

## A MAPS VACCINE TARGETING SALMONELLA ENTERICA SEROVARS

### FIELD OF THE INVENTION

[001] The present invention relates to technologies, compositions, and methods for the prevention and/or treatment of Salmonella infections.

### CROSS-REFERENCED TO RELATED APPLICATIONS

[002] This application is a 371 National Phase Entry of International Patent Application which claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/285,463 filed December 2, 2021, the contents of which is incorporated herein by reference in its entirety.

### SEQUENCE LISTING

[003] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on November 29, 2022, is named 701039-000106WOPT\_SL.xml and is 51,561 bytes in size.

### BACKGROUND OF INVENTION

[004] Diseases caused by Salmonella bacteria range from a mild, self-limiting diarrhea to serious gastrointestinal and septicemic disease in humans and animals. Salmonella is a gram-negative, rod-shaped, motile bacterium (nonmotile exceptions include *S. gallinarum* and *S. pullorum*) that is non-spore forming. Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry, and raw seafoods.

[005] Salmonella infection is a widespread occurrence in animals, especially in poultry and swine, and is one of the most economically damaging of the enteric and septicemic diseases that affect food producing animals. Although many serotypes of Salmonella have been isolated from animals, *S. choleraesuis* and *S. typhimurium* are the two most frequently isolated serotypes associated with clinical salmonellosis in pigs. In swine, *S. typhimurium* typically causes an enteric disease, while *S. choleraesuis* (which is host-adapted to swine) is often the etiologic agent of a fatal septicemic disease with little involvement of the intestinal tract. *S. dublin* and *S. typhimurium* are common causes of infection in cattle; of these, *S. dublin* is host adapted to cattle and is often the etiologic agent of a fatal septicemic disease. Other serotypes such as *S. gallinarum* and *S. pullorum* are important etiologic agents of salmonellosis in avian and other species. Although these serotypes primarily infect animals, *S. dublin* and *S. choleraesuis* also often cause human disease.

[006] Various Salmonella species have been isolated from the outside of egg shells, including *S. enteritidis* which may even be present inside the egg yolk. It has been suggested that the presence of the organism in the yolk is due to transmission from the infected layer hen prior to shell deposition. Foods other than eggs have also caused outbreaks of *S. enteritidis* disease in humans.

[007] Infections caused by *Salmonella enterica* serovars are a significant problem worldwide. For example, *Salmonella enterica* serovars *Typhimurium* and *Enteritidis* are the predominant causes of invasive non-typhoidal *Salmonella* (iNTS) disease, a bloodstream infection with high prevalence in sub-Saharan Africa, especially among young children and HIV-infected individuals.

[008] *S. typhi* and *S. paratyphi* A, B, and C produce typhoid and typhoid-like fever in humans. Although the initial infection with salmonella typically occurs through the gastrointestinal tract, typhoid fever is a systemic disease that spreads throughout the host and can infect multiple organ sites. The fatality rate of typhoid fever can be as high as 10% (compared to less than 1% for most forms of salmonellosis). *S. dublin* has a 15% mortality rate when the organism causes septicemia in the elderly, and *S. enteritidis* has an approximately 3.6% mortality rate in hospital/nursing home outbreaks, with the elderly being particularly affected.

[009] Numerous attempts have been made to protect humans and animals by immunization with a variety of vaccines. Many of the current vaccines provide only poor to moderate protection and require large doses to be completely efficacious. Previously used vaccines against salmonellae and other infectious agents have generally fallen into four categories: (i) specific components from the etiologic agent, including cell fractions or lysates, intact antigens, fragments thereof, or synthetic analogs of naturally occurring antigens or epitopes (often referred to as subunit vaccines); (ii) antiidiotypic antibodies; (iii) the whole killed etiologic agent (often referred to as killed vaccines); or (iv) an avirulent (attenuated) derivative of the etiologic agent used as a live vaccine.

[0010] Reports in the literature have shown that attenuated live vaccines are more efficacious than killed vaccines or subunit vaccines for inducing protective immunity, however, high doses of live vaccines are often required for efficacy and few live-attenuated *Salmonella* vaccines are commercially available. Ideally, a vaccine retains the ability to infect the host without causing serious disease and is also capable of stimulating humoral (antibody-based) immunity and cell-mediated immunity sufficient to provide resistance to any future infection by virulent bacteria.

[0011] There remains a medical need for a vaccine that provides immunity against a broad range of serotypes of *Salmonella*.

## SUMMARY OF INVENTION

[0012] The present disclosure addresses the lack of suitable technologies for the prevention and/or treatment of *Salmonella* infections.

[0013] In some embodiments, a vaccine comprises 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 comprises a polysaccharide of *Salmonella enterica*, and further wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex.

[0014] In some embodiments, the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica* selected from Vi polysaccharide of Typhi, and O-specific polysaccharide of Typhimurium, Enteritidis, and Paratyphi.

[0015] In some embodiments, the at least one polypeptide antigen of the fusion protein is or comprises a polypeptide antigen from *Salmonella*, *Shigella*, or *Streptococcus pneumoniae*.

[0016] In some embodiments, the at least one polypeptide antigen of the fusion protein comprises a *Salmonella* SseB polypeptide or antigenic fragment thereof.

[0017] In some embodiments, the at least one polypeptide antigen of the fusion protein comprises a *Shigella* IpaB polypeptide or antigenic fragment thereof.

[0018] In some embodiments, the at least one polypeptide antigen of the fusion protein comprises a *Streptococcus pneumoniae* SP1500 polypeptide or antigenic fragment thereof; a *Streptococcus pneumoniae* SP0785 polypeptide or antigenic fragment thereof, or both.

[0019] In some embodiments, the at least one polypeptide antigen of the fusion protein 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: 4.

[0020] In some embodiments, the at least one polypeptide antigen of the fusion protein 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: 5.

[0021] In some embodiments, the at least one polypeptide antigen of the fusion protein 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 either SEQ ID NO: 8 or SEQ ID NO: 9, or a combination of SEQ ID NO: 8 and SEQ ID NO: 9.

[0022] In some embodiments, the biotin-binding moiety is a polypeptide 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: 3 or a biotin-binding fragment thereof.

[0023] In some embodiments, an immunogenic composition (e.g., a vaccine) comprises a plurality of different species of immunogenic complexes, wherein the different species comprise: a plurality of biotinylated polysaccharide antigens comprising polysaccharide antigens of one or more *Salmonella enterica* serotypes; and a plurality of fusion proteins, each fusion protein comprising: a biotin-binding moiety; and a polypeptide antigen, 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.

[0024] In some embodiments, the different species each comprise a distinct polysaccharide antigen of one or more *Salmonella enterica* serotypes and/or a distinct polypeptide antigen.

[0025] In some embodiments, the one or more *Salmonella enterica* serotypes is or comprises S. Typhimurium, S. Enteritidis, S. Typhi Vi, S. Paratyphi A, or combinations thereof.

[0026] In some embodiments, the polypeptide antigen is or comprises a polypeptide antigen from *Salmonella*, *Shigella*, and/or *Streptococcus pneumoniae*.

[0027] In some embodiments, the polypeptide antigen is or comprises: an SseB polypeptide antigen of *Salmonella*, an IpaB polypeptide antigen of *Shigella*, and/or a polypeptide antigen comprising an SP1500 polypeptide and/or an SP0785 polypeptide, of *S. pneumoniae*.

[0028] In some embodiments, the immunogenic composition comprises at least two different species of immunogenic complexes, wherein the different species comprise: a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein, wherein the first fusion protein and the second fusion protein each independently comprise: a biotin-binding moiety; and a polypeptide antigen, wherein each of the first and second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

[0029] In some embodiments, the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*.

[0030] In some embodiments, the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof.

[0031] In some embodiments, the immunogenic composition comprises at least two different species of immunogenic complexes, wherein the different species comprise: a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi* Vi non-covalently complexed with a first fusion protein; and a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a second fusion protein, wherein the first fusion protein and the second fusion protein each independently comprise: a biotin-binding moiety; and a polypeptide antigen, wherein each of the first and second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

[0032] In some embodiments, the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SP1500 polypeptide, an SP0785 polypeptide, or both, of *S. pneumoniae* with Rhizavidin (Rhavi), and/or the fusion protein is CP1.

[0033] In some embodiments, the SP1500 polypeptide antigen of *S. pneumoniae* 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: 9, or an immunogenic fragment thereof; the SP0785 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; and the CP1 fusion protein 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, or an immunogenic fragment thereof.

**[0034]** In some embodiments, the immunogenic composition as disclosed herein, comprises at least four different species of immunogenic complexes, wherein the different species comprise: a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein, a third biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi Vi* non-covalently complexed with a third fusion protein; and a fourth biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a fourth fusion protein, wherein the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein each independently comprise: a biotin-binding moiety; and a polypeptide antigen, wherein each of the first, second, third, and fourth biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

**[0035]** In some embodiments, the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*, and the polypeptide antigen of the third fusion protein and of the fourth fusion protein is or comprises an SP1500 polypeptide, an SP0785 polypeptide, or both, of *S. pneumoniae* with Rhavi, and/or the fusion protein is CP1.

**[0036]** In some embodiments, the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof; the SP1500 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; the SP0785 polypeptide antigen of *S. pneumoniae* 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: 9, or an immunogenic fragment thereof; and the CP1 fusion protein 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, or an immunogenic fragment thereof.

**[0037]** In some embodiments, the immunogenic composition comprises at least four different species of immunogenic complexes, wherein the different species comprise: a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein, a third biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi Vi* non-covalently complexed with a third fusion protein; and a

fourth biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a fourth fusion protein, wherein the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein each independently comprise: a biotin-binding moiety; and a polypeptide antigen, wherein each of the first, second, third, and fourth biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

[0038] In some embodiments, the polypeptide antigen of the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*.

[0039] In some embodiments, the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof.

[0040] In some embodiments, a vaccine comprises a plurality of different species of immunogenic complexes, wherein the different species comprise: a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein; wherein each fusion protein comprises (a) a biotin-binding moiety; and (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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, 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.

[0041] In some embodiments, the first polypeptide antigen 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: 4.

[0042] In some embodiments, the first polypeptide antigen 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: 5.

[0043] In some embodiments, the first polypeptide antigen 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9.

[0044] In some embodiments, a vaccine comprises a plurality of different species of immunogenic complexes, wherein the different species comprise: a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein; a biotinylated polysaccharide

antigen of *Salmonella* enterica serotype Paratyphi non-covalently complexed with a fusion protein; wherein each fusion protein comprises (a) a biotin-binding moiety; and (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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, 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.

**[0045]** In some embodiments, the first polypeptide antigen 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: 4.

**[0046]** In some embodiments, the first polypeptide antigen 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: 5.

**[0047]** In some embodiments, the first polypeptide antigen 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9.

**[0048]** In some embodiments, a vaccine comprises a plurality of different species of immunogenic complexes, wherein the different species comprise: a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Typhimurium non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Enteritidis non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Typhi non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Paratyphi non-covalently complexed with a fusion protein; wherein each fusion protein comprises (a) a biotin-binding moiety; and (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% SEQ ID NO: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, 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.

**[0049]** In some embodiments, the first polypeptide antigen 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: 4.

**[0050]** In some embodiments, the first polypeptide antigen 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: 5.

[0051] In some embodiments, the first polypeptide antigen 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9.

[0052] In some embodiments, a vaccine comprises a plurality of different species of immunogenic complexes, wherein the different species comprise: a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Typhimurium non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Enteritidis non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Typhi non-covalently complexed with a fusion protein; a biotinylated polysaccharide antigen of *Salmonella* enterica serotype Paratyphi 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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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.

[0053] In some embodiments, the vaccine comprises a stoichiometrically equal ratio, by weight, of each of the polysaccharide antigens of the different species.

[0054] In some embodiments, the vaccine comprises at least one of the polysaccharide antigens of the different species at a stoichiometrically different ratio, by weight.

[0055] In some embodiments, the vaccine comprises a stoichiometrically different ratio, by weight, of each of the polysaccharide antigens of the different species.

[0056] In some embodiments, the biotin-binding moiety is a polypeptide 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: 3.

[0057] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Salmonella* enterica non-covalently associated with a fusion protein, wherein the fusion protein comprises a biotin-binding moiety and at least one polypeptide antigen.

[0058] In some embodiments, the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella* enterica having a serotype selected from Typhimurium, Enteritidis, Typhi, and Paratyphi.

[0059] In some embodiments, the fusion protein comprises SseB, IpaB, or an SP1500 polypeptide, an SP0785 polypeptide, or both.

[0060] In some embodiments, 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: 4 or an antigenic fragment thereof; (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; or (c) 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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, or an antigenic fragment thereof.

[0061] In some embodiments, the immunogenic complex comprises 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.

[0062] In some embodiments, a vaccine comprises one or more of the above immunogenic complexes.

[0063] In some embodiments, a pharmaceutical composition comprises a vaccine and a pharmaceutically acceptable carrier.

[0064] In some embodiments, a pharmaceutical composition comprises an immunogenic complex, and a pharmaceutically acceptable carrier.

[0065] In some embodiments, the pharmaceutical composition further comprises one or more adjuvants.

[0066] In some embodiments, the one or more adjuvants is or comprises a co-stimulation factor.

[0067] In some embodiments, the one or more adjuvants are selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphated aluminum hydroxide.

[0068] In some embodiments, the one or more adjuvants is or comprises aluminum phosphate.

[0069] In some embodiments, the pharmaceutical composition is formulated for injection.

[0070] In some embodiments, upon administration to a subject, the pharmaceutical composition induces an immune response.

[0071] In some embodiments, 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.

[0072] In some embodiments, a method of making a vaccine, comprises 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 SseB, IpaB, SP0785 or SP1500; wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Salmonella* enterica serotypes selected from Typhimurium, Enteritidis, Typhi, and Paratyphi.

[0073] In some embodiments, a method of immunizing a subject against *Salmonella* enterica infection and/or colonization comprises administering to the subject an immunologically effective amount of the vaccine.

[0074] In some embodiments, a method of immunizing a subject against *Salmonella* enterica infection and/or colonization comprises administering to the subject an immunologically effective amount of the immunogenic complex.

[0075] In some embodiments, a method of immunizing a subject against *Salmonella* enterica infection and/or colonization comprises administering to the subject an immunologically effective amount of the pharmaceutical composition.

