9-15
Y	CHI-JEN LEE ET AL: "Production, Binding Characteristics and Protective Immunity of Monoclonal Antibody to Pneumococcal Type-9V Conjugate", MICROBIOLOGY AND IMMUNOLOGY, vol. 40, no. 11, 4 June 1996 (1996-06-04), pages 857-865, XP002758930, JP ISSN: 0385-5600, DOI: 10.1111/j.1348-0421.1996.tb01151.x the whole document	1-15
Y	Shousun C. Szu ET AL: "Cross-Immunogenicity of Pneumococcal Group 9 Capsular Polysaccharides in Adult Volunteers", Infection and Immunity, 1 March 1982 (1982-03-01), pages 777-782, XP055139982, UNITED STATES Retrieved from the Internet: URL:http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=351116&tool=pmcentrez&rendertype=abstract [retrieved on 2016-06-16] the whole document	1-15

-/--

Petitioner Merck Ex-1015, 1369

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SZU S ET AL: "IMMUNOCHEMICAL CHARACTERIZATION OF CROSS-REACTIVITY OF PNEUMOCOCCAL GROUP 9 CAPSULAR POLYSACCHARIDE TYPES 9N, 9A, 9L, AND 9V", INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 31, no. 1, 1 January 1981 (1981-01-01), pages 371-379, XP001109015, ISSN: 0019-9567 the whole document --- CATHERINE WEIL-OLIVIER ET AL: "Prevention of pneumococcal diseases in the post-seven valent vaccine era: A European perspective", BMC INFECTIOUS DISEASES, BIOMED CENTRAL, LONDON, GB, vol. 12, no. 1, 7 September 2012 (2012-09-07), page 207, XP021106929, ISSN: 1471-2334, DOI: 10.1186/1471-2334-12-207 figure 1 --- WO 2015/110941 A2 (PFIZER [US]) 30 July 2015 (2015-07-30) the whole document ---	1-15 1-15 1-15

Petitioner Merck Ex-1015, 1370

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2016/050036

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

2(completely); 1, 3-15(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Petitioner Merck Ex-1015, 1371

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 3-7, 9-15(all partially)

   An immunogenic composition comprising a least one glycoconjugate from S. pneumoniae serotype 9V for use in a method of immunising a subject against infection by S.pneumoniae serotype 9N.

   ---

2. claims: 1, 3-7, 9-15(all partially)

   An immunogenic composition comprising a least one glycoconjugate from S. pneumoniae serotype 9V for use in a method of immunising a subject against infection by S.pneumoniae serotype 9A

   ---

3. claims: 1, 3-7, 9-15(all partially)

   An immunogenic composition comprising a least one glycoconjugate from S. pneumoniae serotype 9V for use in a method of immunising a subject against infection by S.pneumoniae serotype 9L.

   ---

4. claims: 1, 3-15(all partially)

   An immunogenic composition comprising a least one glycoconjugate from S. pneumoniae serotype 9V for use in a method of immunising a subject against infection by S.pneumoniae serotype 9N, 9A and 9L

   ---

5. claims: 2(completely); 1, 3-15(partially)

   An immunogenic composition comprising a least one glycoconjugate from S. pneumoniae serotype 9V for use in a method of immunising a subject against infection by S.pneumoniae serotype 9N, 9A and/or 9L wherein said composition does not comprise capsular saccharide from S.pneumoniae serotypes 9N, 9A and 9L.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/050036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014092377 A1	19-06-2014	AU 2013360596 A1	02-07-2015
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Petitioner Merck Ex-1015, 1373

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/050036

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		WO 2015110941 A2	30-07-2015

Form PCT/ISA/210 (patent family annex) (April 2005)

Petitioner Merck Ex-1015, 1374

DOCKET NO.: P1143.70000US05

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	17/347,435
Confirmation No.:	4020
Filed:	June 14, 2021
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Khatol S. S. Shah
Art Unit:	1645

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 20, 2024 Electronic Signature for Abigail S. Feldman: /Abigail S. Feldman/

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98

Commissioner:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action on the merits in the above-referenced Application. Therefore, no fee or certification is required.

PART II: Information Cited

The undersigned hereby makes of record in the present Application the information listed on the attached Form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

Petitioner Merck Ex-1015, 1375

The undersigned would also like to bring to the Examiner's attention the following applications that may contain subject matter related to the present Application:

Serial No.	Filing Date	Inventors	Docket No.
†16/089,040	09-27-2018	Forrest *et al.*	P1143.70000US04
†16/753,597	04-03-2020	Forrest *et al.*	P1143.70001US02

†A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

These applications may include subject matter which is related to subject matter of the present Application and may contain claims to such subject matter. During prosecution, the examiners of these applications will search for relevant art and, in some circumstances, take positions with respect to the art and/or the interpretation of the claims that may be material to the examination of the present Application. Accordingly, the undersigned respectfully requests that the Examiner review the file histories of these applications to determine whether there is any information that the Examiner believes is relevant to the prosecution of the present Application. It is assumed that the Examiner has access to the file histories of these applications, and the undersigned does not plan to provide copies of documents from these file histories to the Examiner. However, if the Examiner would like copies of any or all of the information included in the file histories of any or all of these applications, or any other information related to these applications, the Examiner is respectfully requested to contact the undersigned.

The undersigned would also like to bring to the Examiner's attention the enclosed search reports and other communication from corresponding and related international and foreign patent applications:

Serial No.	Date of Mailing	Type of Communication	Docket No.
EP 17776881.9	11-04-2019	*Extended European Search Report	P1143.70000EP00
PCT/US2017/025621	09-03-2017	*International Search Report and Written Opinion	P1143.70000WO00
PCT/US2017/025621	10-11-2018	*International Preliminary Report on Patentability	P1143.70000WO00

EP 18864637.6	06-07-2021	Extended European Search Report	P1143.70001EP00
PCT/US2018/054391	02-11-2019	*International Search Report and Written Opinion	P1143.70001WO00
PCT/US2018/054391	04-16-2020	*International Preliminary Report on Patentability	P1143.70001WO00

*a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 16/089,040, filed 09-27-2018, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed Form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of the present Application; and

3. The citations for the information be printed on any patent which issues from the present Application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency in the fees due, or credit any overpayment, by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825, under Docket No. P1143.70000US05, from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: P1143.70000US05
Date: May 20, 2024

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 338 265 B1**

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **04.05.94** (51) Int. Cl.5: **A61K 39/102**

(21) Application number: **89104996.7**

(22) Date of filing: **21.03.89**

(54) Haemophilus influenzae type B polysaccharide-outer membrane protein conjugate vaccine.

(30) Priority: **19.04.88 US 183206**

(43) Date of publication of application:
25.10.89 Bulletin 89/43

(45) Publication of the grant of the patent:
04.05.94 Bulletin 94/18

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI NL SE

(56) References cited:
EP-A- 0 088 303
EP-A- 0 276 516
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INFECTION AND IMMUNITY, vol. 49, no. 3, September 1985, American Society for Microbiology; R.S. MUNSON, Jr. et al., pp. 544-549&NUM;

JOURNAL OF BACTERIOLOGY, vol. 162, no. 3, June 1985, American Society for Microbiology; V. VACHON et al., pp. 918-924&NUM;

JOURNAL OF CLINICAL INVESTIGATION, vol. 72, August 1983, American Society for Clinical Investigation Inc.; R.S. MUNSON et al., pp. 677-684&NUM;

(73) Proprietor: **AMERICAN CYANAMID COMPANY**
One Cyanamid Plaza
Wayne, NJ 07470-8426(US)

(72) Inventor: **Kuo, Joseph S.-C.**
67 Constitution Drive
Tappan, NY 10983(US)
Inventor: **Bristol, James Edwin**
58 North Serven Street
Pearl River, NY 10965(US)

(74) Representative: **Wächtershäuser, Günter, Dr.**
Tal 29
D-80331 München (DE)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

Rank Xerox (UK) Business Services
(3.10/3.09/3.3.3)

EP 0 338 265 B1

Petitioner Merck Ex-1015, 1379

Description

BACKGROUND OF THE INVENTION

The Haemophilus influenzae type b polysaccharide vaccine currently in use, which consists of the capsular polysaccharide, polyribosyl-ribitol-phosphate ("PRP"), confers immunity in children 18 months of age or older, but not in younger children, who are at greatest risk of Haemophilus influenzae type b caused diseases.

Attempts have been made to modify the immunological characteristics and enhance the immunogenicity of the polysaccharide by preparing polysaccharide-protein conjugate vaccines in which the PRP is coupled to a protein carrier.

The following references discuss the related art:

(1) Chu, et al., Further Studies on the Immunogenicity of Haemophilus influenzae Type b and Pneumococcal Type 6A Polysaccharide-Protein Conjugates, Infect. Immun., 40, 245-256, (1983), relates to the preparation of conjugates of each of H. influenzae type b and pneumococcal 6A polysaccharides to tetanus toxoid by a complex procedure including activation of the polysaccharide with cyanogen bromide, derivatization of the activated polysaccharide with a spacer molecule, adipic acid dihydrazide ("ADH"), and then conjugation to the protein (tentanus toxoid) with a water soluble carbodiimide to form an amido type of linkage to the protein and a complex variety of linkages from the adipic acid spacer to the polysaccharide.

(2) Jennings et al. U.S. Patent 4,356,170 relates to the formation of polysaccharide-protein conjugates by the introduction of reactive aldehyde groups into the terminal residues of antigenic bacterial polysaccharides, specifically those of E. coli, meningococci, pneumococci, β-hemolytic streptococci and H. influenzae, by controlled oxidation and reacting the said aldehyde residues with the free amino groups of proteins, specifically those of tetanus toxoid, diphtheria toxoid, and immunogenic protein such as β-hemolytic streptococci, E. coli, H. influenzae, meningococci and pneumococci by reductive amination to covalently link said polysaccharide and protein.

(3) Anderson U.S. Patent 4,673,574 relates to the formation of immunogenic conjugates comprising the reductive amination product of an immunogenic capsular polysaccharide fragment derived from the capsular polymer of Streptococcus pneumoniae or H. influenzae and containing a reducing end, prepared by means such as oxidative cleavage with periodate or by hydrolysis of a glycosidic linkage, and a bacterial toxin or toxoid as a protein carrier.

(4) Hillman et al. U.S Patent 4,459,286 relates to the preparation of a polysaccharide-protein conjugate by activation of the H. influenzae type b polysaccharide with cyanogen bromide, derivatization of the activated polysaccharide with a spacer molecule, 6-aminocaproic acid, and the conjugation of the major outer membrane protein of Neisseria meningitidis with a water soluble carbodiimide to form an amido type of linkage to the protein and a complex variety of linkages from the 6-aminocaproic acid spacer to the polysaccharide.

(5) Gordon U.S. Patent 4,496,538 relates to the production of a water-soluble covalent polysaccharide-diphtheria toxoid conjugate, wherein a pure H. influenzae type b polysaccharide is activated with cyanogen bromide and intimately mixed with diphtheria toxoid which has been derivatized with an ADH spacer.

(6) Munson, et al., Purification and Comparison of Outer Membrane Protein P2 from H. influenzae Type b Isolates, J. Clin. Invest., 72, 677-684 (1983), relates to the purification of a major outer membrane protein from H. influenzae type b which has an indicated molecular weight of 37,000 daltons and is designated P2. This major membrane protein is immunogenic and the antibody against P2 confers passive protection in young animals.

(7) Vachon, et. al., Transmembrane Permeability Channels across the Outer Membrane of H. influenzae type b, J. Bacteriol., 162, 918-924 (1985), relates to the isolation of a major protein of molecular weight 40,000 daltons ("40K") from the outer membrane H. influenzae type b. The 40K protein acts as a porin in reconstituted vesicles by forming water-filled channels that allow the permeation of low molecular weight solutes.

SUMMARY OF THE INVENTION

This invention is directed to the preparation of immunogenic conjugates of an oxidized form of the capsular polysaccharide (PRP) and a major outer membrane protein of H. influenzae type b and the use of such conjugate(s) in vaccine(s) to confer protection against H. influenzae type b disease.

Petitioner Merck Ex-1015, 1380

Among the aspects of this invention are processes for the isolation and purification of the major outer membrane proteins (P2 proteins) of H. influenzae type b with an indicated molecular weight of 38,000 daltons ("38K") or 40,000 daltons ("40K") using hydroxylapatite column chromatography, as defined in claim 5. The amino acid compositions, terminal amino acid sequences and immunological reactivities of the 38K and 40K proteins have been established.

Still another aspect of this invention is a process for the covalent coupling of the fragments of the polysaccharide ([O]PRP) to the protein carrier (the 38K or 40K protein) to form conjugates as defined in Claim 6.

Further, this invention teaches the use in vaccines of the aforementioned conjugates.

The conjugate vaccines of this invention are highly immunogenic in the animal model. They elicit substantially higher antibody response to PRP than those reported previously. The conjugate vaccines also induce the antibody to the major protein (the 38K or 40k protein), of H. influenzae type b.

The immunoconjugates prepared by this invention have distinct advantages over those disclosed previously, in that the protein carrier is derived from the outer membrane protein of H. influenzae type b. The conjugate vaccine elicits antibodies to both PRP and the outer membrane protein from H. influenzae type b and confers immunity to invasive diseases caused by H. influenzae type b. In addition to permitting the vaccine to confer immunity in children younger than 18 months of age, the carrier protein itself may confer immunity and not merely act as a carrier for the [O]PRP. As described hereinafter, the carrier protein induced antibody response in the same animal model as [O]PRP. Finally, because the conjugate vaccine does not include the use of the H. influenzae type b bacterium, administration of the vaccine will not induce H. influenzae type b caused diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the analysis on SDS-PAGE of the major outer membrane proteins as follows: Lane A: Molecular weight standards (typsin inhibitor, 21K; carbonic anhydrase, 31K; ovalbumin, 45K, serum albumin, 66K; phosphorylase b, 92K); Lanes B, C, D: partially purified outer membrane proteins from H. influenzae b (Lederle CK strain and Lederle WK strain) and H. influenzae nontypable strain; Lane E: purified 40K protein from H. influenzae b (Lederle CK strain); Lane F: purified 38K protein from H. influenzae b (Lederle WK strain).

Figure 2 depicts the results of hydroxylapatite column chromatography on a sample of the 40K protein using a 100 ml linear gradient of 50 mM to 1M sodium phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside. Fractions were monitored at 562nm absorbance for protein content by Pierce BCA Protein Assay.

Figure 3 depicts the results of hydroxylapatite column chromatography on a sample of the 40K protein using a stepwise phosphate gradient of (a) 0.2M phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside, then (b) 1.0 M phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside. Fractions were monitored as in Figure 2.

Figure 4 depicts the results of hydroxylapatite column chromatography on a sample of the 38K protein using the stepwise phosphate gradient of Figure 3. Fractions were monitored as in Figure 2.

DETAILED DESCRIPTION OF THE INVENTION

Isolation and purification of the 38K and 40K major outer membrane proteins of H. influenzae b.

The protein used in this invention is an outer membrane protein of H. influenzae b, whose molecular weight depends on the H. influenzae b strain used to produce it. Specifically, it is the major membrane protein with an indicated molecular weight of 38K or 40K on sodium dodecyl sulfate polyacrylamide gel electrophoresis ("SDS-PAGE") using the method described in Munson, et al. and Vachon, et al., supra.

The 38K protein is produced by the strain of H. influenzae type b designated Lederle WK, which has been deposited with the American Type Culture Collection, Rockville, Maryland and has been assigned ATCC accession number 53,763. The 40K protein is produced by the strain of H. influenzae type b designated Lederle CK, which has been deposited with the American Type Culture Collection and has been

Petitioner Merck Ex-1015, 1381

assigned ATCC accession number 31,441.

The 38K and 40K major outer membrane proteins are part of the group of six major outer membrane proteins that have been isolated from H. influenzae type b. The 38E and 40K proteins have been referred to as the P2 or b/c proteins based on their forming a discrete band during SDS-PAGE. Loeb et al., Cross-Reactivity of Surface-Exposed Epitopes of Outer Membrane Antigens of Haemophilus influenzae Type b, Infect. Immun., 55, 2977-2983 (1987). The molecular weights observed may vary slightly depending on the technique used in performing the analysis.

In one embodiment, the isolation and purification of the 38K or 40K dalton outer membrane protein utilizes an improved method based on that described by Blake and Gotschlich, Purification and partial characterization of the major outer membrane protein of Neisseria gonorrhoea, Infect. Immunol., 36, 277-283 (1982) and Vachon, et al. supra.

A 20 gram portion (wet weight) of H. influenzae b, which had been inactivated with 0.01% thimerosal, was suspended in 9 volumes of distilled water. The bacterial suspension was brought to a final concentration of 2% (w/v) hexadecyltrimethylammonium bromide and was shaken vigorously for 1 hour at 37 °C. After centrifugation at 17,000 x G for 30 minutes at 4 °C, the pellet was removed and resuspended in 9 volumes of distilled water, followed by the addition of calcium chloride to a final concentration of 0.25M. This solution was stirred for 1 hour at room temperature and then brought to a final concentration of 20% (v/v) with ethanol. The preparation was allowed to precipitate for 3 hours at 4 °C.