[0076] In some embodiments, the vaccine, immunogenic composition, or pharmaceutical composition induces an immune response.

[0077] In some embodiments, the immune response is to at least one polysaccharide antigen or at least one polypeptide of a fusion protein.

[0078] In some embodiments, the subject is immunized against *Salmonella* enterica infection and/or colonization with one dose of a vaccine.

[0079] In some embodiments, the subject is immunized against *Salmonella* enterica infection and/or colonization with two doses of a vaccine.

[0080] In some embodiments, the subject is immunized against *Salmonella* enterica infection and/or colonization with three doses of a vaccine.

[0081] In some embodiments, a fusion protein comprising a rhizavidin protein and at least one peptide or polypeptide antigen, wherein the rhizavidin protein comprises amino acids of SEQ ID NO: 3, or 85% sequence identity to amino acids of SEQ ID NO: 3, and *Salmonella* peptide or polypeptide comprises a fragment of at least 20 amino acids of the SseB protein, or the *Shigella* peptide or polypeptide comprises a fragment of at least 20 amino acids of the IpaB protein.

[0082] In some embodiments, the SseB protein comprises at least SEQ ID NO: 4 or a protein of at least 20 amino acids that has at least 85% sequence identity to SEQ ID NO: 4.

[0083] In some embodiments, the IpaB protein comprises at least SEQ ID NO: 5 or a protein of at least 20 amino acids that has at least 85% sequence identity to SEQ ID NO: 5.

[0084] In some embodiments, the fusion protein comprises at least SEQ ID NO: 1.

[0085] In some embodiments, the fusion protein comprises at least SEQ ID NO: 2.

[0086] In some embodiments, an immunogenic composition is or comprises a vaccine

[0087] The summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

#### BRIEF DESCRIPTION OF DRAWINGS

[0088] FIG. 1 is a schematic illustrating the application of the MAPS platform through combination of a polysaccharide (PS) with pathogen-specific proteins.

[0089] FIGS. 2A-2B shows results of ELISA for carrier proteins selection. FIG. 2A shows the antibody against the *S. Enteritidis* O-specific polysaccharide (OSP) (strain J73) and FIG. 2B show the antibody

titer to the specific carrier protein selected from Rhavi-IpaB, Rhavi-SseB and CP1. Sera was tested prior to first immunization (Pre), three weeks after the first immunization (P1), and three weeks after the second immunization (P2). Rhavi-SseB and Rhavi-IpaB carrier proteins were purified without their chaperone proteins.

[0090] FIG. 3 shows the bactericidal activity for MAPS-Salmonella made with one of the three carriers, Rhavi-IpaB, Rhavi-SseB or CP1. Sera were tested three weeks after the first immunization (P1), and three weeks after the second immunization (P2).

[0091] FIG. 4 shows that ELISA (shown in FIG. 2A-2B) and bactericidal titers (shown in FIG. 3) correlated, regardless of carrier protein.

[0092] FIG. 5 shows OSP antibody, SseB antibody and bactericidal titers of *S. Typhimurium* OSP-SseB MAPS. Sera was tested prior to first immunization (Pre), three weeks after the first immunization (P1), and three weeks after the second immunization (P2).

[0093] FIG. 6. shows screening of OSP purified from different *S. Typhimurium* and *S. Enteritidis* strains to identify the strain for purification of the OSP for use in a *Salmonella*-MAPS Complex. Small scale purifications of OSPs from *S. Typhimurium* strains Q55, Q65, LT2, P104, S11 and S12 and from *S. Enteritidis* strains I15, S-1, D82, J73 and R27 were performed, and analyzed by size exclusion column. S12 strain of *S. Typhimurium* and J73 strain of *S. Enteritidis* were selected for large scale purification.

[0094] FIG. 7A and 7B show results of *S. Typhimurium* S12 immunogenicity. FIG. 7A shows antibody titer against *S. Typhimurium* O-specific polysaccharide (OSP) (strain S12) as detected by ELISA with the different carrier proteins Rhavi-IpaB, Rhavi-SseB or CP1. FIG. 7B shows the bactericidal assay with different carrier proteins Rhavi-IpaB, Rhavi-SseB or CP1. Rhavi-SseB and Rhavi-IpaB were purified with their respective chaperone proteins, SseA and IpgC. Chaperone proteins removed by washing with (LDAO) or sodium Deoxycholate (SDOC)). Sera was tested prior to first immunization (Pre), three weeks after the first immunization (P1), and three weeks after the second immunization (P2). Rhavi-SseB and Rhavi-IpaB carrier proteins were purified without their chaperone proteins.

[0095] FIG. 8A and 8B shows the bactericidal activity for *S. Typhimurium* MAPS or *S. Enteritidis*. FIG. 8A shows the antibody titer to OSP of monovalent *S. Typhimurium* MAPS or *S. Enteritidis*-MAPS, each comprising the Rhavi-SseB antigen. FIG. 8B shows the bactericidal killing by *S. Typhimurium* MAPS or *S. Enteritidis*-MAPS, each comprising the Rhavi-SseB antigen.

[0096] FIGS. 9A and 9B shows results of two bivalent *Salmonella*-MAPS compositions that generate robust polysaccharide antibody titers as detected by ELISA. FIG. 9A shows ELISA results for a bivalent *Salmonella*-MAPS complex comprising *S. Typhimurium* (S12) and *S. Enteritidis* OSPs (J73) and the Rhavi-SseB antigen. FIG. 9B shows ELISA for a bivalent *Salmonella*-MAPS complex comprising *S. Typhi* Vi and Paratyphi OSP, and the CP1 antigen. Sera was tested prior to first immunization (Pre), two weeks after the first immunization (P1), and two weeks after the second immunization (P2).

[0097] FIG. 10 shows titers to *S. Typhi* Vi polysaccharide in guinea pigs after immunization with conjugate of DT (Diphtheria toxin) and Vi or Vi-MAPS comprising the *S. Typhi* Vi polysaccharide and an

antigen selected from CP1, Rhavi-rEPA or Rhavi-CRM197. Sera was tested prior to first immunization (Pre), three weeks after the first immunization (Post1), and three weeks after the second immunization (Post2), and three weeks after the third immunization (Post3).

**[0098]** FIG. 11 shows duration of antibody titers to *S. Typhi* Vi polysaccharide. Guinea pigs were immunized with conjugate of DT (Diphtheria toxin) and Vi or Vi-MAPS comprising the *S. Typhi* Vi polysaccharide and an antigen selected from CP1, Rhavi-rEPA or Rhavi-CRM197. Sera was tested three weeks after the first immunization (Post1), and three weeks after the third immunization (Post3), and 6, 10, 14 and 18 weeks after the third immunization (*n* wks P3).

**[0099]** FIG. 12A and FIG. 12B show *S. Typhi*-MAPS comprising the Vi polysaccharide induce Vi-Memory B-cells. FIG. 12A is a schematic of the methodology to assess immunological memory using adoptive transfer. Whole splenocytes from wild type mice immunized with Vi-DT conjugate or Rhavi-rEPA, or Vi MAPS are transferred into RAG-/- immunodeficient mice and evaluated for response to immunization with the antigens DT, Vi or rEPA. FIG 12B shows that both conventional conjugate Vi-DT and *S. Typhi*-MAPS comprising the Vi polysaccharide and Rhavi-rEPA antigen generate Vi-memory B cells. Sera from the recipient RAG-/- immunodeficient were tested prior to first immunization (Pre), and two weeks after the first immunization (Post1).

**[00100]** FIG. 13 shows that *S. Paratyphi*-MAPS comprising the OSP from *S. Paratyphi* requires alum phosphate (aluminum phosphate or "AP"). Antibody titers to *S. Paratyphi* OSP were assessed in the presence and absence of AP, and tested prior to first immunization (Pre), and two weeks after the first immunization (P1) and two weeks after the second immunization (P2).

**[00101]** FIGS. 14A-14B shows the immunogenicity of monovalent and bivalent Salmonella-MAPS, comprising either monovalent *S. Typhi*-MAPS comprising the Vi polysaccharide or monovalent *S. Paratyphi*-MAPS comprising the OSP polysaccharide, or a bivalent Salmonella-MAPS vaccine comprising both. FIG. 14A shows IgG antibodies to Vi after immunization with *S. Typhi*-MAPS comprising CP1 and the Vi polysaccharide or monovalent *S. Paratyphi*-MAPS comprising the OSP polysaccharide, or a bivalent Salmonella-MAPS vaccine comprising both. FIG. 14B shows IgG antibodies to *S. Paratyphi* OSP after immunization with *S. Typhi*-MAPS comprising the Vi polysaccharide or monovalent *S. Paratyphi*-MAPS comprising the OSP polysaccharide, or a bivalent Salmonella-MAPS vaccine comprising both. Sera were tested prior to first immunization (Pre), and two weeks after the first immunization (P1) and two weeks after the second immunization (P2).

**[00102]** FIG. 15A-15D shows the dose response of bivalent Salmonella-MAPS, comprising the *S. Typhi*-MAPS comprising the Vi polysaccharide and the CP1 polypeptide (CP1-Vi) and *S. Paratyphi*-MAPS comprising the OSP polysaccharide and the CP1 polypeptide (CP1-ParaOSP MAPS). FIG. 15A shows the IgG antibodies to Vi, and FIG. 15B shows the IgG antibodies to OSP after administration of the bivalent vaccine at doses of 1µg, 5µg or 25µg. FIG. 15C shows the IgG antibodies to Vi, and FIG. 15D shows the IgG antibodies to OSP after administration of the bivalent vaccine at doses of 0.02µg, 0.1µg or

0.4µg. Sera were tested prior to first immunization (Pre), and two weeks after the first immunization (P1) and two weeks after the second immunization (P2).

**[00103] FIGS. 16A-16B** shows the duration of antibodies in rabbits. Rabbits were immunized with bivalent Salmonella-MAPS, comprising CP1-Vi and CP1-ParaOSP MAPS. **FIG. 16A** shows the IgG antibodies to Vi at varying time period after immunization with bivalent vaccine. **FIG. 16B** shows IgG antibodies to OSP after administration of the bivalent vaccine. Sera were tested prior to first immunization (Pre), and 2, 4, 8, 12, 16, 20 and 24 weeks after the first immunization (P1), and 2, 6 and 10 weeks after the second immunization (P2).

**[00104] FIG. 17A-17B** shows the affinity maturation after boosting with the second immunization (P2) with the bivalent Salmonella-MAPS, comprising CP1-Vi and CP1-ParaOSP MAPS. **FIG. 17A** shows the affinity index (AI) to Vi after the first immunization (P1) and the second (or boost) immunization (P2) with bivalent vaccine. **FIG. 17B** shows the affinity index (AI) to *S. Paratyphi* OSP after the first immunization (P1) and the second (or boost) immunization (P2) with bivalent vaccine. AI is determined by the concentration of sodium thiocyanate to elute 50% of IgG bound to the polysaccharide by ELISA.

**[00105] FIG. 18A-18B** shows the functional assays for *S. Typhi* and *S. Paratyphi*. **FIG. 18A** shows Vi OPA titer, and **FIG. 18B** shows *S. Paratyphi* OSP serum bactericidal killing (SBA) titer. Typhi killing was done with HL-60 cells using a *S. Typhimurium* strain expressing Vi. *S. Paratyphi* killing was done with bactericidal assays (complement only). Sera were tested prior to first immunization (Pre), and after two immunizations with the bivalent Salmonella-MAPS, comprising CP1-Vi and CP1-ParaOSP MAPS.

**[00106] FIGS. 19A and 19B** show that quadrivalent (4V) Salmonella-MAPS generates robust polysaccharide antibody titers. **FIG. 19A** shows results of ELISA for a quadrivalent MAPS comprising: Rhavi-SseB MAPS made with Typhimurium and Enteritidis OSP, CP1 MAPS with Vi and Paratyphi OSP MAPS. **FIG. 19B** shows results of ELISA of a quadrivalent MAPS containing Rhavi-SseB MAPS made with Typhimurium and Enteritidis OSP, CP1 MAPS made with Vi and Paratyphi OSP MAPS. The carrier protein is shown on top for each MAPS. Sera were tested prior to first immunization (Pre), and two weeks after the first immunization (P1).

**[00107] FIG. 20A and 20B** shows immunogenicity of multivalent (2V), and Quadrivalent (4V) Salmonella-MAPS in rabbits. **FIG. 20A** shows ELISA results of protein antibodies multivalent (2V) Salmonella-MAPS comprising polysaccharides from both *S. Typhi* (comprising the Vi polysaccharide) and *S. Paratyphi* (comprising the OSP polysaccharide) and comprising the carrier protein Rhavi-SseB or CP1, as compared to the quadrivalent MAPS containing Rhavi-SseB and polysaccharides from: *S. Typhimurium* (S12), *S. Enteritidis* (J73), *S. Typhi* and *S. Paratyphi*. **FIG. 20B** shows ELISA results of OSP and Vi antibodies from the quadrivalent MAPS containing Rhavi-SseB and polysaccharides from: *S. Typhimurium* (S12), *S. Enteritidis* (J73), *S. Typhi* and *S. Paratyphi*. Sera were tested prior to first immunization (Pre), and two weeks after the first immunization (P1), and two weeks after the second immunization (P2).

[00108] FIG. 21A-21C shows BCA and OPA titers for bivalent (2V) or quadrivalent (4V) Salmonella-MAPS. BCA was done by the incubation of sera with bacteria and complement while OPA was done by the incubation of sera with bacteria, complement and differentiated HL-60 cells. FIG. 21A shows the killing titer of sera from rabbits received Salmonella-MAPS comprising the Rhavi-SseB carrier protein and polysaccharides from both *S. Typhimurium* (S12), *S. Enteritidis* (J73). FIG. 21B shows the results from the killing assay of *S. Typhi* and *S. Paratyphi* by Salmonella-MAPS comprising the CP1 carrier protein and polysaccharides from Vi and ParaOSP polysaccharides. FIG. 21C shows the results from the killing assay by the quadrivalent Salmonella-MAPS comprising the Rhavi-SseB carrier protein and polysaccharides from *Typhimurium* (S12), *S. Enteritidis* (J73), *S. Typhi* and *S. Paratyphi*.

[00109] FIG. 22A-22B shows the OPA titer by SseB antisera. FIG. 22A shows killing of *Typhimurium* (S12) by SseB antisera. FIG. 22B shows killing of *S. Enteritidis* by SseB antisera. Bacteria (either *S. Typhimurium* or *S. Enteritidis*) were incubated with heat-inactivated antisera for 20 minutes at room temperature. Differentiated HL-60 and baby rabbit complement were added, and the killing was carried out at 37°C for 1 hour. Cells were lysed with Saponin and plated for CFU count. Opsonophagocytic killing (OPK) titer was defined as the reciprocal serum dilution required to mediate 50% bacterial killing.

[00110] FIG. 23A-23B shows the addition of SseB antisera enhanced killing by the OSP antisera. FIG. 23A shows OPK assay results of *Typhimurium* by OSP sera, or OSP sera plus SseB sera. FIG. 23B shows OPK assay results of *S. Enteritidis* by OSP sera, or OSP sera plus SseB sera. OSP antisera were either mixed with pre-sera or SseB antisera and then used in the OPK assay. OPK titer was defined as the reciprocal serum dilution required to mediate 50% bacterial killing.

[00111] FIG. 24A-24B shows the bactericidal activity for Monovalent (1V), Bivalent (2V) and quadrivalent (4V) MAPS comprising Rhavi-SseB and OSPs against *S. Typhimurium* and *S. Paratyphi*. FIG. 24A shows bactericidal titers of P2 sera against *S. Typhimurium*. FIG. 24B shows bactericidal titers of P2 sera against *S. Paratyphi*.

[00112] FIG. 25A-25B shows OPA titers of sera against *S. Enteritidis* (J73) or *S. Typhi* with quadrivalent (4V) and bivalent (2V) Salmonella MAPS. FIG 25A shows OPA titer of sera from the immunization of bivalent (2V) or quadrivalent (4V) Salmonella-MAPS against Vi containing bacteria. FIG 25B shows OPA titers of sera from the immunization of the bivalent (2V) or quadrivalent (4V) Salmonella-MAPS against *S. Enteritidis* (J73). No killing was observed for pre-sera.

## DETAILED DESCRIPTION OF INVENTION

[00113] The present disclosure relates, generally, to compositions, systems, and methods that include novel complexed proteins and polysaccharides, e.g., vaccines of complexed proteins and polysaccharides. Such complexes can be used, e.g., to induce and/or increase an immunoprotective response in subjects at risk of or suffering from *S. enterica* infection.

### *Immunogenic Complexes*

[00114] The present disclosure encompasses immunogenic complexes that include one or more polysaccharides and/or polypeptides of *S. enterica*.

[00115] In some embodiments, immunogenic complexes are, or are based on, Multiple Antigen Presenting System (MAPS) complexes. Aspects of the MAPS platform have been previously described in WO2012/155007, the contents of which are herein incorporated by reference in their entirety, and are shown schematically in Figure 1. See also Zhang et al, 2013.

[00116] As described herein, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one or more antigenic polysaccharides. In some embodiments, one or more antigenic polypeptides are complexed via affinity interaction with one or more antigenic polysaccharides. In some embodiments, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one or more antigenic polysaccharides using one or more affinity molecule/complementary affinity molecule pairs. In some embodiments, an immunogenic complex includes (i) a first affinity molecule described herein conjugated to one or more antigenic polysaccharides, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. In some embodiments, an immunogenic complex includes (i) a plurality of a first affinity molecule described herein conjugated to one or more antigenic polysaccharides, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. Upon association of the first affinity molecule and the complementary affinity molecule, the one or more antigenic polypeptides are non-covalently complexed to the one or more antigenic polysaccharides.

[00117] In some embodiments, one or more antigenic polypeptides are complexed via affinity interaction with one antigenic polysaccharide. In some embodiments, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one antigenic polysaccharide using one affinity molecule/complementary affinity molecule pair. In some embodiments, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one antigenic polysaccharide using one or more affinity molecule/complementary affinity molecule pairs. In some embodiments, each of the affinity molecule/complementary affinity molecule pairs is the same, e.g., biotin/biotin-binding moiety pairs. In some embodiments, an immunogenic complex includes (i) a first affinity molecule described herein conjugated to one antigenic polysaccharide, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and at least one immunogenic polypeptide. In some embodiments, an immunogenic complex includes (i) a plurality of a first affinity molecule described herein conjugated to one antigenic polysaccharide, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. Upon association of the first affinity molecule and the complementary affinity molecule, the one or more antigenic polypeptides are non-covalently complexed to the one antigenic polysaccharide.

[00118] In some embodiments, the affinity molecule/complementary affinity molecule pair is selected from one or more of biotin/biotin-binding moiety, antibody/antigen, enzyme/substrate, receptor/ligand,

metal/metal-binding protein, carbohydrate/carbohydrate binding protein, lipid/lipid-binding protein, and His tag/His tag-binding molecule.

[00119] In some embodiments, the first affinity molecule is biotin (or a derivative or fragment thereof), and the complementary affinity molecule is a moiety, e.g., a biotin-binding protein, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is rhizavidin, avidin, streptavidin, bradavidin, tamavidin, lentiavidin, zebavidin, NeutrAvidin, CaptAvidin™, or a biotin-binding domain or biotin-binding fragment thereof, or a combination thereof. In some embodiments, the biotin-binding moiety is rhizavidin, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin binding moiety is or comprises a polypeptide of SEQ ID NO: 3, or is or comprises a polypeptide that has at least 80%, or at least 85% or at least 90% or more than 90% sequence identity to SEQ ID NO: 3, or a biotin-binding domain or biotin-binding fragment thereof.

[00120] In some embodiments, the one or more antigenic polysaccharides are, or are derived from Gram-negative bacteria and/or Gram-positive bacteria. In some embodiments, one or more bacterial antigenic polysaccharides are, or are derived from *S. enterica*. In some embodiments, one or more antigenic polysaccharides are, or are derived from one or more pathogens. In some embodiments, one or more antigenic polysaccharides are, or are derived from 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 or more than 30 serotypes or strains of a pathogen. In some embodiments, one or more antigenic polysaccharides are, or are derived from more than 25 serotypes or strains of a pathogen, e.g., 26, 27, 28, 29, 30, 35, 40, 45, or 50 serotypes or strains. In some embodiments, one or more antigenic polysaccharides are, or are derived from more than 60, 70, 80, 90, or 100 serotypes or strains of a pathogen.

[00121] In some embodiments, the one or more antigenic polysaccharides comprise one or more affinity molecules conjugated to the antigenic polysaccharides. In some embodiments, the one or more affinity molecules comprise biotin or biotin derivatives.

[00122] In some embodiments, the antigenic polysaccharides comprise a plurality of affinity molecules conjugated to the antigenic polysaccharides. In some embodiments, the affinity molecules comprise biotin or biotin derivatives.

[00123] In some embodiments, one or more antigenic polypeptides are covalently linked (e.g., fused) to a complementary affinity molecule described herein. In some embodiments a fusion protein comprises one or more antigenic polypeptides and a complementary affinity molecule disclosed herein. In some embodiments, the complementary affinity molecule is or comprises a biotin-binding moiety. In some embodiments, the biotin-binding moiety comprises rhizavidin or a biotin-binding portion thereof. In some embodiments, antigenic polysaccharides and/or antigenic polypeptides that may be included in immunogenic complexes are recombinantly or synthetically produced.

[00124] In some embodiments, antigenic polysaccharides and/or antigenic polypeptides that may be included in immunogenic complexes are isolated and/or derived from natural sources. In some

embodiments antigenic polysaccharides and/or antigenic polypeptides that may be included in immunogenic complexes are isolated from bacterial cells. Exemplary polysaccharides and/or polypeptides are described below.

### Antigenic Polypeptides

**[00125]** In some embodiments, an immunogenic complex described herein comprises one or more polypeptide antigens. In some embodiments, a polypeptide antigen is a bacterial polypeptide, a fungal polypeptide, and/or a viral polypeptide. In some embodiments, a polypeptide antigen is a polypeptide of, or derived from *S. enterica*, *S. pneumoniae*, or *Shigella flexneri*. In some embodiments, the one or more polypeptide antigen is a polypeptide of, or derived from, a pathogen other than *S. enterica*, *S. pneumoniae*, or *S. flexneri*. In some embodiments, an immunogenic complex includes one or more of the following *S. enterica*, *S. pneumoniae*, or *S. flexneri* antigenic polypeptides, or portions thereof.

#### **[00126]** *SseB Polypeptides*

**[00127]** The SseB polypeptide is a *S. enterica* type 3 secretion system protein conserved across *Salmonella* strains. In some embodiments, an SseB polypeptide is or comprises a full-length SseB polypeptide. For example, in some embodiments, a full-length SseB polypeptide is represented by the amino acid sequence as set forth in SEQ ID NO: 4. In some embodiments, an SseB polypeptide includes a portion of an SseB polypeptide (e.g., a portion of the SseB polypeptide of SEQ ID NO: 4, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150 or more contiguous amino acids of SEQ ID NO: 4). In some embodiments, an SseB polypeptide contains one or more amino acid alterations (e.g., deletion, substitution, and/or insertion) from a naturally-occurring wild-type SseB polypeptide sequence. For example, an SseB polypeptide may contain an amino acid sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 4 or a portion thereof (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or more consecutive amino acids of the sequence shown in SEQ ID NO: 4). Alternatively, an SseB polypeptide may contain a portion (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or more consecutive amino acids) of a sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 4.

**[00128]** Purification of SseB from *E. Coli* (either on its own or as a fusion protein as disclosed herein) needs co-expression of chaperone SseA. Accordingly, SseB can be co-expressed from *E. Coli* with SseA. The SseB polypeptide can be separated from its SseA chaperone using detergent wash (Dodecyltrimethylammonium (LDAO) or sodium Deoxycholate (SDOC)). In some embodiments, SseB is co-expressed with His-tagged SseA in *E. Coli*, the SseB and SseA complex is isolated by using the His-tagged SseA protein, the complex is then washed with LDAO or SDOC to elute the SseB polypeptide.

#### **[00129]** *IpaB Polypeptides*

[00130] The IpaB polypeptide is a *S. flexneri* type 3 secretion system protein conserved across *Shigella* strains. In some embodiments, an IpaB polypeptide is or comprises a full-length IpaB polypeptide. For example, in some embodiments, a full-length IpaB polypeptide is represented by the amino acid sequence as set forth in SEQ ID NO: 5. In some embodiments, an IpaB polypeptide includes a portion of an IpaB polypeptide (e.g., a portion of the IpaB polypeptide of SEQ ID NO: 5, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more contiguous amino acids of SEQ ID NO: 5). In some embodiments, an IpaB polypeptide contains one or more amino acid alterations (e.g., deletion, substitution, and/or insertion) from a naturally-occurring wild-type IpaB polypeptide sequence. For example, an IpaB polypeptide may contain an amino acid sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 5 or a portion thereof (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more consecutive amino acids of the sequence shown in SEQ ID NO: 5). Alternatively, an IpaB polypeptide may contain a portion (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more consecutive amino acids) of a sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 5. An exemplary IpaB polypeptide is provided herein as the amino acid of SEQ ID NO: 5.

[00131] SseB and IpaB are components of the type III secretion system and their purification from *E. Coli* needs co-expression of chaperones SseA and IpgC, respectively.

[00132] Purification of IpaB from *E. Coli* (either on its own or as a fusion protein as disclosed herein) needs co-expression of chaperone IpgC. Accordingly, IpaB can be co-expressed from *E. Coli* with IpgC. The IpaB polypeptide can be separated from its IpgC chaperone using detergent wash (Dodecyltrimethylammonium bromide (LDAO) or sodium Deoxycholate (SDOC)). In some embodiments, IpaB is co-expressed with His-tagged IpgC in *E. Coli*, the IpaB and IpgC complex is isolated by using the His-tagged IpgC protein, the complex is then washed with LDAO or SDOC to elute the IpaB polypeptide.

#### [00133] SP0785 Polypeptides

[00134] SP0785 is a conserved hypothetical *S. pneumoniae* protein described in WO2014/124228, which is incorporated herein in its entirety by reference. In some embodiments, an SP0785 polypeptide is an efflux transporter protein conserved across *S. pneumoniae* strains. In some embodiments, an SP0785 polypeptide is or comprises a full-length SP0785 polypeptide. For example, in some embodiments, a full-length SP0785 polypeptide has 399 amino acids (38 kDa). In some embodiments, an SP0785 polypeptide includes a portion of an SP0785 polypeptide (e.g., a portion which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more contiguous amino acids of SEQ ID NO: 8). In some embodiments, an SP0785 polypeptide contains one or more amino acid alterations (e.g., deletion, substitution, and/or

insertion) from a naturally-occurring wild-type SP0785 polypeptide sequence. For example, an SP0785 polypeptide may contain an amino acid sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 8 or a portion thereof (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more consecutive amino acids of the sequence shown in SEQ ID NO: 8). Alternatively, an SP0785 polypeptide may contain a portion (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or 400 consecutive amino acids) of a sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 8.

#### [00135] *SP1500 Polypeptides*

[00136] SP1500 is a *S. pneumoniae* protein described in WO 2014/124228, which is incorporated herein in its entirety by reference. In some embodiments, an SP1500 polypeptide is an Amino Acid ABC Transporter, amino acid-binding polypeptide conserved across *S. pneumoniae* strains. In some embodiments, an SP1500 polypeptide is or comprises a full-length SP1500 polypeptide. For example, in some embodiments, a full-length SP1500 polypeptide has 278 amino acids (28 kDa). In some embodiments, an SP1500 polypeptide includes a portion of an SP1500 polypeptide (e.g., a portion of the SP1500 polypeptide of SEQ ID NO: 9, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, or more contiguous amino acids of SEQ ID NO: 9). In some embodiments, an SP1500 polypeptide contains one or more amino acid alterations (e.g., deletion, substitution, and/or insertion) from a naturally-occurring wild-type SP1500 polypeptide sequence. For example, an SP1500 polypeptide may contain an amino acid sequence that is at least 60% or more (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 9

#### *Complementary Affinity Molecules*

[00137] In some embodiments, a complementary affinity molecule comprises a biotin-binding moiety. In some embodiments, a fusion protein of the immunogenic complex comprises a biotin-binding moiety, and one or more polypeptide antigens. In some embodiments, a fusion protein comprises a biotin-binding moiety and two or more polypeptide antigens. As used herein, a “biotin-binding moiety” refers to a biotin-binding protein, a biotin-binding fragment thereof, or a biotin-binding domain thereof.