The precipitate was subsequently removed by centrifugation, then the protein in the supernatant was precipitated by the addition of ethanol to a final concentration of 80%. The major outer membrane protein-containing material was recovered by centrifugation at 17,000 x G for 30 minutes at 4 °C and extracted with 25mM imidazole buffer (pH 7.0) containing 0.01% thimerosal and 0.1% octyl-β-D-glucopyranoside to remove soluble contaminant PRP and nucleic acids. The major outer membrane protein-containing material was recovered by centrifugation and then solubilized in 25mM imidazole buffer (pH 7.0) containing 2% octyl-β-D-glucopyranoside for 1 hour at room temperature. Any insoluble protein was removed by centrifugation. The supernatant, which contains the major outer membrane protein (38K or 40k), was then passed through a 1x60cm anion exchange column of DEAE-Sephacel® (Pharmacia, Inc.,Piscataway, New Jersey) to remove contaminating lipopolysaccharide.

Fractions were assayed for the presence of protein using the Lowry protein assay. One hundred μl samples of fractions were used. To each sample was added one ml of a solution prepared by mixing one part of 0.5% $CuSO_4.5H_2O$ (w/v) in water with 50 parts of 2% Na_2CO_3 (w/v), 0.02% sodium potassium tartrate (w/v) and 0.4% NaOH (w/v) in water. After 15 minutes, 100 μl of 1N phenol reagent was added. Absorbance of the sample was measured at 750 nm using a Gilford, 260 UV-visible spectrophotometer after incubation for 20 minutes at ambient temperature. Protein concentrations were determined by comparison with a standard protein solution of bovine serum albumin. For samples containing octyl-β-D-glucopyranoside, protein was measured using the BCA Protein Assay (Pierce Chemical Co., Rockford, Illinois, catalog no. 23225).

About 50% of the 38K or 40K protein was eluted from the column with imidazole buffer, pH 7.0. The remaining 50% of the 38K or 40K protein (about 20 mg) was recovered from the column by elution with 0.5M potassium chloride. Figure 1 shows the SDS-PAGE bands corresponding to the 38K and 40K proteins compared to standards of known molecular weight.

The 40K protein purified in the manner just described was utilized in Examples 1-5 and 7 set forth below.

In an improved embodiment, the 40K protein was purified by solubilizing with sodium phosphate buffer containing octyl-β-D-glucopyranoside, followed by hydroxylapatite ("HA") column chromatography. A sample of 40K protein, which had been partially purified prior to column chromatographic purification, using the techniques described above, contained contaminating material such as lipopolysaccharide ("LPS") and PRP.

Specifically, a sample of partially purified 40K protein from H. influenzae type b (CK strain) was first precipitated with 2 volumes of ethanol (final concentration of ethanol was 66%) at 0-4 °C for at least ten minutes. The sample was centrifuged at 23,000 x G for 15 minutes. Virtually all of the 40K protein (>90%) was recovered in the pellet. The pellet was then dissolved in a buffer solution of either 50mM or 200mM sodium phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside at 8 mg protein/ml. The mixture was stirred at room temperature for 1 hour and the insoluble material was removed by centrifugation.

HA column chromatography was performed at pH 6.5, using a Bio-Gel HA column (Bio-Rad Laboratories, Richmond, California). The HA column contained calcium and phosphate ions that formed the adsorption sites. The gel (40ml) was pretreated with 50mM sodium phosphate buffer (pH 6.5) and 2% octyl-β-D-glucopyranoside and then equilibrated with at least 2 volumes of the buffer. Subsequently, a 10 ml (80

Petitioner Merck Ex-1015, 1382

mg) portion of the 38K or 40K protein was applied to a HA column (3x8 cm). The sample was eluted with a 100 ml linear gradient of 50mM to 1 M sodium phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside. The column eluate was collected in fractions of 2 ml per tube at a flow rate of approximately 10 ml/hour. Fractions were monitored at 562nm absorbance for protein content by Pierce BCA Protein Assay. Fractions containing the 40K protein were pooled and were analyzed for protein concentration and purity by Pierce BCA Protein Assay and by SDS-PAGE.

The LPS and PRP contents in the purified preparation were also determined.

The 40K protein was eluted from the column as two peaks (see Figure 2) at approximately 0.45-0.5 M phosphate concentration. The eluted protein was nearly homogeneous by SDS-PAGE and appeared virtually free of contaminating LPS and PRP. The protein from both peaks had a similar N-terminal sequence.

The 38K and 40K proteins were also purified by HA chromatography with a stepwise phosphate gradient on an HA column. A sample of partially purified 40K protein from H. influenzae type b (CK Strain) was applied onto a column of HA and first eluted with 1.5 volumes of 0.2 M phosphate buffer (pH 6.5) and 2% octyl-β-D-glucopyranoside, and then with 1.0 M phosphate buffer (pH 6.5) and 2% octyl-β-D-glucopyranoside. By using this procedure, the majority of the 40K protein was eluted as a single peak at a phosphate concentration of approximately 0.5-0.7M (see Figure 3).

The stepwise phosphate gradient procedure was also used to purify a partially purified 38K protein from H. influenzae type b (WK Strain). The majority of the 38K protein was eluted from an HA column as a single peak at a phosphate concentration of approximately 0.5-0.7M (see Figure 4).

At low phosphate concentrations (200mM), small amounts of the 38K protein or the 40K protein co-eluted with PRP (first peak in Figures 3 and 4). The protein-containing fractions of each protein were pooled and in both cases found to be nearly homogeneous by SDS-PAGE. Densitometric scans of each stained gel (with coomassie brilliant blue) showed that the 38K and 40K proteins purified by HA chromatography accounted for at least 90% of the total protein. This 90% or more yield of protein obtained through the use of HA chromatography is a significant improvement over the 50% yield obtained by anion exchange chromatography.

The amounts of PRP and endotoxin contents in the final preparations were very low. The final protein preparations had <1.5μg PRP/100μg protein and <5.0 endotoxin units/100μg protein. They passed the rabbit pyrogen test at 2.5μg protein/kg rabbit weight.

The purified 38K and 40K outer membrane proteins were characterized in several ways. Western blot analysis revealed that the 38K and 40K proteins were cross-reactive. Therefore, a finding that one protein would elicit an antibody response in warm-blooded animals (as shown in Example 6 below) would mean that the other protein would also elicit a cross-reactive antibody response.

An amino acid analysis was performed on a sample of each protein which had been hydrolyzed in 6N hydrochloric acid for 24 hours at 110 °C, using a Beckman 121/MB amino acid analyzer. The amino acid compositions of the 38K and 40K proteins are shown in Table I.

Petitioner Merck Ex-1015, 1383

Table I

Amino Acid Composition of the 40K and 38K Major Outer Membrane Proteins of H. influenzae b

Amino Acid	Amino Acid Content (Mole of Residue per Mole of Protein*)	
	40K Protein	38K Protein
Basic	54 (0.15**)	51 (0.15)
Lysine	32	29
Histidine	7	7
Arginine	15	15
Dicarboxylic	88 (0.24)	85 (0.24)
Aspartic, Asparagine	47	45
Glutamic, Glutamine	41	40
Neutral	226 (0.61)	210 (0.61)
Threonine	26	26
Serine	16	13
Proline	3	3
Glycine	50	44
Alanine	27	25
Valine	29	27
Methionine	1	1
Isoleucine	15	14
Leucine	26	24
Tyrosine	20	20
Phenylalanine	13	13

* Based on indicated molecular weight of the proteins in SDS-PAGE.

** Fraction of the total moles of amino acid residues the group represents.

Amino-terminal sequence analyses were carried out on an Applied Biosystems Gas Phase Sequencer Model 470, in conjunction with PTH amino acid analysis on a Hewlett Packard Model 1090 HPLC equipped with an IBM cyano column. Carboxy-terminal amino acid sequences were determined by enzymatic methods. Digestion was accomplished by using both carboxypeptidase A and B; amino acid analyses of aliquots taken at various times were performed on a Beckman 6300 amino acid analyzer.

The amino-termini of the 38K and 40K proteins are identical, as are the carboxy-termini. The sequences are shown in Table II.

Petitioner Merck Ex-1015, 1384

Table II

Amino- and Carboxy-Terminal Sequences of 40K and 38K Protein from H. Influenzae b

Amino-Terminal Sequences for Type b 40K and 38K Protein:

NH_2-Alanine-Valine-Valine-Tyrosine-Asparagine-Asparagine-Glutamic acid-Glycine-Threonine-Asparagine-Valine-Glutamic acid-Leucine-Glycine-Glycine-Arginine-Leucine-Serine-Isoleucine-Isoleucine-Alanine--Glutamic Acid-Glutamine-Serine-Asparagine-

Carboxy-Terminal Sequences for Type b-40K and 38K Protein:

Leucine-Arginine-[Phenylalanine-Tyrosine-Valine]-COOH

Preparation of H. influenzae b capsular polysaccharide, polyribosyl-ribitol-phosphate (PRP)

The H. influenzae b PRP used in this invention has been described in commonly-assigned Kuo U.S. Patent 4,220,717, which is incorporated by reference. Using the procedure described in this patent, a H. influenzae b organism was grown in a culture, centrifuged, and the cell free supernatant collected. The supernatant was then precipitated with ethanol, the precipitate was treated with hexadecyltrimethyl ammonium bromide, and then again with ethanol to recover partially purified PRP. That PRP was then treated with hydroxylapatite, which adsorbed contaminants, so that PRP remained in the supernatant. If any further purification is necessary, the PRP may be applied to a Sepharose 4B-CL® column (Pharmacia, Piscataway, New Jersey). The PRP so obtained was a relatively high molecular size of PRP, with more than 50% having an elution coefficient (K_{av}) value less than 0.30 on a column of Sepharose 4B-CL. This value corresponds to a molecular weight greater than $1x10^6$ daltons.

Preparation of PRP fragments by periodate oxidation of PRP

In its native form, PRP has no reducing ends. In order to create PRP fragments which contain reducing ends (in the form of terminal reactive aldehyde groups), the cis-vicinal hydroxyl group of the polysaccharides was oxidized by a periodate such as sodium periodate, potassium periodate or periodic acid to generate aldehyde functions following the procedure described by Parikh et al., Methods Enzymol., 34, 77-102 (1974) and Jennings et al. U.S. Patent 4,356,170. The use of sodium periodate is preferred. The purified PRP was treated in the dark with 0.2-50mM of sodium periodate at 4 °C or at room temperature, for various lengths of time. In a preferred embodiment, the PRP was treated at pH 4-7. In a particularly preferred embodiment, the PRP was treated at pH 4.0-4.5. The [O]PRP was then dialyzed extensively against pyrogen-free water to remove small molecular size materials. Alternatively, the [O]PRP may be purified through a gel filtration column [beaded agarose acrylamide copolymer (Sephacryl® S-200 or S-300, Pharmacia)]. When a gel filtration column was used, fractions were assayed for the presence of [O]PRP by the orcinol reaction using D-ribose as the standard. Absorbance of fractions was measured at 672 nm. The purified product was then recovered by concentrating and drying. The resulting [O]PRP fragments had chain lengths of about 8-120 monomeric units. This chain length may also be referred to as the degree of polymerization ("D.P.") The [O]PRP was then coupled with the 38K or 40K protein by reductive amination as will now be described.

Preparation of the [O]PRP-major outer member protein conjugate

The aldehyde functions in the [O]PRP were reacted with a solution of the outer membrane protein which contains amino groups to form Schiff bases. The reaction is carried out at pH 3.5 to 9 and is preferably carried out at pH 5 to 9. These Schiff bases are then reduced with a suitable borohydride at pH 6.0-6.7 to form a stable, covalently-bonded conjugate. Examples of such borohydrides include sodium borohydride, sodium cyanoborohydride, potassium borohydride and lithium borohydride. The use of sodium cyanoborohydride is preferred. The methodology for the coupling of oxidized polysaccharide or polysaccharide fragments to a protein using borohydride has been described by Parikh et al. (supra), and Schwartz and Gray, Arch. Biochem. Biophys., 181, 542-549 (1977). The purified major protein (concentration 1-40

Petitioner Merck Ex-1015, 1385

mg/ml) was mixed with [O]PRP (concentration 10-80 mg/ml) in 0.2M potassium phosphate buffer, pH 6.0-6.7, at room temperature. After 20-30 minutes of incubation with gentle stirring, 0.1-2.0mM of sodium cyanoborohydride dissolved in pyrogen-free water was added.

This mixture was then incubated at 25°-37°C with gentle stirring for 1-6 days to form the [O]PRP-outer membrane protein conjugate. The conjugate was purified on a gel filtration column such as Sephacryl® S-200, S-300 or Sepharose CL-4B (Pharmacia). Fractions were assayed for protein and [O]PRP as described previously. The peak fractions which contained the conjugate were pooled, diafiltered and/or lyophilized. Alternatively, the conjugate may be purified by dialysis.

The conjugates which have been prepared and purified in accordance with this invention are preferably used in the preparation of vaccines to confer protection against H. influenzae type b caused diseases.

The conjugates may be added to immunologically acceptable diluents or carriers in a conventional manner to prepare injectable liquid solutions or suspensions. In addition, the conjugates may be bound to aluminium hydroxide, aluminum phosphate (alum) or other pharmaceutically acceptable adjuvants.

For instance, to prepare a conjugate vaccine containing [O]PRP and the major membrane protein of H. influenzae b, the conjugate preparation was suspended in sodium phosphate-buffered saline ("PBS") (pH 7.0-7.4) at concentrations of 5-100 μg of PRP/ml as measured by the orcinol reaction [Herbert, et al., Methods in Microbiology, 5B, 285-291 (1971)].

The conjugate vaccines of this invention may be administered by injection in a conventional manner such as subcutaneous or intramuscular injection into warm-blooded animals to elict an active immune response for protection against systemic infection caused by the pathogen H. influenzae b. The dosage to be administered is determined by means known to those skilled in the art. Protection may be conferred by a single dose of vaccine, or may require the administration of several booster doses.

The following examples further illustrate the invention but are not to be construed as limiting the scope thereof. Examples 1 and 2 were performed at room temperature. The oxidation steps in Examples 3-5 were performed at 2-8°C; the conjugation steps in Examples 3-5 were performed at 37°C. The [O]PRP prepared had approximate D.P. values of 10 (Ex. 1), 37 (Ex. 2), 8 (Ex. 3), 40 (Ex. 4) and 120 (Ex. 5), as measured by the procedure of Park and Johnson, J. Biol. Chem. 181, 149-151 (1949) for reducing sugars.

Example 1

Preparation of [O]PRP-40K Protein Conjugate

A 500 mg portion of PRP was dissolved in 50 ml of 0.1M sodium acetate buffer (pH 4.5). A 107 mg portion of sodium periodate was added in the dark and the mixture was stirred gently for 30 minutes in a capped brown glass bottle wrapped in aluminum foil. The excess sodium periodate was destroyed by the addition of 500 μl of 3M ribitol solution and gentle stirring for 10 minutes. The reaction mixture containing the resulting [O]PRP was extensively dialyzed against pyrogen-free water in a Spectropor® membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, Calif.) with 3.5K (M.W.) cut-off for 3 days at 4°C, using 2 liters of fresh water each day. The dialyzed [O]PRP was then filtered through a 0.2μm Nalge filter, lyophilized and stored at 4°C. The [O]PRP had a D.P. value of approximately 10. The [O]PRP was found to elute with a K_{av} value of about 0.5 from a column of Sephacryl® S-200. The recovery of [O]PRP from the PRP preparation was approximately 50%.

The 40K membrane protein was concentrated using an Amicon concentrator (10K (M.W.) cut-off filter) with 0.2M potassium phosphate buffer (pH 6.5) plus 2% octyl-β-D-glucopyranoside to a protein concentration of about 20 mg/ml. An 80 mg portion of [O]PRP was dissolved in 4 ml of the 40K membrane protein solution with gentle stirring. After 20 minutes, 0.5mM of sodium cyanoborohydride was added and this mixture was incubated at room temperature with gentle stirring for 3 days. The mixture was chromatographed on a column of Sephacryl S-200 which had been equilibrated with 0.2M ammonium acetate (pH 7.0) plus 1% octyl-β-D-glucopyranoside. The first peak fractions containing the conjugate were pooled and used for the preparation of the vaccine. The [O]PRP-40K protein conjugate had a PRP/protein (w/w) ratio of about 1:2.5.

Example 2

Preparation of [O]PRP-40K Protein Conjugate

A 500 mg portion of PRP was dissolved in 50 ml of 0.1M sodium acetate buffer (pH 4.5). A 20.4 mg portion of sodium periodate was added in the dark and the mixture was stirred gently for 30 minutes in a

Petitioner Merck Ex-1015, 1386

capped brown glass bottle wrapped in aluminum foil. The excess sodium periodate was destroyed by reaction with 500 µl of 3M ribitol for 10 minutes. The reaction mixture containing the resulting [O]PRP was extensively dialyzed against pyrogen-free water in a Spectrapor® membrane tubing with 12K (M.W.) cut-off for 3 days at 4 °C. The dialyzed [O]PRP was then filtered through a 0.2µm Nalge filter, lyophilized and then stored at 4 °C. This [O]PRP had a D.P. value of approximately 37. The [O]PRP was found to elute with a K_{av} value of about 0.5-0.6 from a column of Sephacryl® S-300. The recovery of [O]PRP from the PRP preparation was approximately 68%.

The 40K membrane protein was concentrated using an Amicon concentrator (10K M.W. cut-off filter) with 0.2M potassium phosphate buffer (pH 6.5) plus 2% octyl-β-D-glucopyranoside to a protein concentration of about 16 mg/ml. A 40 mg portion of [O]PRP was dissolved in 1 ml of the 40K protein solution with gentle stirring. After 20 minutes, 0.5mM of sodium cyanoborohydride was added and the mixture was incubated with gentle stirring at room temperature for 3 days. The mixture was chromatographed on a column of Sephacryl S-300 which had been equilibrated with 0.2M ammonium acetate (pH 7.0) plus 1% octyl-β-D-glucopyranoside. The conjugate material was eluted with the same buffer. Peak fractions containing the conjugate were pooled, characterized and used for the vaccine preparations. The recovery of the [O]PRP and the membrane protein in the conjugate were about 20% and 60% respectively. The [O]PRP-40K membrane protein conjugate had a PRP/protein ratio of about 1:2.

Example 3

Preparation of [O]PRP-40K Protein Conjugate

All reactions were performed at 2-8 °C unless otherwise stated.

A 300 mg portion of PRP was dissolved in 30 ml of pyrogen-free water, then 24 ml of cold 0.2M sodium acetate buffer (pH 4.0) was added, followed by addition of 6 ml of a 50 mg/ml solution of sodium periodate in sodium acetate buffer (pH 4.0). The final solution was 23.4mM sodium periodate and 5 mg/ml PRP. The mixture was then incubated in the dark, in a capped bottle wrapped in aluminum foil, in an ice-water bath for 30 minutes. The excess sodium periodate was destroyed by reaction with 90 µl of 16.7 M ethylene glycol for 10 minutes. The mixture was then extensively dialyzed at 4 °C in a Spectrapor® membrane tubing with 3.5K (M.W.) cut-off, first against sterile PBS (4 liters, 2 changes, 18 hours/change), then against pyrogen-free water (same regimen). The dialyzed [O]PRP was then transferred to a container and stored at -20 °C until used for the preparation of the conjugate. This [O]PRP had a D.P. value of approximately 8. The [O]PRP was found to elute with a K_{av} value of about 0.9 on Sepharose 4B-CL®. A 100 mg portion of the [O]PRP in pyrogen-free water, at a concentration of 5 mg/ml was placed into a Spectrapor® membrane tubing having a M.W. cut-off of 3.5K and reequilibrated by dialysis against 1 liter of 0.2M potassium phosphate buffer (pH 6.7) containing 1% octyl-β-D-glucopyranoside. The [O]PRP was then concentrated by placing the dialysis tubing in a shallow container filled with dried polyethylene glycol. The final [O]PRP concentration was adjusted to approximately 33 mg/ml with 0.2M potassium phosphate buffer (pH 6.7).

To 100 mg (ca. 3.0 ml) of the [O]PRP solution was added 1.3 ml of 40K protein solution (1.53 mg/ml) in phosphate buffer containing 1% octyl-β-D-glucopyranoside and 37µl of sodium cyanoborohydride (1 mg/µl). The final volume was adjusted to 5 ml with phosphate buffer containing 1% octyl-β-D-glucopyranoside. This mixture was incubated with gentle stirring at 37 °C for 5 days. The conjugate was purified by extensive dialysis against phosphate buffered saline at 4 °C (4 liters, 2 changes, 18 hours/change) during which the conjugate precipitated and was collected by centrifugation. The pellet was then dissolved in 0.2M potassium phosphate buffer (pH 6.7) containing 1% octyl-β-D-glucopyranoside, characterized by SDS-PAGE and used in the animal experiments described hereinafter. The [O]PRP-40K membrane protein conjugate had a PRP/protein ratio of about 1:4.

Example 4

Preparation of [O]PRP-40K Protein Conjugate

All reactions were performed at 2-8 °C unless otherwise stated.

A 300 mg portion of PRP was dissolved in 30 ml of pyrogen-free water. A 29.4 ml portion of cold 0.2M sodium acetate buffer (pH 4.0) was added, followed by addition of 0.6 ml of a 50 mg/ml solution of sodium periodate in sodium acetate buffer (pH 4.0). The final solution was 2.34mM sodium periodate and 5 mg/ml PRP. This mixture was then incubated in a capped bottle wrapped in aluminum foil, in the dark, in an ice water bath for 30 minutes. The excess sodium periodate was destroyed by reaction with 90 µl of 16.7M

Petitioner Merck Ex-1015, 1387

ethylene glycol for 10 minutes. The reaction mixture containing the resulting [O]PRP was then extensively dialyzed in a Spectrapor® membrane tubing with 3.5K (M.W.) cut-off, first against sterile PBS (4 liters, 2 changes, 18 hours/change), then against pyrogen-free water (same regimen). The dialyzed [O]PRP was then transferred to a container and stored at -20 °C until used for preparation of the conjugate. The [O]PRP had a D.P. value of about 40. The [O]PRP was found to elute with a K_{av} of about 0.5-0.7 on Sepharose 4B-CL®. A 100 mg portion of the [O]PRP in pyrogen-free water at a concentration of 5 mg/ml was placed in a Spectrapor® membrane tubing having a M.W. cut-off of about 3500 daltons and reequilibrated by dialysis for 8 hours at room temperature against 1 liter of 0.2M potassium phosphate buffer (pH 6.7) containing 1% octyl-β-D-glucopyranoside. The [O]PRP was then concentrated by placing the dialysis tubing in a shallow container filled with dried polyethylene glycol. The final [O]PRP concentrate was adjusted to approximately 33 mg/ml with 0.2M potassium phosphate buffer (pH 6.7).

To 100 mg (ca. 3.0 ml) of the [O]PRP was added 1.3 ml of the 40K protein solution (1.53 mg/ml) in phosphate buffer containing 1% octyl-β-D-glucopyranoside and 37 µl of sodium cyanoborohydride (1 mg/µl). The final volume was adjusted to 5 ml with phosphate buffer containing 1% octyl-β-D-glucopyranoside and the mixture was incubated with gentle stirring for 5 days at 37 °C. The mixture was then chromatographed on a column of Sephacryl® S-300 which had been equilibrated with 0.2M ammonium acetate (pH 7.0). The conjugate was eluted at the void volume with the same buffer. The peak fractions were pooled, characterized and used for the vaccine preparations. The [O]PRP-40K membrane protein conjugate had a PRP/protein ratio of about 1:2.

Example 5

Preparation of [O]PRP-40K Protein Conjugate

All reactions were performed at 2-8 °C unless otherwise noted.

A 300 mg portion of PRP was dissolved in 30 ml of pyrogen-free water. A 29.94 ml portion of 0.2M sodium acetate buffer (pH 4.0) was added, followed by addition of 0.06 ml of sodium periodate (50 mg/ml) in sodium acetate buffer (pH 4.0). The final solution was 0.234mM sodium periodate and 5 mg/ml PRP. This mixture was incubated in the dark, in a capped bottle wrapped in aluminum foil, in an ice-water bath for 30 minutes. The excess sodium periodate was destroyed by reaction with 90 µl of 16.7M ethylene glycol for 10 minutes. The mixture containing the resulting [O]PRP was then extensively dialyzed at 4 °C in a Spectrapor® membrane tubing with 3.5K (M.W.) cut-off, first against sterile PBS (4 liters, 2 changes, 18 hours/change), then against pyrogen-free water (same regimen). The [O]PRP had a D.P. value of about 120. The [O]PRP was found to elute with a K_{av} value of about 0.3-0.6 on Sepharose 4B-CL®. A 100 mg portion of the [O]PRP in pyrogen-free water (5 mg/ml) was placed in a Spectrapor® membrane tubing having a M.W. cut-off of 3.5K and reequilibrated by dialysis for 8 hours at room temperature against 1 liter of 0.2M potassium phosphate buffer (pH 6.7) containing 1% octyl-β-D-glucopyranoside. The [O]PRP was then concentrated by placing the dialysis tubing in a shallow container filled with dried polyethylene glycol. The final [O]PRP concentration was adjusted to approximately 33 mg/ml with 0.2M potassium phosphate buffer (pH 6.7).

To a 100 mg portion (ca. 3.0 ml) of the [O]PRP solution was added 1.3 ml of the 40K protein solution (1.53 mg/ml) in phosphate buffer containing 1% octyl-β-D-glucopyranoside and 37 ml of sodium cyanoborohydride (1mg/ml). The final volume was adjusted to 5 ml with phosphate buffer containing 1% octyl-β-D-glucopyranoside and the mixture was incubated with gentle stirring for 5 days at 37 °C. The mixture was chromatographed on a column of Sepharose 4B-CL® which had been equilibrated with 0.2M ammonium acetate (pH 7.0). The conjugate was eluted with the same buffer. The peak fractions were pooled, characterized and used for the vaccine preparations. The [O]PRP-40K membrane protein conjugate had a PRP/protein ratio of about 2:1.

Example 6

Preparation of [O]PRP-38K Protein Conjugate

A one gram portion of PRP was dissolved in 100 ml of 0.1M sodium acetate buffer (pH 4.5). A 107 mg portion of sodium periodate was added in the dark and the mixture was stirred gently for 30 minutes in a capped brown glass bottle wrapped in aluminum foil. The excess sodium periodate was destroyed by the addition one ml of 3M ribitol solution and gentle stirring for 10 minutes. The reaction mixture containing the resulting [O]PRP was extensively dialyzed against pyrogen-free water in a Spectropor® membrane tubing

Petitioner Merck Ex-1015, 1388

with 3.5K (M.W.) cut-off for 3 days at 4°C. The dialyzed [O]PRP was then filtered through a 0.2μ Nalge filter, lyophilized and stored at 4°C. The [O]PRP had a D.P. value of approximately 35. The [O]PRP was found to elute with a K_{av} value of about 0.5 from a column of Sephacryl S-300. The recovery of [O]PRP from the PRP preparation was approximately 78%.

The purified 38K protein was concentrated with an Amicon concentrator using 0.2M potassium phosphate buffer (pH 6.5) plus 2% octyl-β-D-glucopyranoside to a protein concentration of about 10 mg/ml. A 30 mg portion of [O]PRP was dissolved in 3 ml of the 38K protein solution (30 mg protein) with gentle stirring. After 20 minutes, 0.3 ml of sodium cyanoborohydride (30 mg/0.3 ml water) was added and this mixture was incubated at 37°C (shaking water bath) with gentle swirling for 3 days. The reaction material was diluted with 2% octyl-β-D-glucopyranoside (in water) to a protein concentration of approximately 2 mg/ml and extensively dialyzed against 50mM NaCl buffer without detergent in a Spectropor® membrane tubing with a M.W. cut-off of 3.5K for 2 days at 4°C, using 50 mM NaCl (500 ml) and two changes. The dialyzed material was centrifuged at 12,000 x G for 10 minutes to remove insoluble material. The supernatant (5-15 ml) was applied to a column of Sepharose 4B-CL® (1.5x90 cm) pre-equilibrated with 50mM NaCl and eluted with the same buffer. Two distinct peaks were obtained for [O]PRP; the [O]PRP-38K protein conjugate was eluted at V_0. The peak fractions which contained the conjugate (PRP and protein) were pooled and stored at 4°C until use. The [O]PRP-38K membrane protein conjugate had a PRP/protein ratio of about 1:5.

Example 7

Antibody Response to Conjugate Vaccines

The conjugates prepared by the procedures of Examples 1-5 were diluted in sterile PBS at pH 7.0, such that a 0.5 ml dose contained 5 or 10 μg of PRP. The conjugate vaccines were sterilized by membrane filtration through a 0.20 μm Gelman filter. The sterile vaccine was stored at 4°C until needed.

The conjugate vaccine (0.5 ml per dose) was injected subcutaneously into young rats once each week for three weeks.

Table III sets forth the antibody response to PRP of vaccines made from the conjugates of Examples 1-5 hereto in comparison to PBS (as a control), PRP alone and two known conjugates. Table IV sets forth the antibody response to the 40K protein after receiving vaccines made from the conjugates of Examples 1, 3 and 4 hereto in comparison to PBS (as a control).

The radioimmunoassay (RIA) method used for the measurement of PRP antibody is described by Kuo, et al., J. Immunol. Methods., 43, 35-47 (1981) which is incorporated herein by reference.

The 40K protein antibody was determined by the enzyme-linked immunosorbent assay of Voller, et al., Lancet, 1, 469 (1975).

The results in Tables III and IV show that rats injected with PRP alone showed no significant increase in the PRP antibody level over the control sera after three immunizations. The rats injected with the conjugates containing diptheria toxoid as a protein carrier had increased levels of the antibodies. Rats injected with the conjugate vaccine consisting of [O]PRP and 40K protein had an excellent antibody response as shown by significantly higher levels of antibodies to PRP (Table III) and to the 40K protein (Table IV).

Petitioner Merck Ex-1015, 1389

Table III

Antibody Response of Young Rats to [O]PRP-40K Membrane Protein Conjugate

Vaccine	Dose μg PRP/0.5 ml	GMT Antibody to PRP(ng/ml)* WEEKS POST ADMINISTRATION 4	5
PBS (Control)	-	<40	<40
PRP	10	<40	<40
PRP (ADH)-Diphtheria toxoid conjugate **	5	2505	2273
	10	1562	1119
[O]PRP-Diphtheria toxoid conjugate	5	189	114
	10	1474	655

* Five rats per group were injected with vaccine. The geometric-mean titer (GMT) of antibody was computed from the five discrete titers.

** This conjugate consists of PRP (ADH)-diphtheria toxoid prepared by the procedure of Chu, et al., Inf. Immun., 40, 245-256 (1983). The PRP is coupled to the diphtheria toxoid through the spacer adipic dihydride (ADH) using cyanogen bromide and water soluble carbodiimide.

Petitioner Merck Ex-1015, 1390

Table III (continued)

Antibody Response of Young Rats to [O]PRP-40K Membrane Protein Conjugate

Vaccine	Dose μg PRP/0.5 ml	GMT Antibody to PRP(ng/ml)* WEEKS POST ADMINISTRATION 4	5
[O]PRP-40K Protein Conjugate			
Example 1 (D.P. = 10)	5	50617	46900
	10	21585	19920
Example 2 (D.P. = 37)	5	18425	32004
	10	18059	33677
Example 3 (D.P. = 8)	5	4148	4140
	10	2347	3814
Example 4 (D.P. = 40)	5	69199	93581
	10	49355	58730
Example 5 (D.P. = 120)	5	19585	20411
	10	23263	18486

* Five rats per group were injected with vaccine. The geometric-mean titer (GMT) of antibody was computed from the five discrete titers.

Petitioner Merck Ex-1015, 1391

Table IV

Antibody Level to the 40K Protein in Young Rats After Receiving [O]PRP-40K Protein Conjugate

Vaccine	Dose (0.5 ml) μg PRP	Dose (0.5 ml) μg protein	Rat No.	Serum Antibody Titers* (A_{492} at 1:320 dilution)
PBS (Control)	-	-	1	0.380
			2	0.391
			3	0.450
[O]PRP-40K Protein Conjugate				
Example 1 (D.P. = 10)	5	12.5	8	1.357
	5	12.5	9	1.626
	10	25	10	1.238
	10	25	11	1.343
Example 3 (D.P. = 8)	5	20	4	1.402
	5	20	5	1.186
	10	40	6	1.176
	10	40	7	1.280

* Fourth week post initial injection.

Petitioner Merck Ex-1015, 1392

Table IV (continued)

Antibody Level to the 40K Protein in Young Rats After Receiving [O]PRP-40K Protein Conjugate

Vaccine	Dose (0.5 ml) µg PRP	µg protein	Rat No.	Serum Antibody Titers* (A_{492} at 1:320 dilution)
Example 4 (D.P. = 40)	5	10	12	1.183
	5	10	13	0.703
	10	20	14	1.082
	10	20	15	0.677

* Fourth week post initial injection.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, NL, SE

1. An immunogenic polysaccharide-protein conjugate comprising the reductive amination product of (a) an oxidized polyribosyl-ribitol-phosphate polysaccharide fragment derived from the capsular polysaccharide of Haemophilus influenzae type b, and (b) the major outer membrane protein of Haemophilus influenzae type b having a molecular weight of about 38,000 daltons or 40,000 daltons.

Petitioner Merck Ex-1015, 1393

2. The conjugate of Claim 1, wherein the 38,000 daltons or 40,000 daltons outer membrane protein of Haemophilus influenzae type b has the N-terminal amino acid sequence NH_2-alanine-valine-valine-tyrosine-asparagine-asparagine-glutamic acid-glycine-threonine-asparagine-valine-glutamic acid-leucine-glycine-glycine-arginine-leycine-serine-isoleucine-isoleucine-alanine-glutamic acid-glutamine-serine-asparagine-.