[00138] In some embodiments, MAPS complexes disclosed herein utilize the high affinity (dissociation constant  $[K_D] \approx 10^{-15}M$ ) 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 is 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],

[00139] In some embodiments, the biotin-binding moiety of the fusion protein comprises rhizavidin or a biotin-binding domain or biotin-binding fragment thereof, as further described in WO 2012/155053, the contents of which are herein incorporated by reference in their entirety. In some embodiments, a biotin-binding moiety is or comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to rhizavidin, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety comprises a polypeptide of SEQ ID NO: 3 or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is or comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 3, or biotin-binding domain or biotin-binding fragment thereof.

#### *Fusion Proteins that Include Antigenic Polypeptides*

[00140] Antigenic polypeptides described herein can be part of a fusion protein. For example, in some embodiments, an immunogenic complex described herein comprises a fusion protein that is or comprises a complementary affinity molecule and one or more antigenic polypeptides described herein. In some embodiments, a fusion protein of the immunogenic complex has carrier properties. In some embodiments, a fusion protein of the immunogenic complex has antigenic properties. In some embodiments, a fusion protein of the immunogenic complex has carrier properties and antigenic properties.

[00141] In some embodiments, the fusion protein is or comprises a complementary affinity molecule described herein (e.g., a biotin-binding moiety described herein), and one or more polypeptides of or derived from *S. enterica*, *S. pneumoniae*, and/or *S. flexneri*.

[00142] In some embodiments, the fusion protein of the immunogenic complex comprises a biotin-binding moiety that is or comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 3 (rhizavidin), or biotin-binding fragment thereof.

[00143] In some embodiments, the fusion protein of the immunogenic complex comprises SseB. In some embodiments, the fusion protein comprises a complementary affinity molecule described herein (e.g., a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO: 4 or an antigenic fragment thereof.

[00144] In some embodiments, the fusion protein of the immunogenic complex comprises IpaB. In some embodiments, the fusion protein comprises a complementary affinity molecule described herein (e.g., a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO: 5 or an antigenic fragment thereof.

[00145] In some embodiments, the fusion protein of the immunogenic complex is CP1, further described in PCT Application WO2021/17016516 entitled “Pneumococcal Fusion Protein Vaccines” and filed September 12, 2019, the contents of each of which are incorporated herein by reference in their entirety. Aspects of the CP1 have also been previously described in WO2020/056127, the contents of which are herein incorporated by reference in their entirety. In some embodiments, the fusion protein comprises a complementary affinity molecule described herein (e.g., a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO: 6 or an antigenic fragment thereof.

**[00146] *SseB-Fusion proteins***

[00147] Another aspect of the present invention relates to a fusion protein comprising, in any order: (a) a biotin-binding moiety and (b) a SseB polypeptide, wherein the fusion protein comprises (i) an amino acid sequence that is at least 80% identical to SEQ ID NO: 3 (Rhavi) and (ii) an amino acid sequence that is at least 80% identical to SEQ ID NO: 4 (SseB). An exemplary SseB polypeptide is provided herein as the amino acid of SEQ ID NO: 4.

[00148] One aspect of the present invention relates to a fusion protein comprising, in any order: (a) a biotin-binding moiety and (b) a SseB polypeptide, wherein the fusion protein comprises (i) an amino acid sequence that is at least 80% identical to SEQ ID NO: 3 (Rhavi) and (ii) an amino acid sequence that is at least 80% identical to SEQ ID NO: 4 (SseB).

[00149] In all aspects of the fusion protein as disclosed herein, the biotin-binding moiety comprises an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:3 or a biotin binding portion thereof. In all aspects of the fusion protein as disclosed herein, SseB polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:4.

[00150] In some embodiments, the fusion protein comprises, in order of N- to C-terminal: a biotin-binding moiety comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:3 or a biotin binding portion thereof, and a SseB polypeptide comprising an amino acid sequence of SEQ ID NO: 4, or an amino acid sequence an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:4.

[00151] In some embodiments, the fusion protein comprises, in order of N- to C-terminal: a SseB polypeptide comprising an amino acid sequence of SEQ ID NO: 4, or an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO: 4, and a biotin-binding moiety comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:3 or a biotin binding portion thereof.

[00152] In some embodiments, fusion protein comprises an amino acid sequence that is at least 80%, 85%, or 90% identical to SEQ ID NO: 1. In some embodiments, fusion protein comprises an amino acid sequence that consists of SEQ ID NO: 1 (Rhavi-SseB). In some embodiments, fusion protein comprises an amino acid sequence that is at least 80%, 85%, or 90% identical to SEQ ID NO: 11. In some

embodiments, fusion protein comprises an amino acid sequence that consists of SEQ ID NO: 11 (SseB-Rhavi).

**[00153]** In some embodiments, one aspect of the present invention relates to a fusion protein comprising (i) a SseB polypeptide having an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO: 4 or an antigenic fragment thereof, and (ii) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 3 (rhizavidin), or biotin-binding fragment thereof.

**[00154] *IpaB Fusion proteins***

**[00155]** One aspect of the present invention relates to a fusion protein comprising, in any order: (a) a biotin-binding moiety and (b) a IpaB polypeptide, wherein the fusion protein comprises (i) an amino acid sequence that is at least 80% identical to SEQ ID NO: 3 (Rhavi) and (ii) an amino acid sequence that is at least 80% identical to SEQ ID NO: 5 (IpaB).

**[00156]** In all aspects of the fusion protein as disclosed herein, the biotin-binding moiety comprises an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:3 or a biotin binding portion thereof. In all aspects of the fusion protein as disclosed herein, IpaB polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:5.

**[00157]** In some embodiments, the fusion protein comprises, in order of N- to C-terminal: a biotin-binding moiety comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:3 or a biotin binding portion thereof, and a IpaB polypeptide comprising an amino acid sequence of SEQ ID NO: 5, or an amino acid sequence an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:5.

**[00158]** In some embodiments, the fusion protein comprises, in order of N- to C-terminal: a IpaB polypeptide comprising an amino acid sequence of SEQ ID NO: 5, or an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:5, and a biotin-binding moiety comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence that is at least 80%, 85%, 90% identical to SEQ ID NO:3 or a biotin binding portion thereof.

**[00159]** In some embodiments, fusion protein comprises an amino acid sequence that is at least 80%, 85%, or 90% identical to SEQ ID NO: 2. In some embodiments, fusion protein comprises an amino acid sequence that consists of SEQ ID NO: 2 (Rhavi-IpaB). In some embodiments, fusion protein comprises an amino acid sequence that is at least 80%, 85%, or 90% identical to SEQ ID NO: 10. In some embodiments, fusion protein comprises an amino acid sequence that consists of SEQ ID NO: 10 (IpaB-Rhavi).

**[00160]** In some embodiments, one aspect of the present invention relates to a fusion protein comprising (i) a IpaB polypeptide having an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%,

98%, 99%, or 100% identity to the sequence of SEQ ID NO: 5 or an antigenic fragment thereof, and a biotin-binding moiety that is or comprises a polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 3 (rhizavidin), or biotin-binding fragment thereof.

**[00161]** *CPI*

**[00162]** In some embodiments, the complex comprises a fusion protein comprising Rhazavidin (Rhavi), SP1500 and SP785 as disclosed herein, which has been previously described in WO2020056127, which is incorporated herein in its entirety by reference. In some embodiments, the fusion protein useful in the MAPS complex comprises a CPI fusion protein having an amino acid sequence of SEQ ID NO: 6, which is Rhavi-SP1500-SP785 fusion protein and corresponds to acids of SEQ ID NO: 23 as disclosed in WO2020/056127. In some embodiments, the fusion protein useful in the MAPS complex comprises a CPI fusion protein having an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 6. In some embodiments, the fusion protein useful in the MAPS complex comprises a CPI fusion protein having an amino acid sequence selected from any of SEQ ID NO: 19, 20, 21, 22, 23, 24, 25 or 26 as disclosed in WO2020/056127, which is incorporated herein in its entirety. In some embodiments, the fusion protein useful in the MAPS complex comprises a CPI fusion protein having an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to a sequence selected from any of SEQ ID NO: 19, 20, 21, 22, 23, 24, 25 or 26 as disclosed in WO2020/056127, which is incorporated herein in its entirety.

**[00163]** In some embodiments, the fusion protein useful in the MAPS complex comprises a CPI fusion protein having an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to any of the sequences selected from: SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 19 or 20 as disclosed herein.

***Linkers or Spacers***

**[00164]** In some embodiments, the fusion protein of the immunogenic complex comprises one or more linkers and/or tags, e.g., a histidine tag. In some embodiments, the linker comprises a polypeptide comprising an amino acid sequence of SEQ ID NO: 7 (GGGSS). In some embodiments, the linker comprises a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of SEQ ID NO: 7. In some embodiments, the linker comprises the amino acid sequence AAA. In some embodiments, the fusion protein of the immunogenic complex comprises a first linker comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 7 (GGGSS), and a second linker comprising the amino acid sequence AAA (SEQ ID NO. 51). In some embodiments, such a linker may be synthesized, or derived from amino acid residues from a restriction site (e.g., a Not I restriction site).

**[00165]** In some embodiments, a fusion protein comprises one or more linkers. In some embodiments, a linker is or comprises one or more amino acids. In some embodiments, a fusion protein comprises an

antigenic polypeptide joined to a biotin-binding moiety by a linker. In some embodiments, a fusion protein comprises at least a first antigenic polypeptide and a biotin-binding moiety, and at least one linker.

**[00166]** In some embodiments, a linker interposes a structure between two protein moieties. In some embodiments, the structure is or comprises an  $\alpha$ -helix. In some embodiments the structure is or comprises a  $\beta$ -strand. In some embodiments, the structure is or comprises a coil/bend. In some embodiments, the structure is or comprises a turn. In some embodiments, a linker decreases steric hindrance between two protein moieties joined by the linker. In some embodiments, a linker decreases unfavorable interactions between two protein moieties joined by the linker. In some embodiments, a linker comprises a mixture of glycine and serine residues. In some embodiments, the linker may additionally comprise threonine, proline, and/or alanine residues. In some embodiment a linker is hydrophilic. In some embodiments a linker is hydrophobic. In some embodiments a linker increases the stability of the fusion protein containing the linker.

**[00167]** In some embodiments, a linker does not interfere with the folding of an antigenic polypeptide to which it is joined. In some embodiments, a linker does not interfere with the antigenicity of an antigenic polypeptide to which it is joined. In some embodiments, a linker does not reduce the antigenicity of an antigenic polypeptide to which it is joined. In some embodiments, a linker does not eliminate the antigenicity of an antigenic polypeptide to which it is joined. In some embodiments the effect of the linker is determined by comparing the polypeptide with the polypeptide joined to the linker.

**[00168]** In some embodiments, a linker does not interfere with the folding of a biotin-binding moiety to which it is joined. In some embodiments, a linker does not interfere with the biotin-binding ability of a biotin-binding moiety to which it is joined. In some embodiments, a linker does not reduce the biotin-binding ability of a biotin-binding moiety to which it is joined. In some embodiments, a linker does not eliminate the biotin-binding ability of a biotin-binding moiety to which it is joined. In some embodiments the effect of the linker is determined by comparing the biotin-binding moiety with the biotin-binding moiety joined to the linker.

**[00169]** In some embodiments, a linker is not antigenic. In some embodiments, a linker does not elicit a T cell response. In some embodiments, a linker does not elicit a B cell response. In some embodiments, a linker does not induce a T cell or a B cell response.

**[00170]** In some embodiments, a linker comprises two or more amino acids. In some embodiments, a linker may be 3-100, 5-100, 10-100, 20-100 30-100, 40-100, 50-100, 60-100, 70-100, 80-100, 90-100, 5-55, 10-50, 10-45, 10-40, 10-35, 10-30, 10-25, 10-20, 10-15, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, or 2-3 amino acids in length. In some embodiments, a linker comprises between 10-100, 10-90, 10-80, 10-70, 10-60, 10-50, 10-40, 10-30, 10-20, 10-15 amino acids. In some embodiments, the linker comprises at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 amino acids. In some embodiments, a linker is or comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids, or more than 100 amino acids in length.

[00171] In some embodiments, a linker is a flexible linker. Flexible linkers may be useful for joining domains that require a certain degree of movement or interaction and may include small, non-polar (e.g. Gly) or polar (e.g. Ser or Thr) amino acids. Incorporation of Ser or Thr can also maintain the stability of the linker in aqueous solutions by forming hydrogen bonds with the water molecules, and therefore reduce unfavorable interactions between the linker and the protein moieties. In some embodiments a linker comprises small non-polar (e.g. Gly) or polar (e.g. Ser or Thr) amino acids. In some embodiments, a linker is a Gly-Ser linker (SEQ ID NO. 52).

[00172] In some embodiments, a linker is or comprises an amino acid sequence of GGGGSSS (SEQ ID NO:21). In some embodiments, a linker is or comprises a sequence of (GGGGS)<sub>n</sub> (SEQ ID NO:22), where n represents the number of repeating GGGGS (SEQ ID NO: 22) units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a polypeptide linker may have an amino acid sequence that is or comprises GGGGSGGGGSGGGGS (SEQ ID NO:24) (i.e., (GGGGS)<sub>3</sub> (SEQ ID NO: 24)) or GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:25) (i.e., (GGGGS)<sub>6</sub> (SEQ ID NO: 25)). In some embodiments, a linker comprises one or more of Gly, Ser, Thr, Ala, Lys, and Glu. In some embodiments, a linker is or comprises KESGSVSSEQLAQFRSLD (SEQ ID NO: 26). In some embodiments, a linker is or comprises EGKSSSGSGESKST (SEQ ID NO: 27). In some embodiments, a linker is or comprises (Gly)<sub>n</sub> (SEQ ID NO:28) where n represents the number of repeating Gly residues and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments a linker is or comprises GGG (SEQ ID NO. 53). In some embodiments, a linker is or comprises (Gly)<sub>6</sub> (SEQ ID NO:47). In some embodiments, a linker is or comprises (Gly)<sub>8</sub> (SEQ ID NO:48). In some embodiments, a linker is or comprises GSAGSAAGSGEF (SEQ ID NO:30). In some embodiments, a linker is or comprises an amino acid sequence of AAA (SEQ ID NO:21).