3. A vaccine comprising the immunogenic conjugate of Claim 1.

4. The vaccine of Claim 3 which further comprises one or more of an immunologically acceptable diluent, carrier or adjuvant.

5. A process for the isolation and purification of the 38,000 daltons or 40,000 daltons major outer membrane protein of Haemophilus influenzae type b which comprises inactivating the Haemophilus influenzae type b, suspending the bacteria in water, bringing the suspension to a final concentration of 2% (w/v) hexadecyltrimethylammonium chloride, shaking the suspension vigorously for 1-2 hours, centrifuging the suspension, removing the pellet and resuspending the pellet in water, bringing the suspension to a final concentration of 0.25M calcium chloride,
bringing the suspension to a concentration of 20% (v/v) with ethanol, allowing the preparation to precipitate, centrifuging to remove the precipitate, diluting the supernatant with ethanol to a concentration of 80%(v/v), centrifuging to precipitate the 38,000 daltons or 40,000 daltons outer membrane protein in the pellet, resolubilizing the protein in imidazole buffer (pH 7.0) or phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside, centrifuging to remove insoluble protein and diluting the supernatant with ethanol to a concentration of 66% (v/v), centrifuging to precipitate the 38,000 daltons or 40,000 daltons outer membrane protein in the pellet, resolubilizing in 200 mM sodium phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside, centrifuging to remove insoluble protein and eluting with sodium phosphate buffer (pH 6.5) over an hydroxylapatite chromatography column to recover the purified 38,000 daltons or 40,000 daltons protein.

6. A process for the preparation of an immunogenic conjugate according to Claim 1, which comprises covalently coupling fragments of oxidized polyribosyl-ribitolphosphate polysaccharide to the 38,000 daltons or 40,000 daltons mayor outer membrane protein of Haemophilus influenzae type b, wherein the oxidized polyribosyl-ribitol-phosphate polysaccharide fragments are prepared by treating purified polyribosyl-ribitol-phosphate polysaccharide with a periodate selected from the group consisting of sodium periodate, potassium periodate, and periodic acid at 4-25 °C in the dark, dialyzing against pyrogen-free water or purifying by gel filtration column chromatography, followed by concentrating and drying the oxidized polyribosyl-ribitol-phosphate polysaccharide.

7. A process for the preparation of an immunogenic conjugate as defined in Claim 6, wherein the immunogenic polysaccharide-protein conjugate is prepared by mixing the purified major outer membrane protein and the oxidized polyribosyl-ribitol-phosphate polysaccharide in buffer, at pH 3.5 to 9, to form Schiff base linkages between the oxidized polyribosylribitol-phosphate polysaccharide and the major outer membrane protein, reducing said linkages with a suitable borohydride reducing agent and purifying the resulting conjugate by gel filtration column chromatography or dialysis.

Claims for the following Contracting States : ES, GR

1. A process for the isolation and purification of the 38,000 daltons or 40,000 daltons major outer membrane protein of Haemophilus influenzae type b which comprises inactivating the Haemophilus influenzae type b, suspending the bacteria in water, bringing the suspension to a final concentration of 2% (w/v) hexadecyltrimethylammonium chloride, shaking the suspension vigorously for 1-2 hours, centrifuging the suspension, removing the pellet and resuspending the pellet in water, bringing the suspension to a final concentration of 0.25M calcium chloride,
bringing the suspension to a concentration of 20% (v/v) with ethanol, allowing the preparation to precipitate, centrifuging to remove the precipitate, diluting the supernatant with ethanol to a concentration of 80%(v/v), centrifuging to precipitate the 38,000 daltons or 40,000 daltons outer membrane protein in the pellet, resolubilizing the protein in imidazole buffer (pH 7.0) or phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside, centrifuging to remove insoluble protein and diluting the supernatant with ethanol to a concentration of 66% (v/v), centrifuging to precipitate the 38,000 daltons

Petitioner Merck Ex-1015, 1394

or 40,000 daltons outer membrane protein in the pellet, resolubilizing in 200 mM sodium phosphate buffer (pH 6.5) containing 2% octyl-β-D-glucopyranoside, centrifuging to remove insoluble protein and eluting with sodium phosphate buffer (pH 6.5) over an hydroxylapatite chromatography column to recover the purified 38,000 daltons or 40,000 daltons protein.

2. A process for the preparation of an immunogenic polysaccharide-protein conjugate comprising the reductive amination product of (a) an oxidized polyribosyl-ribitol-phosphate polysaccharide fragment derived from the capsular polysaccharide of Haemophilus influenzae type b, and (b) the major outer membrane protein of Haemophilus influenzae type b having a molecular weight of about 38,000 daltons or 40,000 daltons, which comprises covalently coupling fragments of oxidized polyribosyl-ribitol-phosphate polysaccharide to the 38,000 daltons or 40,000 daltons mayor outer membrane protein of Haemophilus influenzae type b, wherein the oxidized polyribosyl-ribitol-phosphate polysaccharide fragments are prepared by treating purified polyribosyl-ribitol-phosphate polysaccharide with a periodate selected from the group consisting of sodium periodate, potassium periodate and periodic acid at 4-25 °C in the dark, dialyzing against pyrogen-free water or purifying by gel filtration column chromatography, followed by concentrating and drying the oxidized polyribosyl-ribitol-phosphate polysaccharide.

3. A process for the preparation of an immunogenic conjugate as defined in Claim 2, wherein the immunogenic polysaccharide-protein conjugate is prepared by mixing the purified major outer membrane protein and the oxidized polyribosyl-ribitol-phosphate polysaccharide in buffer, at pH 3.5 to 9, to form Schiff base linkages between the oxidized polyribosylribitol-phosphate polysaccharide and the major outer membrane protein, reducing said linkages with a suitable borohydride reducing agent and purifying the resulting conjugate by gel filtration column chromatography or dialysis.

4. The process of Claim 2 wherein the 38,000 daltons or 40,000 daltons outer membrane protein of Haemophilus influenzae type b has the N-terminal amino acid sequence NH_2-alanine-valine-valine-tyrosine-asparagine-asparagine-glutamic acid-glycine-threonine-asparagine- valine-glutamic acid-leucine-glycine-glycine-arginine-leucine-serine-isoleucine-isoleucine-alanine-glutamic acid-glutamine-serine-asparagine-.

5. A vaccine comprising the immunogenic conjugate prepared in Claim 2.

6. The vaccine of Claim 5 which further comprises one or more of an immunologically acceptable diluent, carrier of adjuvant.

Patentansprüche
Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, NL, SE

1. Immunogenes Polysaccharid-Protein-Konjugat, umfassend das Produkt der reduktiven Aminierung von (a) einem oxidierten Polyribosylribitphosphatpolysaccharid-Fragment, das von dem kapsulären Polysaccharid von Haemophilus influenzae Typ b abgeleitet ist, und (b) dem Haupt-Außenmembranprotein von Haemophilus influenzae Typ b mit einem Molekulargewicht von ungefähr 38000 Dalton oder 40000 Dalton.

2. Konjugat nach Anspruch 1, worin das Außenmembranprotein von Haemophilus influenzae Typ b mit 38000 Dalton oder 40000 Dalton die N-terminale Aminosäuresequenz NH_2-Alanin-Valin-Valin-Tyrosin-Asparagin-Asparagin-Glutaminsäure-Glycin-Threonin-Asparagin-Valin-Glutaminsäure-Leucin-Glycin-Glycin-Arginin-Leucin-Serin-Isoleucin-Isoleucin-Alanin-Glutaminsäure-Glutamin-Serin-Asparagin- aufweist.

3. Impfstoff, umfassend das immunogene Konjugat nach Anspruch 1.

4. Impfstoff nach Anspruch 3, weiter umfassend eines oder mehrere eines immunologisch annehmbaren Verdünnungsmittels, Trägers oder Adjuvans.

5. Verfahren zur Isolierung und Reinigung des Haupt-Außenmembranproteins von Haemophilus influenzae Typ b mit 38000 Dalton oder 40000 Dalton, umfassend das Inaktivieren des Haemophilus influenzae

Petitioner Merck Ex-1015, 1395

Typ b, das Suspendieren der Bakterien in Wasser, das Bringen der Suspension auf eine Endkonzentration von 2% (Gew./Vol.) Hexadecyltrimethylammoniumchlorid, das heftige Schütteln der Suspension über 1-2 Stunden, das Zentrifugieren der Suspension, das Entfernen des Pellets und Resuspendieren des Pellets in Wasser, das Bringen der Suspension auf eine Endkonzentration von 0,25 M Calciumchlorid, das Bringen der Suspension auf eine Konzentration von 20% (Vol./Vol.) mit Ethanol, das Ausfallenlassen des Präparats, das Zentrifugieren, um den Niederschlag zu entfernen, das Verdünnen des Überstands mit Ethanol auf eine Konzentration von 80% (Vol./Vol.), das Zentrifugieren, um das Außenmembranprotein mit 38000 Dalton oder 40000 Dalton im Pellet auszufällen, das Wiederlösen des Proteins in Imidazolpuffer (pH 7,0) oder Phosphatpuffer (pH 6,5), der 2% Octyl-β-D-glucopyranosid enthält, das Zentrifugieren, um unlösliches Protein zu entfernen, und das Verdünnen des Überstands mit Ethanol auf eine Konzentration von 66% (Vol./Vol.), das Zentrifugieren, um das Außenmembranprotein mit 38000 Dalton oder 40000 Dalton im Pellet auszufällen, das Wiederlösen in 200 mM Natriumphosphatpuffer (pH 6,5), der 2% Octyl-β-D-glucopyranosid enthält, das Zentrifugieren, um unlösliches Protein zu entfernen, und das Eluieren mit Natriumphosphatpuffer (pH 6,5) über eine Hydroxylapatit-Chromatographiesäule, um das gereinigte Protein mit 38000 Dalton oder 40000 Dalton zu gewinnen.

6. Verfahren zur Herstellung eines immunogenen Konjugats nach Anspruch 1, umfassend das kovalente Kuppeln von Fragmenten von oxidiertem Polyribosylribitphosphatpolysaccharid an das Haupt-Außenmembranprotein von Haemophilus influenzae Typ b mit 38000 Dalton oder 40000 Dalton, worin die oxidierten Polyribosylribitphosphatpolysaccharid-Fragmente hergestellt werden durch Behandeln von gereinigtem Polyribosylribitphosphatpolysaccharid mit einem Periodat, das aus der aus Natriumperiodat, Kaliumperiodat und Periodsäure bestehenden Gruppe ausgewählt ist, bei 4-25 °C im Dunkeln, Dialysieren gegen Pyrogen-freies Wasser oder Reinigen durch Gelfiltrations-Säulenchromatographie, gefolgt vom Konzentrieren und Trocknen des oxidierten Polyribosylribitphosphatpolysaccharids.

7. Verfahren zur Herstellung eines immunogenen Konjugats nach Anspruch 6, worin das immunogene Polysaccharid-Protein-Konjugat hergestellt wird durch Mischen des gereinigten Haupt-Außenmembranproteins und des oxidierten Polyribosylribitphosphatpolysaccharids in Puffer bei pH 3,5 bis 9, um Schiff-Basen-Verknüpfungen zwischen dem oxidierten Polyribosylribitphosphatpolysaccharid und dem Haupt-Außenmembranprotein zu bilden, Reduzieren der Verknüpfungen mit einem geeigneten Borhydrid-Reduktionsmittel und Reinigen des resultierenden Konjugats durch Gelfiltrations-Säulenchromatographie oder Dialyse.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Isolierung und Reinigung des Haupt-Außenmembranproteins von Haemophilus influenzae Typ b mit 38000 Dalton oder 40000 Dalton, umfassend das Inaktivieren des Haemophilus influenzae Typ b, das Suspendieren der Bakterien in Wasser, das Bringen der Suspension auf eine Endkonzentration von 2% (Gew./Vol.) Hexadecyltrimethylammoniumchlorid, das heftige Schütteln der Suspension über 1-2 Stunden, das Zentrifugieren der Suspension, das Entfernen des Pellets und Resuspendieren des Pellets in Wasser, das Bringen der Suspension auf eine Endkonzentration von 0,25 M Calciumchlorid, das Bringen der Suspension auf eine Konzentration von 20% (Vol./Vol.) mit Ethanol, das Ausfallenlassen des Präparats, das Zentrifugieren, um den Niederschlag zu entfernen, das Verdünnen des Überstands mit Ethanol auf eine Konzentration von 80% (Vol./Vol.), das Zentrifugieren, um das Außenmembranprotein mit 38000 Dalton oder 40000 Dalton im Pellet auszufällen, das Wiederlösen des Proteins in Imidazolpuffer (pH 7,0) oder Phosphatpuffer (pH 6,5), der 2% Octyl-β-D-glucopyranosid enthält, das Zentrifugieren, um unlösliches Protein zu entfernen, und das Verdünnen des Überstands mit Ethanol auf eine Konzentration von 66% (Vol./Vol.), das Zentrifugieren, um das Außenmembranprotein mit 38000 Dalton oder 40000 Dalton im Pellet auszufällen, das Wiederlösen in 200 mM Natriumphosphatpuffer (pH 6,5), der 2% Octyl-β-D-glucopyranosid enthält, das Zentrifugieren, um unlösliches Protein zu entfernen, und das Eluieren mit Natriumphosphatpuffer (pH 6,5) über eine Hydroxylapatit-Chromatographiesäule, um das gereinigte Protein mit 38000 Dalton oder 40000 Dalton zu gewinnen.

2. Verfahren zur Herstellung eines immunogenen Polysaccharid-Protein-Konjugats, welches das Produkt der reduktiven Aminierung von (a) einem oxidierten Polyribosylribitphosphatpolysaccharid-Fragment, das von dem kapsulären Polysaccharid von Haemophilus influenzae Typ b abgeleitet ist, und (b) dem Haupt-Außenmembranprotein von Haemophilus influenzae Typ b mit einem Molekulargewicht von ungefähr 38000 Dalton oder 40000 Dalton umfaßt, umfassend das kovalente Kuppeln von Fragmenten

Petitioner Merck Ex-1015, 1396

von oxidiertem Polyribosylribitphosphatpolysaccharid an das Haupt-Außenmembranprotein von Haemophilus influenzae Typ b mit 38000 Dalton oder 40000 Dalton, worin die oxidierten Polyribosylribitphosphatpolysaccharid-Fragmente hergestellt werden durch Behandeln von gereinigtem Polyribosylribitphosphatpolysaccharid mit einem Periodat, das aus der aus Natriumperiodat, Kaliumperiodat und Periodsäure bestehenden Gruppe ausgewählt ist, bei 4-25 °C im Dunkeln, Dialysieren gegen Pyrogenfreies Wasser oder Reinigen durch Gelfiltrations-Säulenchromatographie, gefolgt vom Konzentrieren und Trocknen des oxidierten Polyribosylribitphosphatpolysaccharids.

3. Verfahren zur Herstellung eines immunogenen Konjugats nach Anspruch 2, worin das immunogene Polysaccharid-Protein-Konjugat hergestellt wird durch Mischen des gereinigten Haupt-Außenmembranproteins und des oxidierten Polyribosylribitphosphatpolysaccharids in Puffer bei pH 3,5 bis 9, um Schiff-Basen-Verknüpfungen zwischen dem oxidierten Polyribosylribitphosphatpolysaccharid und dem Haupt-Außenmembranprotein zu bilden, Reduzieren der Verknüpfungen mit einem geeigneten Borhydrid-Reduktionsmittel und Reinigen des resultierenden Konjugats durch Gelfiltrations-Säulenchromatographie oder Dialyse.

4. Verfahren nach Anspruch 2, worin das Außenmembranprotein von Haemophilus influenzae Typ b mit 38000 Dalton oder 40000 Dalton die N-terminale Aminosäuresequenz NH_2-Alanin-Valin-Valin-Tyrosin-Asparagin-Asparagin-Glutaminsäure-Glycin-Threonin-Asparagin-Valin-Glutaminsäure-Leucin-Glycin-Glycin-Atginin-Leucin-Serin-Isoleucin-Isoleucin-Alanin-Glutaminsäure-Glutamin-Serin-Asparagin- aufweist.

5. Impfstoff, umfassend das in Anspruch 2 hergestellte immunogene Konjugat.

6. Impfstoff nach Anspruch 5, weiter umfassend eines oder mehrere eines immunologisch annehmbaren Verdünnungsmittels, Trägers oder Adjuvans.

Revendications
Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, NL, SE

1. Conjugué immunogène polysaccharide-protéine comprenant le produit de l'amination réductrice de
   (a) un fragment de polysaccharide polyribosyl-ribitol-phosphate oxydé dérivé du polysaccharide capsulaire de Haemophilus influenzae type b, et
   (b) la grande protéine de la membrane externe de Haemophilus influenzae type b ayant une masse moléculaire d'environ 38 000 à 40 000 daltons.

2. Conjugué selon la revendication 1, dans lequel la protéine de la membrane externe de Haemophilus influenzae type b de 38 000 ou 40 000 daltons a la séquence d'aminoacides N-terminale NH_2 - alanine - valine - valine - tyrosine - asparagine-asparagine - acide glutamique - glycine - thréonine - asparagine - valine - acide glutamique - leucine - glycine - glycine - arginine - leucine - sérine - isoleucine - isoleucine - alanine - acide glutamique - glutamine - sérine - asparagine -.

3. Vaccin comprenant le conjugué immunogène de la revendication 1.

4. Vaccin selon la revendication 3, qui comprend en outre un ou plusieurs diluants, supports ou adjuvants immunologiquement acceptables.

5. Procédé pour isoler et purifier la grande protéine de la membrane externe de Haemophilus influenzae type b de 38 000 ou 40 000 daltons, dans lequel on inactive Haemophilus influenzae type b, on met les bactéries en suspension dans l'eau, on ajoute à la suspension du chlorure d'hexadécyltriméthylammonium jusqu'à une concentration finale de 2 % (masse/vol.), on agite vigoureusement la suspension pendant 1-2 heures, on centrifuge la suspension, on sépare le culot et on remet le culot en suspension dans l'eau, on ajoute à la suspension du chlorure de calcium jusqu'à une concentration finale de 0,25 M, on ajoute de l'éthanol à la suspension jusqu'à une concentration de 20 % (v/v), on laisse la préparation précipiter, on la centrifuge pour séparer le précipité, on dilue le surnageant avec de l'éthanol jusqu'à une concentration de 80 % (v/v), on centrifuge pour précipiter la protéine de la membrane externe de 38 000 ou 40 000 daltons dans le culot, on resolubilise la protéine dans du tampon d'imidazole (pH 7,0) ou du tampon de phosphate (pH 6,5) contenant 2 % d'octyl-β-D-

Petitioner Merck Ex-1015, 1397

glucopyranoside, on centrifuge pour éliminer les protéines insolubles et on dilue le surnageant avec de l'éthanol jusqu'à une concentration de 66 % (v/v), on centrifuge pour précipiter la protéine de la membrane externe de 38 000 ou 40 000 daltons dans le culot, on resolubilise dans du tampon de phosphate de sodium 200 mM (pH 6,5) contenant 2 % d'octyl-β-D-glucopyranoside, on centrifuge pour éliminer les protéines insolubles et on élue avec du tampon de phosphate de sodium (pH 6,5) sur une colonne de chromatographie d'hydroxyapatite pour récupérer la protéine purifiée de 38 000 ou 40 000 daltons.

6. Procédé de préparation d'un conjugué immunogène selon la revendication 1, qui comprend le couplage covalent de fragments de polysaccharide polyribosyl-ribitol-phosphate oxydé à la grande protéine de 38 000 daltons ou 40 000 daltons de la membrane externe de Haemophilus influenzae type b, dans lequel les fragments de polysaccharide polyribosyl-ribitol-phosphate oxydé sont préparés par traitement d'un polysaccharide polyribosyl-ribitol-phosphate purifié avec un periodate choisi dans le groupe constitué par le periodate de sodium, le periodate de potassium et l'acide periodique, à 4 - 25 °C à l'abri de la lumière, dialyse contre de l'eau apyrogène ou purification par chromatographie sur colonne de filtration sur gel, suivie par une concentration et un séchage du polysaccharide polyribosyl-ribitol-phosphate oxydé.

7. Procédé de préparation d'un conjugué immunogène tel que défini dans la revendication 6, dans lequel on prépare le conjugué polysaccharide-protéine immunogène par mélange de la grande protéine de la membrane externe purifiée et du polysaccharide polyribosyl-ribitol-phosphate oxydé dans un tampon, à un pH de 3,5 à 9, pour former des liaisons de bases de Schiff entre le polysaccharide polyribosyl-ribitol-phosphate oxydé et la grande protéine de la membrane externe, réduction desdites liaisons avec un agent réducteur de type borohydrure convenable et purification du conjugué obtenu par chromatographie sur colonne de filtration sur gel ou dialyse.

Revendications pour les Etats contractants suivants : ES, GR

1. Un procédé pour isoler et purifier la protéine majeure de la membrane externe de Haemophilus influenzae du type b de 38 000 daltons ou de 40 000 daltons, qui consiste à inactiver l'Haemophilus influenzae type b, à mettre en suspension les bactéries dans l'eau, à amener la suspension à une concentration finale de 2% (poids/volume) de chlorure d'hexadécyltriméthylammonium, à secouer la suspension vigoureusement pendant 1-2 h, à centrifuger la suspension, à séparer le culot de centrifugation et à remettre en suspension le culot dans l'eau, à amener la suspension à une concentration finale de chlorure de calcium de 0,25 M, à amener la suspension à une concentration de 20% (volume/volume) avec l'éthanol, à laisser la préparation précipiter, à centrifuger pour séparer le précipité, à diluer le liquide surnageant par de l'éthanol à une concentration de 80% (volume/volume), à centrifuger pour précipiter la protéine de la membrane externe de 38 000 daltons ou de 40 000 daltons dans le culot de centrifugation, à resolubiliser la protéine dans un tampon imidazole (pH 7,0) ou dans un tampon phosphate (pH 6,5) contenant 2% d'octyl-β-D-glucopyranoside, à centrifuger pour séparer la protéine insoluble et à diluer le liquide surnageant par de l'éthanol à une concentration de 66% (volume/volume), à centrifuger pour précipiter la protéine de la membrane externe de 38 000 daltons ou de 40 000 daltons dans le culot de centrifugation, à resolubiliser dans un tampon phosphate de sodium 200 mM (pH 6,5) contenant 2% d'octyl-β-D-glucopyranoside, à centrifuger pour séparer la protéine insoluble et à éluer par un tampon phosphate de sodium (pH 6,5) sur une colonne de chromatographie d'hydroxylapatite pour récupérer la protéine purifiée de 38 000 daltons ou de 40 000 daltons.

2. Un procédé pour la préparation d'un conjugué immunogène de polysaccharide-protéine comprenant le produit d'amination réductrice de

(a) un fragment de polysaccharide polyribosyl-ribitol-phosphate oxydé dérivé du polysaccharide capsulaire de l'Haemophilus influenzae type b, et (b) la protéine majeure de la membrane externe de l'Haemophilus influenzae type b ayant un poids moléculaire d'environ 38 000 daltons ou 40 000 daltons, qui consiste à coupler par covalence des fragments du polysaccharide polyribosyl-ribitol-phosphate oxydé avec la protéine majeure de la membrane externe de l'Haemophilus influenzae type b, de 38 000 daltons ou de 40 000 daltons, où les fragments de polysaccharide polyribosyl-ribitol-phosphate oxydé sont préparés par traitement du polysaccharide polyribosyl-ribitol-phosphate purifié par un periodate choisi dans le groupe comprenant le periodate de sodium, le periodate de potassium

Petitioner Merck Ex-1015, 1398

et l'acide periodique à 4-25 °C dans l'obscurité, dialyse par rapport à l'eau apyrogène ou purification par chromatographie sur colonne de filtration de gel suivie de la concentration et du séchage du polysaccharide polyribosyl-ribitol-phosphate oxydé.

3. Un procédé de préparation d'un conjugué immunogène tel que défini dans la revendication 2, selon laquelle le conjugué immunogène de polysaccharide-protéine est préparé par mélangeage de la protéine majeure purifiée de la membrane externe avec le polysaccharide polyribosyl-ribitol-phosphate oxydé dans un tampon, à pH 3,5 à 9, pour former des liaisons de base de Schiff entre le polysaccharide polyribosyl-ribitol-phosphate oxydé et la protéine majeure de la membrane externe, réduction desdites liaisons par un agent réducteur borohydrure approprié et purification du conjugué résultant par chromatographie sur colonne de filtration sur gel ou dialyse.

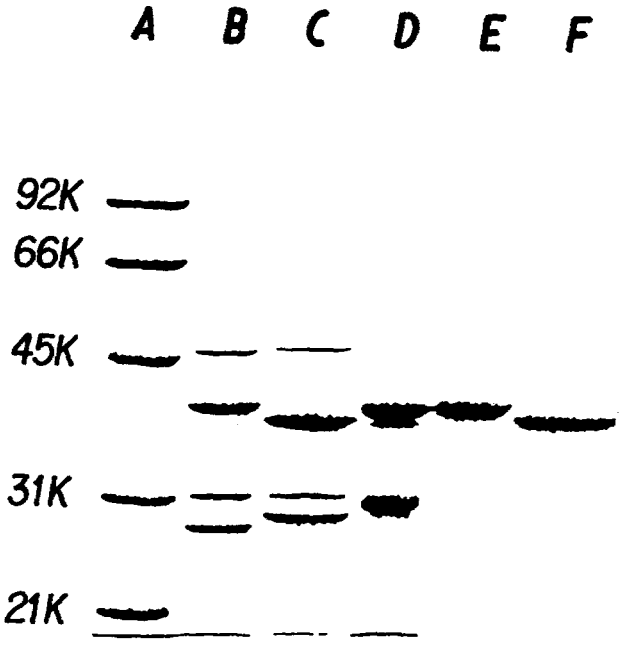
4. Le procédé selon la revendication 2, selon laquelle la protéine de la membrane externe de 38 000 daltons ou de 40 000 daltons de l'Haemophilus influenzae type b a la séquence d'aminoacides N terminale suivante : NH_2-alanine-valine-valine-tyrosine-asparagine-asparagine-acide glutamique-glycine-thréonine-asparagine-valine-acide glutamique-leucine-glycine-glycine-arginine-leucine-sérine-isoleucine-isoleucine-alanine-acide glutamique-glutamine-sérine-asparagine.

5. Un vaccin comprenant le conjugué immunogène préparé selon la revendication 2.

6. Le vaccin selon la revendication 5, qui comprend en outre un ou plusieurs diluants, supports ou adjuvants immunologiquement acceptables.

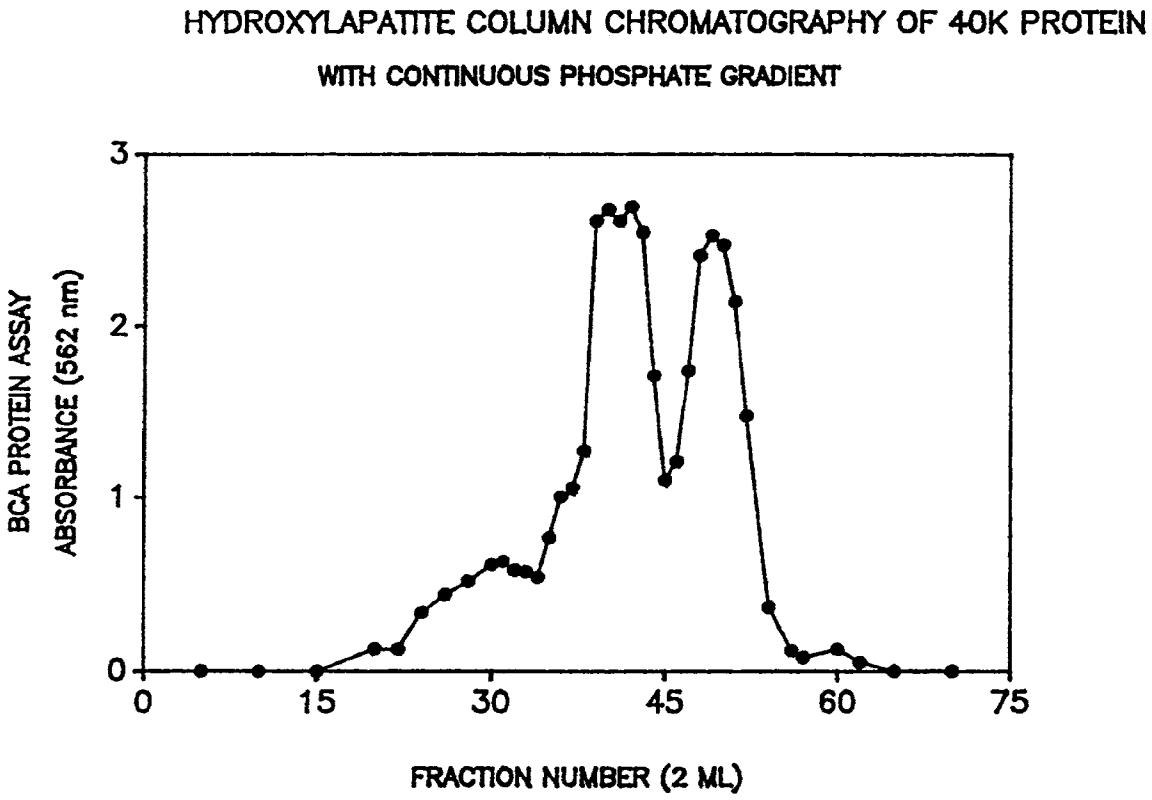
Petitioner Merck Ex-1015, 1399

FIG. 1



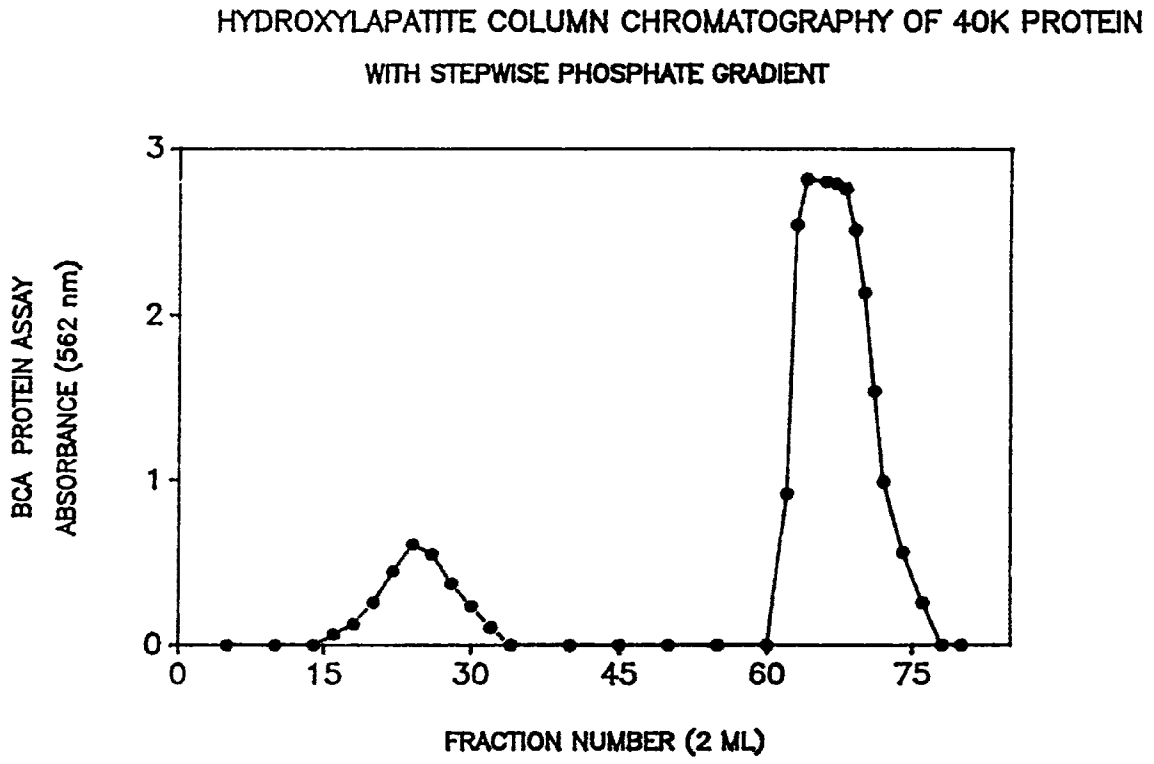
Petitioner Merck Ex-1015, 1400

FIG. 2



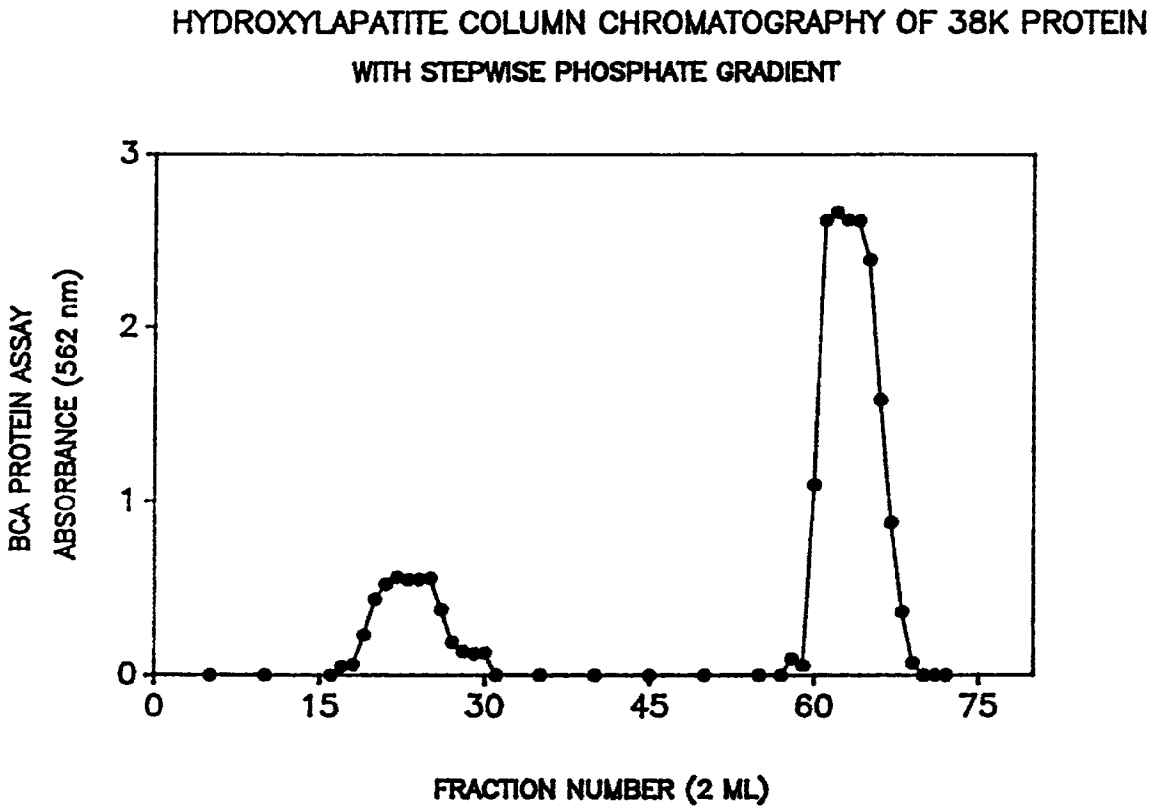
Petitioner Merck Ex-1015, 1401

FIG. 3



Petitioner Merck Ex-1015, 1402

FIG. 4



Petitioner Merck Ex-1015, 1403

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P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
17/347,435	05/20/2024 03:09:29 PM Z ET	P1143.70000US05