[00173] In some embodiments, a linker is a rigid linker. Rigid linkers are useful to keep a fixed distance between domains and to maintain their independent functions. Rigid linkers may also be useful when a spatial separation of the domains is critical to preserve the stability or bioactivity of one or more components in the fusion. In some embodiments, a linker is or comprises (EAAAK)<sub>n</sub> (SEQ ID NO:31) where n represents the number of repeating EAAAK (SEQ ID NO: 31) units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises A(EAAAK)<sub>n</sub>A, (SEQ ID NO:32) where n represents the number of repeating EAAAK (SEQ ID NO: 31) units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises A(EAAAK)<sub>n</sub>A (SEQ ID NO: 32), where n represents the number of repeating EAAAK (SEQ ID NO: 31) units and is 2, 3, 4, or 5. In some embodiments, a linker is or comprises A(EAAAK)<sub>4</sub>ALEA(EAAAK)<sub>4</sub>A (SEQ ID NO:33). In some embodiments, a linker is or comprises [A(EAAAK)<sub>n</sub>A]<sub>m</sub>, (SEQ ID NO:34) wherein n is 2, 3, or 4 and m is 1 or 2. In some embodiments, a linker is or comprises AEAAAKEAAKA (SEQ ID NO:35).

[00174] In some embodiments a linker is or comprises (X-Pro)<sub>n</sub> (SEQ ID NO:36), with X designating any amino acid, where n represents the number of repeating X-Pro units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments a linker is or comprises (Ala-Pro)<sub>n</sub> (SEQ ID NO:50), where n represents the number of repeating Ala-Pro units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments a linker is or comprises (Ala-Pro)<sub>n</sub> (SEQ ID NO: 50), where n represents the number of repeating Ala-Pro units and is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17.

[00175] In some embodiments a linker is or comprises (Lys-Pro)<sub>n</sub> (SEQ ID NO:37), where n represents the number of repeating Lys-Pro units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments a linker is or comprises (Gln-Pro)<sub>n</sub> (SEQ ID NO:38), where n represents the number of repeating Gln-Pro units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises (Ala-Pro)<sub>7</sub> (SEQ ID NO:39).

[00176] In some embodiments a linker is or comprises GAPGGGGGAAAAAGGGGGGAP (GAG linker, SEQ ID NO:41). In some embodiments a linker is or comprises GAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAP (GAG2 linker, SEQ ID NO:42). In some embodiments a linker is or comprises GAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAP (GAG3 linker, SEQ ID NO:43).

[00177] Suitable linkers or spacers also include those having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous or identical to the above exemplary linkers.

[00178] Additional linkers suitable for use with some embodiments may be found in U.S. Patent Publication No. 2012/0232021, filed on March 2, 2012, and [Chen, 2013] the disclosures of which is hereby incorporated by reference in their entireties. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide. A variety of different linker elements that can appropriately be used when engineering polypeptides (e.g., fusion polypeptides) are known in the art (Holliger et al, 1993; Poljak, 1994).

### *Antigenic Polysaccharides*

[00179] In some embodiments, an immunogenic complex described herein includes one or more *S. enterica* polysaccharides. In some embodiments, an immunogenic complex described herein comprises a polysaccharide from the *S. enterica* subspecies *enterica* (*I*). In some embodiments, an immunogenic complex includes one or more *S. enterica* capsular polysaccharides or O-specific polysaccharides (OSP) from, or derived from, one or more *S. enterica* serovars selected from Typhi, Typhimurium, Enteritidis, and Paratyphi.

[00180] In some embodiments, an immunogenic complex described herein comprises a *S. enterica* polysaccharide that is > 60kDa, or > 70kDa, or > 80kDa, or > 90kDa, or > 100kDa, or > 110kDa, or > 120kDa. In some embodiments, an immunogenic complex described herein comprises an OSP polysaccharide from *S. enterica* that is between 90-110kDa.

[00181] There are currently 2,463 serotypes (serovars) of Salmonella. The antigenic formulae of Salmonella serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on Salmonella at the Pasteur Institute, Paris, France (WHO Collaborating Centre), and new serotypes are listed in annual updates of the Kauffmann-White scheme (Popoff M Y, Bockemühl J, Brenner F W. Supplement 1998 (no. 42) to the Kauffmann-White scheme. Res Microbiol. 2000;151:63–65; Popoff M Y, Le Minor L. Antigenic formulas of the Salmonella serovars, 7th revision. World Health Organization Collaborating Centre for Reference and Research on Salmonella. Paris, France: Pasteur Institute; 1997.).

[00182] In some embodiments, an immunogenic complex described herein includes one *S. enterica* polysaccharide, selected from Typhi, Typhimurium, Enteritidis, and Paratyphi. In some embodiments, a MAPS immunogenic complex described can comprise a polysaccharide from other Salmonella serotypes, including, but not limited to Salmonella serotypes that cause salmonellosis, such as, but not limited to *Newport*, *Javiana*, 4, [5], 12:i:-, *Heidelberg*, *Saintpaul*, *Muenchen*, *Montevideo*, and *Infantis*. In some embodiments, a MAPS immunogenic complex described can comprise a polysaccharide from other Salmonella serotypes, selected from the group of *S. choleraesuis*, *S. Dublin*, *S. gallinarum* and *S. pullorum*.

[00183] In some embodiments, an immunogenic complex described herein comprises a Vi polysaccharide from *S. Typhi*. In some embodiments, an immunogenic complex described herein comprises an O-specific polysaccharide (OSP) from any one or more of: *Typhimurium*, *Enteritidis*, and *Paratyphi*.

[00184] In some embodiments, an immunogenic complex described herein comprises a polysaccharide from a type strain of Salmonella enterica selected from any of: ATCC:43971, CCUG:42060, CIP:60.62, NBRC:13245, NCIMB:11450, NCTC:12416, personal::LT2, DSM:17058, ATCC:700720, SGSC:1412.

[00185] *S. Typhimurium*

[00186] In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from Salmonella enterica subsp. enterica serovar Typhimurium, and can be selected from any one or more of S. Typhimurium strains: Q55, Q65, LT2,P104, S11, S12 or ATCC SL1344. In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from Salmonella enterica subsp. enterica serovar Typhimurium, and can be selected from any one or more of S. Typhimurium strains: 00-01036; 08-1736; 101966; 102261; 102690; 102923; 104772; 106041; 108402; 109456; 113599; 114115; 116045; 119016; 128781; 130302; 135497; 135901; 14028s Wu; 34502; 35423; 36618; 4581; 5579; 70031; 798; 85513; 85982; 86-0368;

87541; 92199; 95799; 96521; 98346; 98787; A130; A36; ATCC 14028; AZ 057; CDC 2009K-1640; CDC 2009K-2059; CDC 2010K-1587; CDC 2011K-0870; CDC 2011K-1702; CDC H2662; CDC\_2009K1153; CDC\_2009K1158; CDC\_2009K1277; CDC\_2009K1283; CDC\_2009K1288; CFSAN000629; CFSAN000632; CFSAN000633; CFSAN000634; CFSAN000635; CFSAN000637; CFSAN000638; CFSAN000641; CFSAN000642; CFSAN000643; CFSAN000645; CFSAN000648; CFSAN000649; CFSAN000653; CFSAN001736; CFSAN002047; D23580; DA5803; DA5810 ; DA5816; DA5822; DA5828; DA5884; DA5894; DA5915; DT104; DT2; FDA 03-061782; FDA 71-2164; FDA 76-1071; FDA 78-0204; FDA 85-0189; FDA 86-0023; FDA 86-0353; FDA 88-0085; FDA 93-0158; FDA 93-0429; FDA 95-0328; JB124; JCM 1652; L-3553; L796; L847; L852; L904; L927; L945; LT2; RM10602; RM13674; RM13676; RM14082; RM14083; RM14510; RM14512; RM6835; SARA13; SJ2353; SL1344; ST1489; ST1660/06; ST18563; ST4/74; ST4581; ST4848; ST78896; STm1; STm10; STm11; STm12; STm2; STm3; STm4; STm5; STm6; STm7; STM709; STm8; STm9; T000240; TN061786; TR7095; U288; UC08; UC09; UC14; UC17; UK-1; USDA-ARS-USMARC-1808; USDA-ARS-USMARC-1809; USDA-ARS-USMARC-1810; USDA-ARS-USMARC-1811; USDA-ARS-USMARC-1879; USDA-ARS-USMARC-1880; USDA-ARS-USMARC-1881; USDA-ARS-USMARC-1896; USDA-ARS-USMARC-1897; USDA-ARS-USMARC-1898; USDA-ARS-USMARC-1899; USDA-ARS-USMARC-1911; W2342, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium variants. 5, O 5 (stain 73007); *Salmonella enterica* subsp. *enterica* serovar Typhimurium var. Copenhagen str. 0084; or strain 59802 monophasic; monophasic 4,5,12:i:-; monophasic 4,[5],12:i:-, or *Salmonella enterica* subsp. *enterica* serovar Copenhagen.

**[00187]** In some embodiments, an immunogenic complex described herein comprises at least one LT2 polysaccharide selected from *Salmonella enterica* subsp. *enterica* serovar Typhimurium, from any typhimurium strain disclosed herein or known to a person of ordinary skill in the art.

**[00188]** *S. Enteritidis*

**[00189]** In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from *Salmonella enterica* subsp. *enterica* serovar enteritidis, and can be selected from any one or more of enteritidis strains: I15, S-1, D28, J73, R27, R11, SL1344.

**[00190]** In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from *Salmonella enterica* subsp. *enterica* serovar Enteritidis, and can be selected from any Enteritidis strains, for example, any one or more of Enteritidis strains selected from the group of, but not limited to strain: 02-2966; 04-0307; 0502571; 07-0056; 0701376-4; 08-0047; 08-0128; 08-0627; 08-1080; 0804789B; 0811210F ; 10-101; 10-103; 10-130; 10-134; 10-28670; 10-29153; 10-29949; 10-30147; 10-31528; 10-33213; 10-33369; 10-33371; 10-33603; 10-34213; 10-34587; 10-34599; 10-34601; 10-35178; 10-35179; 10-35180; 10-35181; 10-35182; 10-35183; 10-35184; 10-35417; 10-36119; 10-36319; 10-36979; 10-37723; 10-38792; 10-39087; 11-03844; 11-06235; 11-21079; 11-22186; 11-27690; 11-30508; 11-31312; 11-32014; 1102933A; 12-11922; 12-12016; 12-12071; 12-12205; 12-

12288; 12-14089; 12-14426; 12-14487; 12-14693; 12-14697; 12-14699; 12-14700; 12-14703; 12-14895; 12-14982; 12-15432; 12-15721; 12-16076; 12-16086; 12-16414; 12-16608; 12-17211; 12-17240; 12-17486; 12-17892; 12-17893; 12-18137; 12-18138; 12-18160; 12-18401; 12-18526; 12-18775; 12-19490; 12-19760; 12-19798; 12-19824; 12-2; 12-20008; 12-20343; 12-20418; 12-21190; 12-21313; 12-21314; 12-21567; 12-21569; 12-21687; 12-22120; 12-22891; 12-22983; 12-23418; 12-23426; 12-24078; 12-24683; 12-24729; 12-25457; 12-26550; 12-26681; 12-26778; 12-26898; 12-3; 12-5; 12-7; 12-8; 12-9; 122205; 13-1; 13183-1; 17927; 18569; 20037; 2009K0477; 2009K0479; 2009K0958; 2009K1324; 2009K1651; 2009K1726; 2010-1237; 2010K-0262; 2010K-0263; 2010K-0264; 2010K-0267; 2010K-0268; 2010K-0271; 2010K-0277; 2010K-0284; 2010K-0286; 2010K-0287; 2010K-0297; 2010K-0300; 2010K-0301; 2010K-0302; 2010K-0303; 2010K-0313; 2010K-0329; 2010K-0345; 2010K-0351; 2010K-0669; 2010K-1028; 2010K-1369; 2010K-1554; 2010K-1832; 2010K-1923; 2010K-2029; 2010K-2599; 21027; 21046; 22079; 22087; 22510-1; 22558; 22704; 33944; 3402; 34986; 39997; 436; 48-0811; 485549-17; 50-3079; 50-5646; 53-407; 54-2220; 543463 22-17; 543463 40-18; 543463 42-20; 55795; 561362 1-1; 561362 9-7; 576709; 58-6482; 596866-22; 596866-70; 6.0562-1; 607307-2; 607307-6; 607308-16; 607308-19; 607308-9; 61080; 61979; 62-1976; 622731-39; 629163; 629164-26; 629164-37; 635290-58; 638970-15; 639016-6; 639672-46; 639672-50; 640631; 642044 4-1; 642044 8-1; 642046 4-7; 648898 4-5; 648899 3-17; 648900 1-16; 648901 1-17; 648901 16-16; 648901 39-2; 648901 6-18; 648902 6-8; 648903 1-6; 648904 3-6; 648905 5-18; 653049 13-19; 674470\_11-10; 75-2732; 76-0331; 76-2651; 77-0424; 77-0915; 77-1427; 77-2659; 77320; 78-1757; 78296; 81-2625; 81748; 82631; 84-1226; 85366; 8b-1; 9-135; 9-193; 9-332; 9-531; 9-884; 93-0063; 93-2836A; 93-6175B; 93-7741; 93-7922A; 97569; 98-0467; 98-9534; 9810102B; 98961; ATCC BAA-1045; ATCC BAA-1587; BEAR072987-03; BEAR078296-01; BEAR083469-02; BEAR091751-04; C49; C75; C76; CDC\_2010K\_0895; CDC\_2010K\_0899; CDC\_2010K\_0956; CDC\_2010K\_0968; CDC\_2010K\_1010; CDC\_2010K\_1018; CDC\_2010K\_1441; CDC\_2010K\_1444; CDC\_2010K\_1445; CDC\_2010K\_1455; CDC\_2010K\_1457; CDC\_2010K\_1543; CDC\_2010K\_1558; CDC\_2010K\_1559; CDC\_2010K\_1565; CDC\_2010K\_1566; CDC\_2010K\_1575; CDC\_2010K\_1580; CDC\_2010K\_1594; CDC\_2010K\_1725; CDC\_2010K\_1729; CDC\_2010K\_1745; CDC\_2010K\_1747; CDC\_2010K\_1791; CDC\_2010K\_1795; CDC\_2010K\_1808; CDC\_2010K\_1810; CDC\_2010K\_1811; CDC\_2010K\_1882; CDC\_2010K\_1884; CFSAN000024; CFSAN000027; CFSAN000028; CFSAN000030; CFSAN000034; CFSAN000035; CFSAN000036; CFSAN00004; CFSAN000040; CFSAN000041; CFSAN000043; CFSAN000044; CFSAN000052; CFSAN000053; CFSAN000054 ; CFSAN000059; CFSAN000060; CFSAN000066; CFSAN000067; CFSAN000069; CFSAN000070; CFSAN000072; CFSAN000074; CFSAN000075; CFSAN000081; CFSAN000082; CFSAN000083; CFSAN000085; CFSAN000086; CFSAN000087; CFSAN000089; CFSAN000094; CFSAN000096; CFSAN000099; CFSAN000100; CFSAN000102; CFSAN000103; CFSAN000104; CFSAN000122; CFSAN000568; CFSAN001578; CFSAN001579; CFSAN001580; CFSAN001581; CFSAN001582; CFSAN001583; CFSAN001584; CFSAN001585; CFSAN001586; CFSAN004342; CHS4; CHS44; CVM\_56-3991; CVM\_69-4941; CVM\_76-3618;

CVM\_81-2490; CVM\_N202; EC20090135; EC20090193; EC20090195; EC20090332; EC20090530; EC20090531; EC20090641; EC20090698; EC20090884; EC20100088; EC20100089; EC20100100; EC20100101; EC20100103; EC20100130; EC20100131; EC20100134; EC20100325; EC20110221; EC20110222; EC20110223; EC20110353; EC20110354; EC20110355; EC20110356; EC20110357; EC20110358; EC20110359; EC20110360; EC20110361; EC20111095; EC20111174; EC20111175; EC20111510; EC20111514; EC20111515; EC20111554; EC20111561; EC20111576; EC20120002; EC20120003; EC20120005 ; EC20120007; EC20120008; EC20120009; EC20120051; EC20120200; EC20120213; EC20120219; EC20120229; EC20120240; EC20120356; EC20120469; EC20120496; EC20120497; EC20120498; EC20120505; EC20120528; EC20120544; EC20120548; EC20120555; EC20120580; EC20120581; EC20120590; EC20120597; EC20120677; EC20120685; EC20120686; EC20120687; EC20120697; EC20120722; EC20120734; EC20120738; EC20120765; EC20120773; EC20120774; EC20120775; EC20120776; EC20120916; EC20120917; EC20120918; EC20120925; EC20120927; EC20120929; EC20120963; EC20120968; EC20120969; EC20120970; EC20120994; EC20121004; EC20121175; EC20121176; EC20121177; EC20121178; EC20121179; EC20121180; EC20121541; EC20121542; EC20121671; EC20121672; EC20121689; EC20121744; EC20121746; EC20121747; EC20121748; EC20121750; EC20121751; EC20121753; EC20121765; EC20121812; EC20121825; EC20121826; EC20121969; EC20121970; EC20121976; EC20121986; EC20121989; EC20121990; EC20122022; EC20122026; EC20122031; EC20122033; EC20122045; EC20130345; EC20130346; EC20130347; EC20130348; IEH 300646-11; J0828; J0903; J0915; LA5; LK5; MEI 19721-1; P125109; PT13\_05-6566; PT13\_07-4012; PT13\_07-7071; PT1\_09-1747; PT23; PT8\_08-3405; PT8\_08-8253; RDNC; RM2968; RM4369; S-277; S-380; SA19930684; SA19940857; SA19942384; SA19943269; SA19960848; SA19961622; SA19970510; SA19970769; SA19971331; SA19980677; SA19981522; SA19981857; SA19982831; SA19983126; SA19992322; SA19994216; SA20082034; SA20083456; SA20083636; SA20084384; SA20084644; SA20084824; SA20085285; SA20090419; SA20090435; SA20090877; SA20091739; SA20092320; SA20093266; SA20093421; SA20093430; SA20093538; SA20093543; SA20093784; SA20093788; SA20093950 ; SA20093977; SA20094079; SA20094177; SA20094301; SA20094350; SA20094352; SA20094383; SA20094389; SA20094521; SA20094642; SA20094682; SA20094803; SA20095309; SA20095440; SA20100239; SA20100349; SA20121703; SA20123395; SARB17; SARB19; SE10; SE15-1; SE30663; SE8a; SHSE001; SHSE002; SHSE003; SHSE004; SL909; SL913; UC02; UC03; UC05; UC07; UC10; UC11; UC12; UC13

*Salmonella enterica* subsp. *enterica* serovar *Enteritidis* var. *chaco*; *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* var. *danyasz*; *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* var. *jena* and *Salmonella enterica* subsp. *enterica* strin 47038.

[00191] In some embodiments, an immunogenic complex described herein comprises at least one lipopolysaccharide (LPS) selected from *Salmonella enterica* subsp. *enterica* serovar *enteritidis*, from any *enteritidis* strain disclosed herein or known to a person of ordinary skill in the art. In some embodiments, an immunogenic complex described herein comprises at least one O-specific polysaccharide (OPS)

selected from *Salmonella enterica* subsp. *enterica* serovar *enteritidis*, from any *enteritidis* strain disclosed herein or known to a person of ordinary skill in the art, for example, but not limited to strains: I15, S-1, D28, J73, R27, R11, SL1344.

**[00192]** *S. Typhi*:

**[00193]** In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from *Salmonella enterica* subsp. *enterica* serovar *Typhi*, and can be selected from any one or more of *S. Typhi* strains: 404ty; AG3; BL196; CFSAN000626; CFSAN000628; CR0044; CR0063; CT18; E00-7866; E01-6750; E02-1180; E98-0664; E98-2068; E98-3139; J185; M223; P-stx-12; SGSC2661; ST0208; STH2370; Ty2; Ty21a; UJ308A; UJ816A. In some embodiments, an immunogenic complex described herein comprises at least one Vi polysaccharide selected from *Salmonella enterica* subsp. *enterica* serovar *typhi*, from any *typhi* strain disclosed herein or known to a person of ordinary skill in the art.

**[00194]** In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from a *Salmonella enterica* subsp. *enterica* serovar *Typhisis*, selected from any one or more of *S. Typhisis* strains: CFSAN000654 or CFSAN000655.

**[00195]** *S. paratyphi A, B or C*:

**[00196]** In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from any of serovar *Paratyphi A, B or C* of *Salmonella enterica* subsp. *enterica* *S. paratyphi A, B or C*.

**[00197]** *Paratyphi A*: In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from *Paratyphi A*, e.g., a *Salmonella enterica* subsp. *enterica* serovar *Paratyphi A* strain selected from any of *Paratyphi A* strains: AKU\_12601, ATCC 11511 ; ATCC 9150 ; GXS2268 ; GZ9A00052 ; JX05-19; SA19950809; YN09620 ; ZJ98-53.

**[00198]** *Paratyphi B*: In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from *Paratyphi B*, e.g., a *Salmonella enterica* subsp. *enterica* serovar *Paratyphi B* strain selected from any of *Paratyphi B* strains: ATCC 10719; ATCC 19940; ATCC 51962; ATCC 8759; ATCC BAA-1584; ATCC BAA-1585; CFSAN000524; CFSAN000525; CFSAN000526; CFSAN000527; CFSAN000528; CFSAN000529; CFSAN000530; CFSAN000531; CFSAN000532; CFSAN000533; CFSAN000534; CFSAN000535; CFSAN000536; CFSAN000537; CFSAN000539; CFSAN000540; CFSAN000541; CFSAN000542; CFSAN000545; CFSAN000546; CFSAN000547; CFSAN000548; CFSAN000549; SARA42; SARA56; SARA61; SARA62; SPB7; *Salmonella enterica* subsp. *enterica* serovar *Paratyphi B* var. *L-tartrate* + ; *Salmonella enterica* subsp. *enterica* serovar *Java*.

**[00199]** *Paratyphi C*: In some embodiments, an immunogenic complex described herein comprises at least one polysaccharide selected from *Paratyphi C*, e.g., a *Salmonella enterica* subsp. *enterica* serovar *Paratyphi C* strain selected from any of *Paratyphi C* strains; CFSAN000603; CFSAN000604; CFSAN000605; RKS4594; SARB 49.

[00200] *Other subspecies of Salmonella enterica and serovars of the subspecies of Salmonella enterica subspecies enterica*

[00201] In some embodiments, an immunogenic complex described herein comprises a polysaccharide from a type strain of *Salmonella enterica* subspecies selected from any of the subspecies, including, but not limited to: *arizonae*, *diarizonae*, *houtenae*, *indica*, *salamae*, *subspecies IV*.

[00202] Polysaccharides for use in the immunogenic complexes as disclosed herein can comprise a polysaccharide from any *Salmonella enterica* subspecies, or serovar thereof that is listed in the Taxonomy data based found at world wide website: “ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=28901”, which is incorporated herein in its entirety by reference.

#### *Methods of Isolating and Purifying Polysaccharides*

[00203] In some embodiments, the disclosure provides methods of purifying one or more polysaccharides described herein from one or more of *S. Typhi*, *S. Typhimurium*, *S. Enteritidis*, and *S. Paratyphi* from cellular components of bacteria. In some embodiments, methods comprise purifying capsular polysaccharides from one or more cellular components of bacteria.

[00204] In some embodiments, the bacteria are Gram-negative. In some embodiments, the bacteria are Gram-positive. In some embodiments, the bacteria are *S. enterica*. In some embodiments, the *S. enterica* bacterial serotypes selected from Typhi, Typhimurium, Enteritidis, and Paratyphi. Other polysaccharides from other *Salmonella* serotypes are envisioned for use in the MAPS immunogenic complexes as disclosed herein, including, but not limited to *Salmonella* serotypes that cause salmonellosis, such as, but not limited to Enteritidis, Newport, Typhimurium, Javiana, 4, [5], 12:i:-, Heidelberg, Saintpaul, Muenchen, Montevideo, and Infantis.

[00205] In some embodiments, O-specific polysaccharides purified from *S Paratyphi A* are small (around 40-50 kDa) which limits the cross-linking that can occur with the carrier proteins (e.g., rhizavidin binding to biotin on the polysaccharide). Accordingly, O-specific polysaccharides of between about 90-120 kDa can be purified from modified *S Paratyphi*, which have been modified for at least one of: (i) deletion of the short chain OSP enzyme WZZ, and (ii) overexpression of long chain OSP enzyme Fepe from *S. Paratyphi* or WZZ<sub>2</sub> from *Pseudomonas aeruginosa*.

[00206] In some embodiments, the cellular components include protein. In some embodiments, the cellular proteins include nucleic acid. In some embodiments, the cellular components include lipids. In some embodiments, the cellular components include polysaccharides. In some embodiments, the cellular components are part of a lysate.

[00207] In some embodiments, the polysaccharide purification processes incorporate a series of ethanol precipitations, washes of crude polysaccharide preparations with ethanol, diethyl ether, and/or acetone, and drying under vacuum to furnish purified products. In some embodiments, a phenol extraction step is incorporated for polysaccharide purifications. In some embodiments the purification process employs a CTAB (cetyltrimethyl ammonium bromide) precipitation step in addition to using ethanol and phenol precipitation steps.

*Methods of Biotinylating Polysaccharides*

[00208] In some embodiments, the disclosure provides methods of biotinylating one or more polysaccharides described herein. In some embodiments, the method comprises reacting purified polysaccharides with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) for activation of hydroxyl groups in the polysaccharides followed by the addition of amine PEG biotin under conditions that result in covalent linkage of biotin to the polysaccharides. In some embodiments, the desired level of biotinylation is achieved by varying the ratio of CDAP to polysaccharide. In some embodiments, the method comprises reacting purified polysaccharides with 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide Hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS). In some embodiments, the biotinylated polysaccharides are purified by filtration to remove process residuals such as unreacted biotin, dimethylaminopyridine, acetonitrile, cyanide and unreacted glycine. In some embodiments, the level of polysaccharide biotinylation described herein is optimized to reduce the amount of accessible biotin following MAPS complexation.

*Manufacture of Immunogenic Complexes*

[00209] The present disclosure includes methods for manufacturing immunogenic complexes described herein. In some embodiments, a method of manufacturing immunogenic complexes comprises complexing at least one biotinylated polysaccharide with at least one biotin-binding fusion protein. In some embodiments, the fusion protein comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 1. In some embodiments, the fusion protein comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 2. In some embodiments, the fusion protein comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 6.

[00210] In some embodiments, the average (e.g., the mean) protein (e.g., antigenic protein) to polysaccharide ratio of a 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 protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes

is approximately 7:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average protein:PS ratios are 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.

[00211] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising any one or more of: a Rhavi-SseB, a Rhavi-IpaB, or a CP1 protein and a O-specific polysaccharide, from or derived from *S. enterica* serotype Typhimurium. In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium 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 either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-

IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Typhimurium 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.

[00212] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising any one of: an Rhavi-SseB, a Rhavi-IpaB, or a CP1 protein and a O-specific polysaccharide, from or derived from *S. enterica* serotype Enteritidis. In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis 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 either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific

polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Enteritidis 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.

[00213] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising any one or more of: Rhavi-SseB, a Rhavi-IpaB, or a CP1 protein and a capsular polysaccharide, from or derived from *S. enterica* serotype Typhi. In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi 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 either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica*

serotype Typhi in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to capsular polysaccharide from or derived from *S. enterica* serotype Typhi 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.

[00214] In some embodiments, a vaccine or immunogenic composition comprises a plurality of immunogenic complexes comprising any one or more of: an Rhavi-SseB, a Rhavi-IpaB, or a CP1 protein and a O-specific polysaccharide, from or derived from *S. enterica* serotype Paratyphi. In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi 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 either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of either Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in

the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of Rhavi-SseB, a Rhavi-IpaB, or CP1 protein to O-specific polysaccharide from or derived from *S. enterica* serotype Paratyphi 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.

#### ***Immunogenic and Vaccine Compositions***

**[00215]** 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 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.

#### ***[00216] Vaccine compositions***

**[00217]** 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.

**[00218]** 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.

**[00219]** *Exemplary Salmonella-MAPS vaccine composition*

**[00220]** In some embodiments, a vaccine composition disclosed herein comprises at least 2, or at least 3, or at least 4 species of immunogenic compositions, *e.g.*, at least 2, or 3, or 4 Salmonella-MAPS immunogenic complexes.