Title of Invention

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4020	FILED BY	Katelyn Farmer
PATENT CENTER #	65596110	AUTHORIZED BY	Chelsea Witte-Garcia
CUSTOMER #	23628	FILING DATE	06/14/2021
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Bruce D. Forrest

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 8543	E20245JF10396785	Katelyn Farmer

PRE-AUTHORIZED ACCOUNT	PRE-AUTHORIZED CATEGORY
232825	37 CFR 1.16 (National application filing, search, and examination fees); 37 CFR 1.17 (Patent application and reexamination processing fees)

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2251	EXTENSION FOR RESPONSE WITHIN FIRST MONTH	88.00	1	88.00
			TOTAL AMOUNT:	$88.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement

Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/96 (11-23)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Pogona, LLC

Application No./Patent No.: 17/347,435 Filed/Issue Date: June 14, 2021

Titled: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Pogona, LLC (Name of Assignee) , a Limited Liability Company (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. [x] The assignee of the entire right, title, and interest.

2. [ ] An assignee of less than the entire right, title, and interest (check applicable box):

   [ ] The extent (by percentage) of its ownership interest is _____ %. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   [ ] There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. [ ] The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. [ ] The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. [x] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 058283 , Frame 0489 , or for which a copy thereof is attached*.

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
   The document was recorded in the United States Patent and Trademark Office at
   Reel _____ , Frame _____ , or for which a copy thereof is attached*.

2. From: _____ To: _____
   The document was recorded in the United States Patent and Trademark Office at
   Reel _____ , Frame _____ , or for which a copy thereof is attached*.

12463937.1

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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached*.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached*.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached*.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached*.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ *As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Chelsea E. Witte-Garcia/	May 20, 2024
Signature	Date
Chelsea E. Witte-Garcia, PhD	75,713
Printed or Typed Name	Title or Registration Number

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 20, 2024 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

Docket No.: P1143.70000US05
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	17/347,435
Confirmation No.:	4020
Filed:	June 14, 2021
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	K. S. Shahnan Shah
Art Unit:	1645

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 20, 2024 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

RESPONSE TO RESTRICTION REQUIREMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

In response to the Restriction Requirement set forth in the Office Action mailed February 20, 2024, Applicant hereby provisionally elects Group I, a pharmaceutical composition, for continued examination. The elected invention encompasses at least claims 296-309.

Applicant believes no fee is due with this Response to Restriction Requirement, other than the enclosed fee associated with the extension of time. However, if a fee is due with this Response to Restriction Requirement, please

Petitioner Merck Ex-1015, 1408

charge our Deposit Account No. 23/2825, under Docket No. P1143.70000US05 from which the undersigned is authorized to draw.

Dated: May 20, 2024

Respectfully submitted,

By /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Petitioner Merck Ex-1015, 1409

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Alexandria, VA 22313 - 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
17/347,435	**05/20/2024 03:09:29 PM Z ET**	**P1143.70000US05**

Title of Invention

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4020	FILED BY	Katelyn Farmer
PATENT CENTER #	65596110	FILING DATE	06/14/2021
CUSTOMER #	23628	FIRST NAMED INVENTOR	Bruce D. Forrest
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Chelsea Witte-Garcia

Documents

TOTAL DOCUMENTS: 6

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
P114370000US05-373-CEW.pdf	2	Assignee showing of ownership per 37 CFR 3.73	27 KB
P114370000US05-ERRR-CEW.pdf	2	Response to Election / Restriction Filed	25 KB
P114370000US05-EXT-CEW.pdf	1	Extension of Time	24 KB
P114370000US05-TRN-CEW.pdf	1	Miscellaneous Incoming Letter	25 KB
P114370000US05-POA-CEW.pdf	2	Power of Attorney	99 KB

P114370000US05-FEE-CEW.pdf	1	Fee Worksheet (SB06)	33 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
P114370000US05-373-CEW.pdf	6A39892D76308FE0D4CE121427DA77B3E10E1F07EE2FE4E803377FD6EAA94E7E2D7060AA1EF83503B78DDEC64C6ADAF58C916274793E5DBBC715D7F3B33568CA
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P114370000US05-EXT-CEW.pdf	C7AA514CDEB213DD5B81411CAA68A26C87D122589EB49038F41CE5C577CC809753016EF17C2648BB772266776669258 9AACCA205A7838E8482B7C52706637A76
P114370000US05-TRN-CEW.pdf	E6D8EEC4C3C11159B5DCED08E96462D8E5EFAFBFD061FA49F323204DBBFB9AF3685B5C20AD5F8580FF2D50F86AB13087B4C8F1782D195423322E3A922D4D5983
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P114370000US05-FEE-CEW.pdf	1A4D4AE4B4AF877C3F85010BB07BE66CC4FD590A93BF384481D2AECF3827F3B571023D28B73E2446F43ED36762CBC5A5D94D66BDAE695CC273C2CF1803F5AD90

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/22 (11-23)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) P1143.70000US05
Application Number 17/347,435-Conf. #4020	Filed June 14, 2021
For SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	
Art Unit 1645	Examiner K. S. Shahnan Shah

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

	Fee	Small Entity Fee	Micro Entity Fee	
[x] One month (37 CFR 1.17(a)(1))	$220	$88	$44	$ 88.00
[ ] Two months (37 CFR 1.17(a)(2))	$640	$256	$128	$
[ ] Three months (37 CFR 1.17(a)(3))	$1,480	$592	$296	$
[ ] Four months (37 CFR 1.17(a)(4))	$2,320	$928	$464	$
[ ] Five months (37 CFR 1.17(a)(5))	$3,160	$1,264	$632	$

[x] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[x] Payment by credit card. ~~Form PTO-2038 is attached.~~

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[x] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 23/2825 .

[x] Payment made via USPTO patent electronic filing system.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

[ ] applicant.

[ ] attorney or agent of record. Registration number _____ .

[x] attorney or agent acting under 37 CFR 1.34. Registration number 75,713 .

/Chelsea E. Witte-Garcia/ — Signature
May 20, 2024 — Date

Chelsea E. Witte-Garcia, Ph.D. — Typed or printed name
617.646.8000 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[ ] * Total of 1 forms are submitted.

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 20, 2024 — Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

Petitioner Merck Ex-1015, 1413

Doc Code: TRAN.LET
Document Description: Transmittal Letter

USED IN LIEU OF PTO/SB/21 (07-09)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	17/347,435-Conf. #4020
Filing Date	June 14, 2021
First Named Inventor	Bruce D. Forrest
Art Unit	1645
Examiner Name	K. S. Shahnan Shah
Attorney Docket Number	P1143.70000US05

ENCLOSURES (*Check all that apply*)

- [x] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [x] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

-Response to Restriction Requirement
-Statement Under 37 CFR 3.73(c)

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	May 20, 2024	Reg. No.	75,713

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 20, 2024 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

12459511.1

FEE TRANSMITTAL

Complete if known	
Application Number	17/347,435-Conf. #4020
Filing Date	June 14, 2021
First Named Inventor	Bruce D. Forrest
Examiner Name	K. S. Shahnan Shah
Art Unit	1645
Practitioner Docket No.	P1143.70000US05

[x] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

TOTAL AMOUNT OF PAYMENT ($)88.00

METHOD OF PAYMENT (check all that apply)

[ ] Check [x] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

[ ] Charge fee(s) indicated below

[ ] Charge fee(s) indicated below, **except for the filing fee**

[x] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**

[ ] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17

[x] Credit any overpayment of fee(s)

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**Fees Paid ($)**
Utility	320	128*	64	700	280	140	800	320	160	
Design	220	88	44	160	64	32	640	256	128	
Plant	220	88	44	440	176	88	660	264	132	
Reissue	320	128	64	700	280	140	2,320	928	464	
Provisional	300	120	60	0	0	0	0	0	0	

* The $128 small entity status filing fee for a utility application is further reduced to $64 for a small entity applicant who files the application via Patent Center or EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	**Undiscounted Fee ($)**	**Small Entity Fee ($)**	**Micro Entity Fee ($)**
Each claim over 20 (including Reissues)	100	40	20
Each independent claim over 3 (including Reissues)	480	192	96
Multiple dependent claims	860	344	172

Total Claims		**Extra Claims**	**Fee ($)**		**Fee Paid ($)**
_____	- or HP =	_____	x _____	=	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		**Extra Claims**	**Fee ($)**		**Fee Paid ($)**
_____	- or HP =	_____	x _____	=	_____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $420 ($168 for small entity) ($84 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		**Extra Sheets**		**Number of each additional 50 or fraction thereof**	**Fee ($)**		**Fee Paid ($)**
_____	- 100 =	_____	/50 =	_____ (round **up** to a whole number)	x _____	=	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English specification, $140 fee ($56 small entity) ($28 for micro entity)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity)	
Other (e.g., late filing surcharge): 2251 Extension for response within first month	88.00

SUBMITTED BY

Signature	/Chelsea E. Witte-Garcia/	Registration No. (Attorney/Agent)	75,713	Telephone	617.646.8000
Name (Print/Type)	Chelsea E. Witte-Garcia, Ph.D.			Date	May 20, 2024

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: May 20, 2024 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

12459510.1

Doc Code: PA..
Document Description: Power of Attorney

PTO/AIA/82A (07-13)
Approved for use through 09/30/2025. OMB 0651-0035
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TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	17/347,435
Filing Date	June 14, 2021
First Named Inventor	Bruce D. Forrest
Title	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Art Unit	1645
Examiner Name	K. S. Shahnan Shah
Attorney Docket Number	P1143.70000US05

SIGNATURE of Applicant or Patent Practitioner

Signature	/Chelsea E. Witte-Garcia/	Date (Optional)	May 20, 2024
Name	Chelsea E. Witte-Garcia, Ph.D.	Registration Number	75,713
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ *Total of 1 forms are submitted.

Certificate of Electronic Filing under 37 C.F.R. § 1.8

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Dated: May 20, 2024 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

12463949.1

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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

[X] Practitioners associated with Customer Number: 23628

OR

[ ] Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

[X] The address associated with Customer Number: 23628

OR

[ ]

Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

Assignee name and address:

Pogona, LLC
135 Piermont Avenue
South Nyack, NY 10960

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee.

Signature	Date: 10 Dec 2019
Name Bruce D. Forrest	Telephone +1 845 353 4353
Title Manager Pogona, LLC	

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/347,435	06/14/2021	Bruce D. Forrest	P1143.70000US05

CONFIRMATION NO. 4020

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

POA ACCEPTANCE LETTER

OC000000073297207

Date Mailed: 06/03/2024

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/20/2024.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/lqchau/

Petitioner Merck Ex-1015, 1418

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/347,435	06/14/2021	Bruce D. Forrest	P1143.70000US05	4020

23628 7590 06/27/2024
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

EXAMINER
SHAHNAN SHAH, KHATOL S

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
06/27/2024	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Petitioner Merck Ex-1015, 1419

Office Action Summary	Application No. 17/347,435	Applicant(s) Forrest et al.	
	Examiner KHATOL S SHAHNAN SHAH	Art Unit 1645	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 1/28/2022.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 296-315 is/are pending in the application.

5a) Of the above claim(s) 310-315 is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 296-309 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☑ Claim(s) 296-315 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 6/14/2021 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date 5/20/2024.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Petitioner Merck Ex-1015, 1420

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. Applicants' amendment of 1/28/2022 is acknowledged. Claims 1-295 have canceled. New claims 296-315 have been added.

3. Applicants' response of 5/20/2024 is acknowledged.

Information Disclosure Statement

4. The information disclosure statement (IDS) submitted on 5/20/2024 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner. An initialed copy is attached hereto.

Drawings

5. The drawings submitted 6/14/2021 are accepted by the examiner.

Election/Restrictions

6. Applicant's election of Group I, claims 296-309 in the reply filed on May 20, 2024 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.01(a)).

Claims 310-315 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on May 20, 2024. Claims 296-309 are currently under examination.

Claim Objections

7. Claim 302 is objected to because of the following informalities:

As it pertains to claim 302: CRM^{197} and OMPC are acronyms which should be, on first sight, accompanied by their respective meanings. For example, cross-reacting material 197 (CRM^{197}); outer membrane protein complex (OMPC).

Additionally, CRM^{197} is a non-toxic mutant of diphtheria, which is a type of diphtheria toxoid. The claim recites CRM^{197} as well as a diphtheria toxoid. Diphtheria toxoid encompasses CRM^{197}.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

8. The following is a quotation of 35 U.S.C. 112(b):

> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 296-309 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claim 296 is rendered vague and indefinite by the use of the terms “fragment thereof”. It is unclear what is meant by said term, as it is not explicitly defined in the specification. What constitutes a “fragment” of a capsular polysaccharide? What core features/structures must be maintained? Which structures are Applicant in possession of? As written, it is impossible to determine the metes and bounds of the claimed invention.

Double Patenting

10. The non-statutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A non-statutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have

been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on non-statutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based e-Terminal Disclaimer may be filled out completely online using web-screens. An e-Terminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about e-Terminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

11. Claims 296-309 are rejected on the ground of non-statutory double patenting as being unpatentable over claims 1-10, 12-14 and 15 of U.S. Patent No. 11,058,757 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because:

Pending independent claim 296 is drawn to a pharmaceutical composition comprising at least 2 immunogenic saccharide- polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof, conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group,

wherein a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4,5, 6A, 6B, 6C, 7F, OV, 14, 18C, 19A, 19F, 23F, 8, 9N, LOA, 1LA, 12F, ISA, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, wherein the at least one additional serotype is not the first serotype.

Meanwhile, the patented claims are drawn to pharmaceutical composition comprising a plurality of at least two unique immunogenic saccharide-polypeptide conjugates,

Claim 1 of patent recite a pharmaceutical composition comprising a plurality of at least two unique immunogenic saccharide-polypeptide conjugates, each comprising individually a capsular polysaccharide conjugated to a polypeptide, wherein each of the capsular polysaccharides is from a group consisting of 23A, 23B, and 35B.

Claim 12 of patent recite the pharmaceutical composition of claim 1, further comprising at least one additional immunogenic saccharide 19. A method of making a composition comprising: polypeptide conjugate comprising a capsular polysaccharide contacting the plurality of immunogenic saccharide - poly from a unique Streptococcus pneumoniae serotype selected peptide conjugate of claim 1 with an excipient, and from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, adjuvant, or any combination thereof. 15B, 15C, 16F, 20A, 20B, 22F, and 34.

The claims of the patent and the instant application recite the same pharmaceutical composition and same limitations. The cited patented claims anticipate and/or make obvious the cited pending claims. The depend claims are also recited most of the same limitations.

Double Patenting

12. Claims 296-309 are provisionally rejected on the ground of non-statutory double patenting as being unpatentable over claims 449- 450, 455, 461- 466, 470, 471, 473-478, 481, 482-489, 492-494 of co-pending Application No. 16/753,597 (reference application has not been published). Although the claims at issue are not identical, they are not patentably distinct from each other because:

Claim 296 is drawn to a pharmaceutical composition comprising at least 2 immunogenic saccharide- polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof, conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4,5, 6A, 6B, 6C, 7F, OV, 14, 18C, 19A, 19F, 23F, 8, 9N, LOA, 1LA, 12F, ISA, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, wherein the at least one additional serotype is not the first serotype.

The co-pending Application No. 16/753,597 is drawn to a pharmaceutical composition comprising a plurality of immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein each of the capsular polysaccharide, fragment thereof, or combination thereof is from a *Streptococcus pneumoniae* serotype, wherein the *Streptococcus pneumoniae* serotype in the polarity of immunogenic saccharide-polypeptide conjugate comprise 3, 11A, 12F, 15A, 15B, 19A, 22F, 23A, and 35B and at least one additional saccharide-polypeptide conjugate *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 4, 5, 6A, 6B,6C, 7C, 7F,8, 9,9N, 9V, 10A, 14, 15C, 16F, 17F, 18C, 19F, 20B, 23F, 24B, 24F, 31, 33B, 33F, 34, 35F and 38.

The claims of the application No. 16/753,597 and the instant application recite the same pharmaceutical composition and same limitations The cited claims of the application No. 16/753,597 anticipate and/or make obvious the cited pending claims. The depend claims are also recite the same limitations.

The co-pending claims anticipate and/or make obvious the pending claims.

This is a provisional non-statutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claim Rejections - 35 USC § 102

13. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale, or otherwise available to the public before the effective filing date of the claimed invention.
>
> (a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed before the effective filing date of the claimed invention.

14. Claims 296, 298, 299, 301, 302, 303, 304, 305, 306, 307, 308 and 309 are rejected under 35 U.S.C. 102(a)(1)/102(a)(2) as being anticipated by Porro et al., (WO 2014/118201 A1), priority to 1/31/2013.

Claim 296 is drawn to a pharmaceutical composition comprising at least 2 immunogenic saccharide- polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof, conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4,5, 6A, 6B, 6C, 7F, OV, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, wherein the at least one additional serotype is not the first serotype.

Porro et al. disclose limitations of claims 296, 298, 299 a pharmaceutical or antigenic multivalent molecular construct consisting of a basic unit comprising a helper-T dependent carrier protein covalently bound to a minimum of three carbohydrate structures of different serological specificity, wherein each carbohydrate structure are at least three capsular polysaccharides selected among type 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7F, 8. 9N, 9V,10A, 11A, 11B, 11C, 11F, 12F, 14, 15A, 15B, 15C, 17F, 18C, 19F, 20, 22F, 23A, 23F, 33F and 35B of Streptococcus pneumoniae (see abs, claims specially claims 1, 2, 4, 5, 8, 9, 11. Porro et al. disclose conjugated polypeptides to capsular polysaccharides (see claims). The invention

contains each *S. pneumonia* capsular saccharide (see page 8, 12, 68 and claim 5). Porro et al. disclose that examples of carrier proteins which may be used in the present invention are diphtheria toxoid, tetanus toxoid, CRM197, pneumococcal surface protein (see claims 4, 7 8). meeting the limitation of claims 301, 302, 307). Porro et al. disclose cross-linked polysaccharide to poly peptide limitations of claim 307 see pages 43, 45 and 47. Porro et al. disclose the immunogenic composition may be adjuvanted (pages 26,31, 60, 63 and tables 5,6 and claims 13-14), and may further comprise a buffering agent (pages 36, 38, 43, 47, 52 and fig 4) and a salt (page 38 (meeting the limitation of claim 303). Moreover, the vaccine preparation (claim 11-12; meeting the limitation of claim 304) containing immunogenic compositions may be administered via the intramuscular, intradermal or subcutaneous routes (see page 31,61 and claim 14). As to limitation of claim 305, Porro et al. teach opsonophagocytic response (see pages 20, 61). As to limitation of claims 306 and 309. Porro et al. teach 0.1 to 1.0 microgram saccharide-polypeptide conjugate amount by weight (see claim 12 , page 62). As it pertains to claim 308, the Office takes the position that the prior art meets this limitation, particularly, the polypeptide that is a toxoid based upon Applicant's definition: paragraph 035- The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide. A CRM197 protein or toxoid is inherently, based upon Applicant's definition, 'partly mitigated'.

The prior art anticipates the claimed invention.

Since the Office does not have the facilities for examining and comparing applicants' composition with the composition of the prior art, the burden is on applicant to show a novel or unobvious difference between the claimed product and the prior art. See In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and in re Fitzgerald et al., 205 USPQ 594.

Claim Rejections - 35 USC § 102

15. Claims 296, 298, 299, 301, 302, 303, 304, and 307 are rejected under 35 U.S.C. 102(a)(1)/102(a)(2) as being anticipated by Matur et al., (WO 2016207805 A1, priority to 6/23/2015.

Claim 296 is drawn to a pharmaceutical composition comprising at least 2 immunogenic saccharide- polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof, conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4,5, 6A, 6B, 6C, 7F, OV, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, wherein the at least one additional serotype is not the first serotype.

As to claims 296, 301, 302, 303, 304, 307 Matur et al. abstract recite : The present invention relates to a multivalent Pneumococcal conjugate vaccine (PCV) composition comprising: 1) at least 12 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 15B, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* activated with CDAP and conjugated to carrier protein selected from CRM 191, pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA) or combination thereof and 2) a pharmaceutically acceptable carrier. Also see claims.

Matur et al. teach CRM 197, PsaA, diphtheria toxoid, T.T, (see claims and pages 2, 9, 11, 15 example 2). Matur et al. teach limitations of claims 298, 299 serotype 6C and 15 A (see claim 3 and pages 9, 15). Matur et al. teach limitations of claims 301, 302, 307 teach mixtures of polypeptides, saccharide cross-linked polypeptides (see claims and page 2). Matur et al. teach the immunogenic composition may be adjuvanted (pages 11, 12, 14, 15 claims 6-10), and may further comprise a buffering agent (pages 11, 12, 14, 15 claims 6-10) and a salt (page 12, 14, 15 claims 6-10 (meeting the limitation of claim 303). Matur et al. teach immunogenic compositions

may be administered via the intramuscular, intradermal or subcutaneous routes (see pages 12 , 17).

Claim Rejections - 35 USC § 102

17. Claims 296, 298, 301, 302, 303, 304, 305, 307 and 308 are rejected under 35 U.S.C. 102(a)(1)/102(a)(2) as being anticipated by Bertaud et al., (WO 2017/067962 A1, priority to 10/21/2015, submitted on Applicant's IDS).

Claim 296 is drawn to a pharmaceutical composition comprising at least 2 immunogenic saccharide- polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof, conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4,5, 6A, 6B, 6C, 7F, OV, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, wherein the at least one additional serotype is not the first serotype.

Bertaud et al. disclose pneumococcal capsular saccharide conjugate pharmaceutical composition or vaccines. In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 3, 11A, 12F, 15B, 19A and 22F (see page 9, lines 23-25; page 10; lines 2-8; meeting the limitation of claim 296). Bertaud et al. disclose that in one embodiment the composition comprises a plurality of serotypes selected from 1, 3, 4, 5, 6A, 6B,6C, 7F, 9V, 14, 18C, 19A, 19F and 23F (see page 13, lines 15-20; meeting the limitation of claim 296 and 298). The invention contains each *S. pneumonia* capsular saccharide (see page 26, lines 24-30). Bertaud et al. disclose that examples of carrier proteins which may be used in the present invention are Diphtheria toxoid, tetanus toxoid, CRM197, pneumococcal pneumolysin (see page 11, lines 14, 15, 23-25), detoxified pneumolysin and protein D. The combination comprises two or more different carrier proteins. In some instances, it comprises 2, 3, 4, 5 or 6 different carrier proteins (see page 12; lines 10-23; all meeting the limitation of claims

301, 302, 307). Bertaud et al. disclose that the carrier protein CRM197 having an average weight of between 210-410 or 250-310 kDa. Said polysaccharide is linked to said protein by a linker using CDAP chemistry (see page 22; lines 1-15; meeting the limitation of claim 302, 307. The immunogenic composition may be adjuvanted (page 35/36/42), and may further comprise a surfactant (page 41), a buffering agent (page 41/43) and a salt (page 35/43) (meeting the limitation of claim 303). Moreover, the vaccine preparation (page 43; meeting the limitation of claim 304) containing immunogenic compositions may be administered via the intramuscular, intradermal or subcutaneous routes (see page 42, lines 30-35; meeting the limitation of claim 304). As to limitation of claim 305, Bertaud et al. teach opsonophagocytic response (see pages 60, 67, 70, examples 3 and 6 and fig 3B). As it pertains to claim 308, the Office takes the position that the prior art meets this limitation, particularly, the polypeptide that is a toxoid based upon Applicant's definition: paragraph 035- The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide. A CRM197 protein or toxoid is inherently, based upon Applicant's definition, 'partly mitigated'.

The prior art anticipates the claimed invention.

Since the Office does not have the facilities for examining and comparing applicants' composition with the composition of the prior art, the burden is on applicant to show a novel or unobvious difference between the claimed product and the prior art. See In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and in re Fitzgerald et al., 205 USPQ 594.

Claim Rejections - 35 USC § 103

18. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

19. Claim 296-309 is rejected under 35 U.S.C. 103 as being unpatentable over Bertaud et al., WO 2017/067962 A1, priority to 10/21/2015 as applied to claims 296, 298, 301, 302, 303, 304, 305, 307 and 308 above, and further in view of del Amo et al. Clinical Microbiology and Infection vol. 20, pp. 684-689, 8 November 2013.

Claims are drawn to a pharmaceutical composition comprising at least 2 immunogenic saccharide- polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof, conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein a first serotype is selected from the group consisting of 6C, 15A, 16F, 20B, 24F, 31, and 34 and at least one additional serotype is selected from the group consisting of , 2, 3, 4,5, 6A, 6B, 6C, 7F, OV, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38, wherein the at least one additional serotype is not the first serotype.

Bertaud et al. disclose pneumococcal capsular saccharide conjugate pharmaceutical composition or vaccines. In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 3, 11A, 12F, 15B, 19A and 22F (see page 9, lines 23-25; page 10; lines 2-8; meeting the limitation of claim 296). Bertaud et al. disclose that in one embodiment the composition comprises a plurality of serotypes selected from 1, 3, 4, 5, 6A, 6B,6C, 7F, 9V, 14, 18C, 19A, 19F and 23F (see page 13, lines 15-20; meeting the limitation of claim 296 and 298). The invention contains each *S. pneumonia* capsular saccharide (see page 26, lines 24-30). Bertaud et al. disclose that examples of carrier proteins which may be used in the present invention are Diphtheria toxoid, tetanus toxoid, CRM197, pneumococcal pneumolysin (see page 11, lines 14, 15, 23-25), detoxified pneumolysin and protein D. The combination comprises two or more different carrier proteins. In some instances, it comprises 2, 3, 4, 5 or 6 different carrier proteins (see page 12; lines 10-23; all meeting the limitation of claims 301, 302, 307). Bertaud et al. disclose that the carrier protein CRM197 having an average weight of between 210-410 or 250-310 kDa. Said polysaccharide is linked to said protein by a linker using CDAP chemistry (see page 22; lines 1-15; meeting the

limitation of claim 302, 307. The immunogenic composition may be adjuvanted (page 35/36/42), and may further comprise a surfactant (page 41), a buffering agent (page 41/43) and a salt (page 35/43) (meeting the limitation of claim 303). Moreover, the vaccine preparation (page 43; meeting the limitation of claim 304) containing immunogenic compositions may be administered via the intramuscular, intradermal or subcutaneous routes (see page 42, lines 30-35; meeting the limitation of claim 304). As to limitation of claim 305, Bertaud et al. teach opsonophagocytic response (see pages 60, 67, 70, examples 3 and 6 and fig 3B). As it pertains to claim 308, the Office takes the position that the prior art meets this limitation, particularly, the polypeptide that is a toxoid based upon Applicant's definition: paragraph 035- The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide. A CRM197 protein or toxoid is inherently, based upon Applicant's definition, 'partly mitigated. As to limitation of claims 306 and 309 percentage amount by weight, this would be considered optimization of experimental parameters and would be obvious to one of ordinary skill in the art. However, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235(CCPA 1955).

Bertaud et al. do not teach serotypes recited in claims 297 (20B), 299 (15A) and 300 (16F).

del Amo et al. teach plurality of Streptococcal pneumoniae serotypes included in the new generations of conjugate vaccines or pharmaceutical compositions (see title, abstract page 685 and tables 1 and 2). del Amo et al. teach 40 serotypes/serogroups (1, 2, 3, 4, 5, 6A/6B, 6C, 7C/(7B/ 40), 7F/7A, 8, 9N/9L, 9V/9A, 10A, 10F/(10C/33C), 11A/11D, 12F/(12A/44/46), 13, 14, 15A/15F, 15B/15C, 16F, 17F, 18/(18A/ 18B/18C/18F), 19A, 19F, 20, 21, 22F/22A, 23A, 23B, 23F, 24/ (24A/24B/24F), 31, 33F/(33A/37), 34, 35A/(35C/42), 35B, 35F/ 47F, 38/25F, 39) see page 685.

It would have been obvious before the effective filing date of the presently claimed invention to employ serotypes 20B, 15A and 16F taught by del Amo et al. together with the claimed serotypes recited by Bertaud et al. to obtain the claimed invention. Because the extra serotypes further cover infections associated with

that serotype with a reasonable expectation of success. One of skill in the art would be motivated because one can prevent those infections also. There is an expectation of success because Bertaud et al. specifically teach that you can mix any of the ones listed and the conjugates have been made with various mixtures for a long time. All the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention. See the recent Board decision Ex parte Smith, --USPQ2d--, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007) (citing KSR, 82 USPQ2d at 1396). Accordingly, the subject matter of claims w o u l d have been prima facie obvious to one of ordinary skill in the art before the effective filing date of the presently claimed invention, absent evidence to the contrary.

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to combine known serotypes recited by the claims in a conjugate which function in a predictable manner to yield a reasonable expectation of success along with predictable results to one of ordinary skill in the art at the time of the invention. Thus, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Conclusion

20. No claims are allowed.

21. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KHATOL S SHAHNAN SHAH whose telephone number is (571)272-0863. The examiner can normally be reached on Mon-Tue, Thurs-Fri 12pm-8pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary B. Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Khatol S Shahnan-Shah/
Examiner, Art Unit 1645
June 14, 2024

/JANA A HINES/
Primary Examiner, Art Unit 1645

Notice of References Cited	Application/Control No. 17/347,435	Applicant(s)/Patent Under Reexamination Forrest et al.	
	Examiner KHATOL S SHAHNAN SHAH	Art Unit 1645	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date YYYY-MM-DD	Name	CPC Classification	US Classification
	A					
	B					
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	D					
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	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

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*	O	WO-2014118201-A1	2014-08-07	WO	PORRO et al	A61K39/092
*	P	WO-2016207905-A2	2016-12-29	WO	MATUR et al	A61K39/092
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*	U	del Amo et al. Clinical Microbiology and Infection vol. 20, pp. 684-689, 8 November 2013 (Year: 2013)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in YYYY-MM-DD format are publication dates. Classifications may be US or foreign.

Petitioner Merck Ex-1015, 1435

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	17/347,435	Forrest et al.
	Examiner	Art Unit
	KHATOL S SHAHNAN SHAH	1645

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
P2E2 all databases	06/13/2024	KSS
PALM inventor, biotech, continuity, priority	06/13/2024	KSS
Google scholar	06/13/2024	KSS
NPL	06/13/2024	KSS
Checked co pending applications	06/13/2024	KSS

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/KHATOL S SHAHNAN SHAH/ Examiner, Art Unit 1645	

Petitioner Merck Ex-1015, 1436

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 1 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
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EXAMINER:	DATE CONSIDERED:
/KHATOL S SHAHNAN SHAH/	06/13/2024

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

*a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 16/089,040, filed 09-27-2018, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

Petitioner Merck Ex-1015, 1437

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 2 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

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EXAMINER: /KHATOL S SHAHNAN SHAH/	DATE CONSIDERED: 06/13/2024

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Petitioner Merck Ex-1015, 1438

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 3 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

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/KHATOL S SHAHNAN SHAH/	06/13/2024

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*a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 16/089,040, filed 09-27-2018, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

Petitioner Merck Ex-1015, 1439

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 4 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

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/K.S.S/	*	Extended European Search Report, mailed November 4, 2019, in connection with Application No. EP 17776881 (P1143.70000EP00).	
/K.S.S/	*	International Search Report and Written Opinion, mailed September 13, 2017, in connection with Application No. PCT/US2017/025621 (P1143.70000WO00).	
/K.S.S/	*	International Preliminary Report on Patentability, mailed October 11, 2018, in connection with Application No. PCT/US2017/025621 (P1143.70000WO00).	
/K.S.S/		Extended European Search Report, mailed June 7, 2021, in connection with Application No. EP 18864637.6 (P1143.70001EP00).	
/K.S.S/	*	International Search Report and Written Opinion, mailed February 11, 2019, in connection with Application No. PCT/US2018/054391 (P1143.70001WO00).	
/K.S.S/	*	International Preliminary Report on Patentability, mailed April 16, 2020, in connection with Application No. PCT/US2018/054391 (P1143.70001WO00).	
/K.S.S/		[NO AUTHOR LISTED], Decision Denying Institution of Inter Partes Review 37 C.F.R. § 42.108. Case IPR2017-01223. Patent 9,399,060 B2. Paper 8. 2017 Oct 20. 22 pages.	
/K.S.S/	*	BENTLEY *et al.*, Genetic Analysis of the Capsular Biosynthetic Locus from All 90 Pneumococcal Serotypes. PLoS Genet. 2006 Mar; 2(3): e31. Epub 2006 Mar 10. doi: 10.1371/journal.pgen.0020031.	