**[00221]** In some embodiments, a vaccine composition as described herein comprises at least two immunogenic complexes, wherein the two immunogenic complexes are selected from any of: *S.* Typhimurium-SseB, *S.* Enteritidis-SseB, *S.* Typhi-SseB (also referred to as Vi-SseB), *S.* ParatyphiOSP-SseB (also referred to as ParaOSP-SseB), *S.* Typhimurium-IpaB, *S.* Enteritidis-IpaB, *S.* Typhi Vi-IpaB (also referred to as Vi-IpaB), *S.* ParatyphiOSP-IpaB (also referred to as ParaOSP-IpaB), *S.* Typhimurium-CP1, *S.* Enteritidis-CP1, *S.* Typhi Vi-CP1 (Vi-CP1), *S.* ParatyphiOSP-CP1 (ParaOSP-CP1). Exemplary combinations for a bivalent MAPS-Salmonella vaccine are shown in Table 1A.

**[00222]** **Table 1A.** Exemplary combination of immunogenic complexes for a bivalent (2V) vaccine composition comprising two Salmonella-MAPS immunogenic complexes.

| <b>2V Salmonella-MAPS immunogenic compositions</b> |                               |
|----------------------------------------------------|-------------------------------|
| Typhimurium-SseB, Enteritidis-SseB                 | Enteritidis-SseB, Vi-CP1      |
| Typhimurium-SseB, Vi-SseB                          | Enteritidis-SseB, ParaOSP-CP1 |
| Typhimurium-SseB, ParaOSP-SseB                     | Typhi-SseB, ParaOSP-SseB      |
| Typhimurium-SseB, Typhimurium-IpaB                 | Typhi-SseB, Typhimurium-IpaB  |
| Typhimurium-SseB, Enteritidis-IpaB                 | Typhi-SseB, Enteritidis-IpaB  |
| Typhimurium-SseB, Vi-IpaB                          | Typhi-SseB, Vi-IpaB           |
| Typhimurium-SseB, ParaOSP-IpaB                     | Typhi-SseB, ParaOSP-IpaB      |
| Typhimurium-SseB, Typhimurium-CP1                  | Typhi-SseB, Typhimurium-CP1   |
| Typhimurium-SseB, Enteritidis-CP1                  | Typhi-SseB, Enteritidis-CP1   |

|                                    |                                    |
|------------------------------------|------------------------------------|
| Typhimurium-SseB, Vi-CP1           | Typhi-SseB, Vi-CP1                 |
| Typhimurium-SseB, ParaOSP-CP1      | Typhi-SseB, ParaOSP-CP1            |
| Enteritidis-SseB, Vi-SseB          | Paratyphi-SseB, Typhimurium-IpaB   |
| Enteritidis-SseB, ParaOSP-SseB     | Paratyphi-SseB, Enteritidis-IpaB   |
| Enteritidis-SseB, Typhimurium-IpaB | Paratyphi-SseB, Vi-IpaB            |
| Enteritidis-SseB, Enteritidis-IpaB | Paratyphi-SseB, ParaOSP-IpaB       |
| Enteritidis-SseB, Vi-IpaB          | Paratyphi-SseB, Typhimurium-CP1    |
| Enteritidis-SseB, ParaOSP-IpaB     | Paratyphi-SseB, Enteritidis-CP1    |
| Enteritidis-SseB, Typhimurium-CP1  | Paratyphi-SseB, Vi-CP1             |
| Enteritidis-SseB, Enteritidis-CP1  | Paratyphi-SseB, ParaOSP-CP1        |
| Typhimurium-IpaB, ParaOSP-CP1      | Typhimurium-IpaB, Enteritidis-IpaB |
| Enteritidis-IpaB, Vi-IpaB          | Typhimurium-IpaB, Vi-IpaB          |
| Enteritidis-IpaB, ParaOSP-IpaB     | Typhimurium-IpaB, ParaOSP-IpaB     |
| Enteritidis-IpaB, Typhimurium-CP1  | Typhimurium-IpaB, Typhimurium-CP1  |
| Enteritidis-IpaB, Enteritidis-CP1  | Typhimurium-IpaB, Enteritidis-CP1  |
| Enteritidis-IpaB, Vi-CP1           | Typhimurium-IpaB, Vi-CP1           |
| Enteritidis-IpaB, ParaOSP-CP1      | Paratyphi-IpaB, Typhimurium-CP1    |
| Typhi-IpaB, ParaOSP-IpaB           | Paratyphi-IpaB, Enteritidis-CP1    |
| Typhi-IpaB, Typhimurium-CP1        | Paratyphi-IpaB, Vi-CP1             |
| Typhi-IpaB, Enteritidis-CP1        | Paratyphi-IpaB, ParaOSP-CP1        |
| Typhi-IpaB, Vi-CP1                 | Typhimurium-CP1, Enteritidis-CP1   |
| Typhi-IpaB, ParaOSP-CP1            | Typhimurium-CP1, Vi-CP1            |
|                                    | Typhimurium-CP1, ParaOSP-CP1       |
|                                    | Enteritidis-CP1, Vi-CP1            |
|                                    | Enteritidis-CP1, ParaOSP-CP1       |
|                                    | Typhi-CP1, ParaOSP-CP1             |

**[00223]** In some embodiments, an immunogenic composition or vaccine comprises at least three immunogenic complexes as described herein, wherein the three immunogenic complexes are selected from any of: Typhimurium-SseB, S. Enteritidis-SseB, S. Typhi-SseB (also referred to as Vi-SseB), S. ParatyphiOSP-SseB (also referred to as ParaOSP-SseB), S. Typhimurium-IpaB, S. Enteritidis-IpaB, S. Typhi Vi-IpaB (also referred to as Vi-IpaB), S. ParatyphiOSP-IpaB (also referred to as ParaOSP-IpaB), S. Typhimurium-CP1, S. Enteritidis-CP1, S. Typhi Vi-CP1 (Vi-CP1), S. ParatyphiOSP-CP1 (ParaOSP-CP1). Exemplary combinations of immunogenic complexes for a multivalent Salmonella-MAPS vaccine are shown in Table 1B.

**[00224] Table 1B.** Exemplary combinations of immunogenic complexes for a multivalent (3V) vaccine composition comprising three Salmonella-MAPS immunogenic complexes.

| <b>3V Salmonella MAPS immunogenic compositions</b>   |                                                  |
|------------------------------------------------------|--------------------------------------------------|
| Typhimurium-SseB, Enteritidis-SseB, Vi-SseB          | Enteritidis-SseB, Vi-SseB, ParaOSP-SseB          |
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB     | Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB      |
| Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB | Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB      |
| Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB | Enteritidis-SseB, Vi-SseB, Vi-IpaB               |
| Typhimurium-SseB, Enteritidis-SseB, Vi-IpaB          | Enteritidis-SseB, Vi-SseB, ParaOSP-IpaB          |
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-IpaB     | Enteritidis-SseB, Vi-SseB, Typhimurium-CP1       |
| Typhimurium-SseB, Enteritidis-SseB, Typhimurium-CP1  | Enteritidis-SseB, Vi-SseB, Enteritidis-CP1       |
| Typhimurium-SseB, Enteritidis-SseB, Enteritidis-CP1  | Enteritidis-SseB, Vi-SseB, Vi-CP1                |
| Typhimurium-SseB, Enteritidis-SseB, Vi-CP1           | Enteritidis-SseB, Vi-SseB, ParaOSP-CP1           |
|                                                      | Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB |

|                                                      |                                                      |
|------------------------------------------------------|------------------------------------------------------|
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-CP1      | Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB     |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB              | Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB              |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB          | Enteritidis-SseB, ParaOSP-SseB, ParaOSP-IpaB         |
| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB          | Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB     |
| Typhimurium-SseB, Vi-SseB, Vi-IpaB                   | CP1                                                  |
| Typhimurium-SseB, Vi-SseB, ParaOSP-IpaB              | Enteritidis-SseB, ParaOSP-SseB, Enteritidis-CP1      |
| Typhimurium-SseB, Vi-SseB, Typhimurium-CP1           | Enteritidis-SseB, ParaOSP-SseB, Vi-CP1               |
| Typhimurium-SseB, Vi-SseB, Enteritidis-CP1           | Enteritidis-SseB, ParaOSP-SseB, ParaOSP-CP1          |
| Typhimurium-SseB, Vi-SseB, Vi-CP1                    | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB |
| Typhimurium-SseB, Vi-SseB, ParaOSP-CP1               | Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB          |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB     | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-IpaB     |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB     | Enteritidis-SseB, Typhimurium-IpaB, Typhimurium-CP1  |
| Typhimurium-SseB, ParaOSP-SseB, Vi-IpaB              | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-CP1  |
| Typhimurium-SseB, ParaOSP-SseB, ParaOSP-IpaB         | Enteritidis-SseB, Typhimurium-IpaB, Vi-CP1           |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-CP1      | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-CP1      |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-CP1      | Enteritidis-SseB, Typhimurium-IpaB, Vi-CP1           |
| Typhimurium-SseB, ParaOSP-SseB, Vi-CP1               | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-CP1      |
| Typhimurium-SseB, ParaOSP-SseB, ParaOSP-CP1          | Enteritidis-SseB, Enteritidis-IpaB, Vi-IpaB          |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB | Enteritidis-SseB, Enteritidis-IpaB, ParaOSP-IpaB     |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-IpaB          | Enteritidis-SseB, Enteritidis-IpaB, Typhimurium-CP1  |
| Typhimurium-SseB, Typhimurium-IpaB, ParaOSP-IpaB     | Enteritidis-SseB, Enteritidis-IpaB, Enteritidis-CP1  |
| Typhimurium-SseB, Typhimurium-IpaB,                  | Enteritidis-SseB, Enteritidis-IpaB, Vi-CP1           |
| Typhimurium-CP1                                      | Enteritidis-SseB, Enteritidis-IpaB, ParaOSP-CP1      |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-CP1  | Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB              |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-CP1           | Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1           |
| Typhimurium-SseB, Typhimurium-IpaB, ParaOSP-CP1      | Enteritidis-SseB, Vi-IpaB, Enteritidis-CP1           |
| Typhimurium-SseB, Enteritidis-IpaB, Vi-IpaB          | Enteritidis-SseB, Vi-IpaB, Vi-CP1                    |
| Typhimurium-SseB, Enteritidis-IpaB, ParaOSP-IpaB     | Enteritidis-SseB, Vi-IpaB, ParaOSP-CP1               |
| Typhimurium-SseB, Enteritidis-IpaB, Typhimurium-CP1  | Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1      |
| Typhimurium-SseB, Enteritidis-IpaB, Enteritidis-CP1  | Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1      |
| Typhimurium-SseB, Enteritidis-IpaB, Vi-CP1           | Enteritidis-SseB, ParaOSP-IpaB, Vi-CP1               |
| Typhimurium-SseB, Enteritidis-IpaB, ParaOSP-CP1      | Enteritidis-SseB, ParaOSP-IpaB, ParaOSP-CP1          |
| Typhimurium-SseB, Vi-IpaB, ParaOSP-IpaB              | Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1   |
| Typhimurium-SseB, Vi-IpaB, Typhimurium-CP1           | Enteritidis-SseB, Typhimurium-CP1, Vi-CP1            |
| Typhimurium-SseB, Vi-IpaB, Enteritidis-CP1           | Enteritidis-SseB, Typhimurium-CP1, ParaOSP-CP1       |
| Typhimurium-SseB, Vi-IpaB, Vi-CP1                    | Enteritidis-SseB, Enteritidis-CP1, Vi-CP1            |
| Typhimurium-SseB, Vi-IpaB, ParaOSP-CP1               | Enteritidis-SseB, Enteritidis-CP1, ParaOSP-CP1       |
| Typhimurium-SseB, ParaOSP-IpaB, Typhimurium-CP1      | Enteritidis-SseB, Vi-CP1, ParaOSP-CP1                |
| Typhimurium-SseB, ParaOSP-IpaB, Enteritidis-CP1      | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB     |
| Typhimurium-SseB, ParaOSP-IpaB, Vi-CP1               | ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB              |
| Typhimurium-SseB, ParaOSP-IpaB, ParaOSP-CP1          | ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB         |
| Typhimurium-SseB, Typhimurium-CP1, Enteritidis-CP1   | ParaOSP-SseB, Typhimurium-IpaB,                      |
| Typhimurium-SseB, Typhimurium-CP1, Vi-CP1            | Typhimurium-CP1                                      |
| Typhimurium-SseB, Typhimurium-CP1, ParaOSP-CP1       | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-CP1      |
| Typhimurium-SseB, Enteritidis-CP1, Vi-CP1            | ParaOSP-SseB, Typhimurium-IpaB, Vi-CP1               |