EXAMINER:	DATE CONSIDERED:
/KHATOL S SHAHNAN SHAH/	06/13/2024

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

*a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 16/089,040, filed 09-27-2018, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

Petitioner Merck Ex-1015, 1440

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 5 of 6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

/K.S.S/		CHOCHUA *et al.*, Invasive Serotype 35B Pneumococci Including an Expanding Serotype Switch Lineage, United States, 2015-2016. Emerg Infect Dis. 2017 Jun;23(6):922-930. doi: 10.3201/eid2306.170071. Erratum in: Emerg Infect Dis. 2017 Oct;23(10):1762.	
/K.S.S/		COX *et al.*, Investigating the candidacy of a capsular polysaccharide-based glycoconjugate as a vaccine to combat *Haemophilus influenzae* type a disease: A solution for an unmet public health need. Vaccine. 2017 Oct 27;35(45):6129-6136. doi: 10.1016/j.vaccine.2017.09.055. Epub 2017 Sep 23.	
/K.S.S/	*	DURANDO *et al.*, Experience with pneumococcal polysaccharide conjugate vaccine (conjugated to CRM197 carrier protein) in children and adults. Clin Microbiol Infect. 2013;19 Suppl 1:1-9. doi:10.1111/1469-0691.12320.	
/K.S.S/		FRASCH, Analytical and Manufacturing Challenges: Preparation of Bacterial Polysaccharide Conjugates. Vaccine Technology III. Session IV: Conjugate Vaccines. Albufeira, Portugal. 2008 Jun 1-6. 20 pages.	
/K.S.S/	*	GUO *et al.*, Genetic diversity of fluoroquinolone-nonsusceptible *Streptococcus pneumoniae* clinical isolates and the first identification of serotype 20B in China. Eur J Clin Microbiol Infect Dis. 2014 Mar;33(3):465-70. doi: 10.1007/s10096-013-1979-5. Epub 2013 Oct 4.	
/K.S.S/	*	HU *et al.*, Towards the next generation of biomedicines by site-selective conjugation. Chem Soc Rev. 2016;45(6):1691-1719. doi:10.1039/c4cs00388h.	
/K.S.S/		HUANG *et al.*, Continued impact of pneumococcal conjugate vaccine on carriage in young children. Pediatrics. 2009 Jul;124(1):e1-11. doi: 10.1542/peds.2008-3099. Author Manuscript, 21 pages.	
/K.S.S/	*	JEFFERIES *et al.*, 13-valent pneumococcal conjugate vaccine (PCV13). Hum Vaccin. 2011;7(10):1012-1018. doi:10.4161/hv.7.10.16794.	
/K.S.S/		KAUFFMANN *et al.*, Memorandum on the Nomenclature of the Pneumococcus Group. Int Bull Bacteriol Nomen Tax. 1954 Apr 15;4(2):125-128.	
/K.S.S/	*	KOLKMAN *et al.*, Functional analysis of glycosyltransferases encoded by the capsular polysaccharide biosynthesis locus of *Streptococcus pneumoniae* serotype 14. J Biol Chem. 1997;272(31):19502-19508. doi:10.1074/jbc.272.31.19502.	
/K.S.S/	*	NUNES *et al.*, The impact of private use of PCV7 in 2009 and 2010 on serotypes and antimicrobial resistance of Streptococcus pneumoniae carried by young children in Portugal: Comparison with data obtained since 1996 generating a 15-year study prior to PCV13 introduction. Vaccine. 2016 Mar 29;34(14):1648-56. doi: 10.1016/j.vaccine.2016.02.045. Epub 2016 Feb 23.	
/K.S.S/		PORAT *et al.*, Four antibiotic-resistant *Streptococcus pneumoniae* clones unrelated to the pneumococcal conjugate vaccine serotypes, including 2 new serotypes, causing acute otitis media in southern Israel. J Infect Dis. 2004 Feb 1;189(3):385-92. doi: 10.1086/381183. Epub 2004 Jan 16.	

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Petitioner Merck Ex-1015, 1441

FORM PTO-1449/A and B (modified PTO/SB/08)				APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
				FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet	6	of	6	GROUP ART UNIT: 1645	EXAMINER: Khatol S. S. Shah

/K.S.S/	*	SKINNER *et al.*, Pre-clinical evaluation of a 15-valent pneumococcal conjugate vaccine (PCV15-CRM197) in an infant-rhesus monkey immunogenicity model. Vaccine. 2011;29(48):8870-8876. doi:10.1016/j.vaccine.2011.09.078.	

[NOTE – No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287 OG 163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

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CONFIRMATION NO. 4020

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
17/347,435	06/14/2021 RULE	424	1645	P1143.70000US05

APPLICANTS
Pogona, LLC, South Nyack, NY;

INVENTORS
Bruce D. Forrest, South Nyack, NY;
Jack D. Love, Tampa, FL;

**** CONTINUING DATA ***************************
This application is a CON of 16/089,040 09/27/2018 PAT 11058757
which is a 371 of PCT/US2017/025621 03/31/2017
which claims benefit of 62/373,807 08/11/2016
and claims benefit of 62/372,263 08/08/2016
and claims benefit of 62/330,245 05/02/2016
and claims benefit of 62/316,555 03/31/2016

**** FOREIGN APPLICATIONS ***************************

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06/24/2021

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
❑ Yes ☑ No	❑ Yes ☑ No	/KHATOL S SHAHNAN SHAH/ Examiner's Signature	❑ KS Initials	NY	15	20	1

ADDRESS
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206
UNITED STATES

TITLE
SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

FILING FEE RECEIVED	FEES: Authority has been given in Paper	
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Related searches

antimicrobial properties polypeptide conjugates

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L2105	40	forrest ADJ bruce	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 09:58 AM
L2106	28	L2105 AND saccharide	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 09:59 AM
L2107	26	L2106 AND polypeptide	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 09:59 AM
L2108	26	L2107 AND conjugate	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2024/06/14 10:00 AM

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			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)				
L2109	0	LL2108 AND capsular	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:01 AM
L2110	21	jack ADJ love	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:02 AM
L2111	0	L2110 AND conjugate	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:03 AM
L2112	0	L2110 AND (capsular ADJ polysaccharide)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT,	OR	ON	ON	2024/06/14 10:04 AM

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			AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)				
L2113	21720	conjugate AND (capsular ADJ polysaccharide)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:05 AM
L2114	0	L2113 AND (strepcoccus ADJ pneumoniae)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:06 AM
L2115	9501	L2113 AND (6B OR 6C OR 23A OR 23B)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO;	OR	ON	ON	2024/06/14 10:07 AM

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			JPO; DERWENT)				
L2116	8117	L2115 AND immunogenic	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:07 AM
L2117	5990	L2116 AND pharmaceutical	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:08 AM
L2118	4688	L2117 AND serotype	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:09 AM
L2119	4382	L2118 AND streptococcus	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL,	OR	ON	ON	2024/06/14 10:09 AM

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			PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)				
L2120	4187	L2119 AND pneumoniae	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:09 AM
L2121	3222	L2120 AND polypeptide	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:10 AM
L2122	2248	L2121 AND saccharide	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:10 AM
L2123	1525	L2122 AND CRM197	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB,	OR	ON	ON	2024/06/14 10:11 AM

			HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)				
L2124	592	L2123 AND KLH	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:11 AM
L2125	476	L2124 AND PspA	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:12 AM
L2126	473	L2125 AND PsaA	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:12 AM
L2127	358	L2126 AND OMPC	(US-PGPUB; USPAT; USOCR; FIT (AP, AT,	OR	ON	ON	2024/06/14 10:13 AM

Petitioner Merck Ex-1015, 1452

			AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)				
L2128	13	L2127 AND uspA	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:13 AM
L2129	0	LL2128 AND (20B OR 16F OR 15A)	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO; JPO; DERWENT)	OR	ON	ON	2024/06/14 10:14 AM
L2130	0	LL2128 AND 20B	(US-PGPUB; USPAT; USOCR; FIT (AP, AT, AU, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, MC, MD, MY, NL, NO, NZ, OA, PH, PL, PT, RO, RS, RU, SE, SG, SI, SK, SU, TH, TN, TR, TW, UA, VN, WO); FPRS; EPO;	OR	ON	ON	2024/06/14 10:15 AM

Petitioner Merck Ex-1015, 1453

			JPO; DERWENT)				

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

Petitioner Merck Ex-1015, 1454

Conjugate of structurally reassigned pneumococcal serotype 31 polysaccharide with CRM197 elicited potent immune response

T Sun, S Mai, H Mao, H Li, Y Duan, S Meng, J Bao… - Carbohydrate …, 2022 - Elsevier

… The reaction is faster, and the DMF **conjugate** elicited comparable immune responses with the aqueous **conjugate**. S-31-ps **conjugate** vaccine has the potential of being included in the …

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Development of functional star-shaped polypeptides for biomedical applications

S Stefanovic - 2023 - repository.rosi.com

… For that reason, it is of great importance to design the polymeric **composition** in such a way as … The synthesised star bis-MPA-PLL **conjugates** were examined as gene vectors. Pendant …

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DOCKET NO.: P1143.70000US05

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	17/347,435
Confirmation No.:	4020
Filed:	June 14, 2021
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Khatol S. Shahnan Shah
Art Unit:	1645

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: September 27, 2024 Electronic Signature for Abigail S. Feldman: /Abigail S. Feldman/

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98

Commissioner:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed more than three months after the filing date of the present Application and after the mailing date of a first Office Action, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311, or an action that otherwise closes prosecution in the present Application. Please charge our Credit Card in the amount of $104.00 covering the fee set forth in 37 C.F.R. § 1.17(p).

Petitioner Merck Ex-1015, 1456

PART II: Information Cited

The undersigned hereby makes of record in the present Application the information listed on the attached Form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed Form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of the present Application; and

3. The citations for the information be printed on any patent which issues from the present Application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency in the fees due, or credit any overpayment, by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825, under Docket No. P1143.70000US05, from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Jessica A. von Reyn/
Jessica A. von Reyn
Registration No.: 76,839
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: P1143.70000US05
Date: September 27, 2024

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
17/347,435	**09/27/2024 11:23:42 AM Z ET**	**P1143.70000US05**

Title of Invention

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4020	FILED BY	Abigail Feldman
PATENT CENTER #	67350200	FILING DATE	06/14/2021
CUSTOMER #	23628	FIRST NAMED INVENTOR	Bruce D. Forrest
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Jessica von Reyn

Documents

TOTAL DOCUMENTS: 10

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
No_Author_Listed_Pneumococcal_Polysaccharide_Vaccine_Wikipedia_2015_4pgs-NPL.pdf	4	Non Patent Literature	346 KB
Curry_et_al_Vaccine_2023_v41_p903-NPL.pdf	11	Non Patent Literature	563 KB
Henrichsen_J_Clin_Microbiol_1995_v33_p2759-NPL.pdf	4	Non Patent Literature	105 KB
Robbins_et_al_The_J_Infectious_Disease_1983_v148_p1136-NPL.pdf	24	Non Patent Literature	4904 KB
Schiffman_et_al_Reviews_Infectious_Disease_1981_v3_pS18-NPL.pdf	13	Non Patent Literature	2490 KB

Sih_et_al_Ann_Otol_Rhinol_Laryngol_2001_v110_p662-NPL.pdf	5	Non Patent Literature	1100 KB
P114370000US05-TRN-JXV.pdf	1	Miscellaneous Incoming Letter	26 KB
P114370000US05-FEE-JXV.pdf	1	Fee Worksheet (SB06)	34 KB
P114370000US05-IDSBODY-JXV.pdf	3	Letter specifying the conditions for filing under 37 CFR 1.97	31 KB
P114370000US05-IDS1449-JXV.pdf	1	Information Disclosure Statement (IDS) Form (SB08)	27 KB

Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
No_Author_Listed_Pneumococcal_Polysaccharide_Vaccine_Wikipedia_2015_4pgs-NPL.pdf	49808E153048CF6B3D195D3BE928CBBEBEC9AE58219AB9FC69A82A06B7A5EB504B27B8DA4C2F75C216953FDA1F64C22B4C8145CC9E1DE4E8153D8BFF56B866EC
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Schiffman_et_al_Reviews_Infectious_Disease_1981_v3_pS18-NPL.pdf	ED0AA11613FEE2462C43A20F5F44AF070F37DDD17417013D25E339956371FDEC00CA9A7AE88F382B4DAE994F8815BE8072B2A3B84BBA8983F4004486E84F5102
Sih_et_al_Ann_Otol_Rhinol_Laryngol_2001_v110_p662-NPL.pdf	49B3C8C9B66C9BA52F03A3BFD7204A3D54D84245A4B1D5AD45F1A14E124E7B7837AE80993774F5DAC0CE56CE7A5CBF0D15955B7EA3B120CDC502B3CC1B147B3F
P114370000US05-TRN-JXV.pdf	6DFDE151D64032F1660F4D98B0C0B9A1C8CB610EF2117BBD8F3EF3B6AC27379F4F2AC1610C2A5006F53F68E51652396B513798E485E7033529E59A6C7E7596D7
P114370000US05-FEE-	E510FEDBC143A120C30BB52FE2A7241F1D5D3D336FDF0244

JXV.pdf	3FEE493953FA19363420EA929BD54FA96B9498C14551D3A8D8BB8BE5D6ABBEDFF11D89F7A3ACFAD1
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P114370000US05-IDS1449-JXV.pdf	E608CE269AFAACD3E35EA2B01A140033CF294CC4130B19F142A1D44FC43D2323F0D8113B0A3490D24B509B89E4F2FE0788187DAE46DB75223C5A3F37844106CE

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 17/347,435	ATTY. DOCKET NO.: P1143.70000US05
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: June 14, 2021	CONFIRMATION NO.: 4020
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 1 of 1	GROUP ART UNIT: 1645	EXAMINER: Khatol S. Shahnan Shah

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		

FOREIGN PATENT DOCUMENTS

Examiner's Initials #	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/ Country	Number	Kind Code			

OTHER ART – NON PATENT LITERATURE DOCUMENTS

Examiner's Initials #	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
		[NO AUTHOR LISTED], Pneumococcal polysaccharide vaccine. Wikipedia. 2015. Accessed from <https://web.archive.org/web/20150227211129/https://en.wikipedia.org/wiki/Pneumococcal_polysaccharide_vaccine>. 4 pages.	
		CURRY *et al*., Preclinical evaluation of an investigational 21-valent pneumococcal conjugate vaccine, V116, in adult-rhesus monkey, rabbit, and mouse models. Vaccine. 2023 Jan 23;41(4):903-913. doi: 10.1016/j.vaccine.2022.12.017. Epub 2022 Dec 22.	
		HENRICHSEN, J., Six newly recognized types of *Streptococcus pneumoniae*. J Clin Microbiol. 1995 Oct;33(10):2759-62. doi: 10.1128/jcm.33.10.2759-2762.1995.	
		ROBBINS *et al*., Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J Infect Dis. 1983 Dec;148(6):1136-59. doi: 10.1093/infdis/148.6.1136.	
		SCHIFFMAN, G., Chemistry and immunochemistry of the pneumococcal polysaccharide vaccine with special reference to cross-reactions and immunologic factors. Rev Infect Dis. 1981 Mar-Apr;3 Suppl:S18-30. doi: 10.1093/clinids/3.supplement_1.s18.	
		SIH, T.M., Acute otitis media in Brazilian children: analysis of microbiology and antimicrobial susceptibility. Ann Otol Rhinol Laryngol. 2001 Jul;110(7 Pt 1):662-6. doi: 10.1177/000348940111000712.	

[NOTE – No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287 OG 163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

EXAMINER:	DATE CONSIDERED:

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

13174455.1

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
17/347,435	**09/27/2024 11:23:42 AM Z ET**	**P1143.70000US05**

Title of Invention

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	4020	FILED BY	Abigail Feldman
PATENT CENTER #	67350200	AUTHORIZED BY	Jessica von Reyn
CUSTOMER #	23628	FILING DATE	06/14/2021
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Bruce D. Forrest

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 8543	**E20249QB25317313**	**Abigail Feldman**

PRE-AUTHORIZED ACCOUNT	PRE-AUTHORIZED CATEGORY
232825	**37 CFR 1.17 (Patent application and reexamination processing fees)**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2806	SUBMISSION OF AN INFORMATION DISCLOSURE STATEMENT	104.00	1	104.00
			TOTAL AMOUNT:	**$104.00**

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National Stage of an International Application under 35 U.S.C. 371

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.