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| <p> Typhimurium-SseB, Enteritidis-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Vi-CP1, ParaOSP-CP1<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB<br/> Vi-SseB, ParaOSP-SseB, Vi-IpaB<br/> Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-CP1<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-CP1<br/> Vi-SseB, ParaOSP-SseB, Vi-CP1<br/> Vi-SseB, ParaOSP-SseB, ParaOSP-CP1<br/> Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB<br/> Vi-SseB, Typhimurium-IpaB, Vi-IpaB<br/> Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB<br/> Vi-SseB, Typhimurium-IpaB, Typhimurium-CP1<br/> Vi-SseB, Typhimurium-IpaB, Enteritidis-CP1<br/> Vi-SseB, Typhimurium-IpaB, Vi-CP1<br/> Vi-SseB, Typhimurium-IpaB, ParaOSP-CP1<br/> Vi-SseB, Enteritidis-IpaB, Vi-IpaB<br/> Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB<br/> Vi-SseB, Enteritidis-IpaB, Typhimurium-CP1<br/> Vi-SseB, Enteritidis-IpaB, Enteritidis-CP1<br/> Vi-SseB, Enteritidis-IpaB, Vi-CP1<br/> Vi-SseB, Enteritidis-IpaB, ParaOSP-CP1<br/> Vi-SseB, Vi-IpaB, ParaOSP-IpaB<br/> Vi-SseB, Vi-IpaB, Typhimurium-CP1<br/> Vi-SseB, Vi-IpaB, Enteritidis-CP1<br/> Vi-SseB, Vi-IpaB, Vi-CP1<br/> Vi-SseB, Vi-IpaB, ParaOSP-CP1<br/> Vi-SseB, ParaOSP-IpaB, Typhimurium-CP1<br/> Vi-SseB, ParaOSP-IpaB, Enteritidis-CP1<br/> Vi-SseB, ParaOSP-IpaB, Vi-CP1<br/> Vi-SseB, ParaOSP-IpaB, ParaOSP-CP1<br/> Vi-SseB, Typhimurium-CP1, Enteritidis-CP1<br/> Vi-SseB, Typhimurium-CP1, Vi-CP1<br/> Vi-SseB, Typhimurium-CP1, ParaOSP-CP1<br/> Vi-SseB, Enteritidis-CP1, Vi-CP1<br/> Vi-SseB, Enteritidis-CP1, ParaOSP-CP1<br/> Vi-SseB, Vi-CP1, ParaOSP-CP1<br/> ParaOSP-SseB, Vi-CP1, ParaOSP-CP1<br/> Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1<br/> Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br/> Vi-IpaB, ParaOSP-IpaB, Vi-CP1<br/> Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1<br/> Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1<br/> Vi-IpaB, Typhimurium-CP1, Vi-CP1<br/> Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1<br/> Vi-IpaB, Enteritidis-CP1, Vi-CP1<br/> Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1<br/> Vi-IpaB, Vi-CP1, ParaOSP-CP1<br/> <br/> Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB<br/> Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1<br/> Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1<br/> Enteritidis-IpaB, Vi-IpaB, Vi-CP1<br/> Enteritidis-IpaB, Vi-IpaB, ParaOSP-CP1 </p> | <p> ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-CP1<br/> ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB<br/> ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB<br/> ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1<br/> ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1<br/> ParaOSP-SseB, Enteritidis-IpaB, Vi-CP1<br/> ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-CP1<br/> ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB<br/> ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1<br/> ParaOSP-SseB, Vi-IpaB, Enteritidis-CP1<br/> ParaOSP-SseB, Vi-IpaB, Vi-CP1<br/> ParaOSP-SseB, Vi-IpaB, ParaOSP-CP1<br/> ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1<br/> ParaOSP-SseB, ParaOSP-IpaB, Enteritidis-CP1<br/> ParaOSP-SseB, ParaOSP-IpaB, Vi-CP1<br/> ParaOSP-SseB, ParaOSP-IpaB, ParaOSP-CP1<br/> ParaOSP-SseB, Typhimurium-CP1, Enteritidis-CP1<br/> ParaOSP-SseB, Typhimurium-CP1, Vi-CP1<br/> ParaOSP-SseB, Typhimurium-CP1, ParaOSP-CP1<br/> ParaOSP-SseB, Enteritidis-CP1, Vi-CP1<br/> ParaOSP-SseB, Enteritidis-CP1, ParaOSP-CP1<br/> <br/> Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB<br/> Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB<br/> Typhimurium-IpaB, Enteritidis-IpaB,<br/> Typhimurium-CP1<br/> Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1<br/> Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1<br/> Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-CP1<br/> Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB<br/> Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1<br/> Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1<br/> Typhimurium-IpaB, Vi-IpaB, Vi-CP1<br/> Typhimurium-IpaB, Vi-IpaB, ParaOSP-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB,<br/> Typhimurium-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB, Vi-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB, ParaOSP-CP1<br/> Typhimurium-IpaB, Typhimurium-CP1,<br/> Enteritidis-CP1<br/> Typhimurium-IpaB, Typhimurium-CP1, Vi-CP1<br/> Typhimurium-IpaB, Typhimurium-CP1,<br/> ParaOSP-CP1<br/> Typhimurium-IpaB, Enteritidis-CP1, Vi-CP1 </p> |
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| Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1<br>Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br>Enteritidis-IpaB, ParaOSP-IpaB, Vi-CP1<br>Enteritidis-IpaB, ParaOSP-IpaB, ParaOSP-CP1<br>Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1<br>Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1<br>Enteritidis-IpaB, Typhimurium-CP1, ParaOSP-CP1<br>Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1<br>Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1<br>Enteritidis-IpaB, Vi-CP1, ParaOSP-CP1 | Typhimurium-IpaB, Enteritidis-CP1, ParaOSP-CP1<br>Typhimurium-IpaB, Vi-CP1, ParaOSP-CP1<br><br>ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1<br>ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1<br>ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1<br>ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1<br>ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1<br>ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1<br>Typhimurium-CP1, Enteritidis-CP1, Vi-CP1<br>Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1<br>Typhimurium-CP1, Vi-CP1, ParaOSP-CP1<br>Enteritidis-CP1, Vi-CP1, ParaOSP-CP1 |
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**[00225]** In some embodiments, an immunogenic vaccine comprises at least four immunogenic compositions as described herein, wherein the four immunogenic compositions are selected from any of: Typhimurium-SseB, S. Enteritidis-SseB, S. Typhi-SseB (also referred to as Vi-SseB), S. Paratyphi-OSP-SseB (also referred to as ParaOSP-SseB), S. Typhimurium-IpaB, S. Enteritidis-IpaB, S. Typhi Vi-IpaB (also referred to as Vi-IpaB), S. Paratyphi-OSP-IpaB (also referred to as ParaOSP-IpaB), S. Typhimurium-CP1, S. Enteritidis-CP1, S. Typhi Vi-CP1 (Vi-CP1), S. Paratyphi-OSP-CP1 (ParaOSP-CP1). Exemplary combinations of immunogenic complexes for a quadrivalent (4V) Salmonella-MAPS vaccine are shown in Table 1C.

**[00226]** In one embodiment, a quadrivalent vaccine comprises the four immunogenic complexes of S Typhimurium-SseB, S. Enteritidis-SseB, Typhi-CP1 and Paratyphi-CP1 (referred to herein in the Examples as “Salmonella-MAPS 1”). In one embodiment, a quadrivalent vaccine (4V) comprises the following four immunogenic complexes of: S Typhimurium-SseB, S. Enteritidis-SseB, Typhi-SseB and Paratyphi-SseB (referred to herein in the Examples as “Salmonella-MAPS 2”).

**[00227]** **Table 1C.** Exemplary combination of immunogenic complexes for a quadrivalent (4V) vaccine composition comprising four Salmonella-MAPS immunogenic complexes.

| <b>4V Salmonella-MAPS vaccines</b>                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                 |
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| Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, ParaOSP-SseB<br>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB<br>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB<br>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, Vi-IpaB<br>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, ParaOSP-IpaB<br>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, Typhimurium-CP1 | Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1<br>Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br>Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB, Vi-CP1<br>Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1<br>Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1<br>Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1, Vi-CP1<br>Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1 |

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| <p>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, Enteritidis-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, Vi-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Vi-SseB, ParaOSP-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, ParaOSP-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, Typhimurium-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, Enteritidis-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, Vi-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, ParaOSP-SseB, ParaOSP-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, Typhimurium-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, Vi-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB, Vi-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB, ParaOSP-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB, Typhimurium-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB, Enteritidis-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB, Vi-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Enteritidis-IpaB, ParaOSP-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB</p> <p>Typhimurium-SseB, Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Vi-IpaB, Enteritidis-CP1</p> <p>Typhimurium-SseB, Enteritidis-SseB, Vi-IpaB, Vi-CP1</p> | <p>Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1, Vi-CP1</p> <p>Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, Vi-IpaB, Vi-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1</p> <p>Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1</p> <p>Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1</p> <p>Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1</p> <p>Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1</p> <p>Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1</p> <p>Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1</p> <p>Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1</p> <p>Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1</p> <p>Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1</p> <p>Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1</p> <p>Vi-IpaB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1</p> <p>Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1</p> <p>Vi-IpaB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1</p> <p>Vi-IpaB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1</p> <p>ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1</p> <p>ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1</p> <p>ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1</p> <p>ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1</p> <p>Typhimurium-CP1, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1</p> |
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| Typhimurium-SseB, Enteritidis-SseB, Vi-IpaB, ParaOSP-CP1             | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB                                    |
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1    | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB                               |
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1    | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1                            |
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-IpaB, Vi-CP1             | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1                            |
| Typhimurium-SseB, Enteritidis-SseB, ParaOSP-IpaB, ParaOSP-CP1        | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1                                     |
| Typhimurium-SseB, Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1 | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-CP1                                |
| Typhimurium-SseB, Enteritidis-SseB, Typhimurium-CP1, Vi-CP1          | ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB                                        |
| Typhimurium-SseB, Enteritidis-SseB, Typhimurium-CP1, ParaOSP-CP1     | ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1                                     |
| Typhimurium-SseB, Enteritidis-SseB, Enteritidis-CP1, Vi-CP1          | ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1                                     |
| Typhimurium-SseB, Enteritidis-SseB, Enteritidis-CP1, ParaOSP-CP1     | ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB, Vi-CP1                                              |
| Typhimurium-SseB, Enteritidis-SseB, Vi-CP1, ParaOSP-CP1              | ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-CP1                                         |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB            | ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1                                |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB            | ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1                                |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, Vi-IpaB                     | ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Vi-CP1                                         |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB                | ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB, ParaOSP-CP1                                    |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, Typhimurium-CP1             | ParaOSP-SseB, Typhimurium-IpaB, Typhimurium-CP1, Enteritidis-CP1                             |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, Enteritidis-CP1             | ParaOSP-SseB, Typhimurium-IpaB, Typhimurium-CP1, Vi-CP1                                      |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, Vi-CP1                      | ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-SseB, Typhimurium-IpaB, Typhimurium-CP1, ParaOSP-CP1 |
| Typhimurium-SseB, Vi-SseB, ParaOSP-SseB, ParaOSP-CP1                 | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-CP1, Vi-CP1                                      |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB        | ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-CP1, ParaOSP-CP1                                 |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, Vi-IpaB                 | ParaOSP-SseB, Typhimurium-IpaB, Vi-CP1, ParaOSP-CP1                                          |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB            | ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB                                        |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, Typhimurium-CP1         | ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1                                     |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, Enteritidis-CP1         | ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1                                     |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, Vi-CP1                  | ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB, Vi-CP1                                              |
| Typhimurium-SseB, Vi-SseB, Typhimurium-IpaB, ParaOSP-CP1             | ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-CP1                                         |
| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB, Vi-IpaB                 | ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1                                |
| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB            | ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1                                |

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| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB, Typhimurium-CP1       | ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Vi-CP1             |
| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB, Enteritidis-CP1       | ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB, ParaOSP-CP1        |
| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB, Vi-CP1                | ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1 |
| Typhimurium-SseB, Vi-SseB, Enteritidis-IpaB, ParaOSP-CP1           | ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1          |
| Typhimurium-SseB, Vi-SseB, Vi-IpaB, ParaOSP-IpaB                   | ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1, ParaOSP-CP1     |
| Typhimurium-SseB, Vi-SseB, Vi-IpaB, Typhimurium-CP1                | ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1          |
| Typhimurium-SseB, Vi-SseB, Vi-IpaB, Vi-CP1                         | ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1     |
| Typhimurium-SseB, Vi-SseB, Vi-IpaB, ParaOSP-CP1                    | ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1     |
| Typhimurium-SseB, Vi-SseB, ParaOSP-IpaB, Typhimurium-CP1           | ParaOSP-SseB, Enteritidis-IpaB, Vi-CP1, ParaOSP-CP1              |
| Typhimurium-SseB, Vi-SseB, ParaOSP-IpaB, Enteritidis-CP1           | ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1             |
| Typhimurium-SseB, Vi-SseB, ParaOSP-IpaB, Vi-CP1                    | ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1             |
| Typhimurium-SseB, Vi-SseB, ParaOSP-IpaB, ParaOSP-CP1               | ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB, Vi-CP1                      |
| Typhimurium-SseB, Vi-SseB, Typhimurium-CP1, Enteritidis-CP1        | ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1                 |
| Typhimurium-SseB, Vi-SseB, Typhimurium-CP1, Vi-CP1                 | ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1          |
| Typhimurium-SseB, Vi-SseB, Typhimurium-CP1, ParaOSP-CP1            | ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1, Vi-CP1                   |
| Typhimurium-SseB, Vi-SseB, Enteritidis-CP1, Vi-CP1                 | ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1              |
| Typhimurium-SseB, Vi-SseB, Enteritidis-CP1, ParaOSP-CP1            | ParaOSP-SseB, Vi-IpaB, Enteritidis-CP1, Vi-CP1                   |
| Typhimurium-SseB, Vi-SseB, Vi-CP1, ParaOSP-CP1                     | ParaOSP-SseB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1              |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB | ParaOSP-SseB, Vi-IpaB, Vi-CP1, ParaOSP-CP1                       |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB          | ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1     |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB     | ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1     |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-SseB     | ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1              |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, Typhimurium-CP1  | ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1         |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-CP1  | ParaOSP-SseB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1              |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, Vi-CP1           | ParaOSP-SseB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1         |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-CP1      | ParaOSP-SseB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1                  |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB          | ParaOSP-SseB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1           |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB     | ParaOSP-SseB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1      |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1  | ParaOSP-SseB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1               |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1  | ParaOSP-SseB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1               |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB, Vi-CP1           | ParaOSP-CP1                                                      |

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|-----------------------------------------------------------------------|----------------------------------------------------------------------|
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-CP1         | Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB            |
| Typhimurium-SseB, ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB                 | Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1         |
| Typhimurium-SseB, ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1              | Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1         |
| Typhimurium-SseB, ParaOSP-SseB, Vi-IpaB, Enteritidis-CP1              | Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB, Vi-CP1                  |
| Typhimurium-SseB, ParaOSP-SseB, Vi-IpaB, Vi-CP1                       | Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-CP1             |
| Typhimurium-SseB, ParaOSP-SseB, Vi-IpaB, ParaOSP-CP1                  | Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1    |
| Typhimurium-SseB, ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1         | Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1    |
| Typhimurium-SseB, ParaOSP-SseB, ParaOSP-IpaB, Enteritidis-CP1         | Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB, Vi-CP1             |
| Typhimurium-SseB, ParaOSP-SseB, ParaOSP-IpaB, Vi-CP1                  | Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB, ParaOSP-CP1        |
| Typhimurium-SseB, ParaOSP-SseB, ParaOSP-IpaB, ParaOSP-CP1             | Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1 |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-CP1, Enteritidis-CP1      | Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1          |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-CP1, Vi-CP1               | Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1, ParaOSP-CP1     |
| Typhimurium-SseB, ParaOSP-SseB, Typhimurium-CP1, ParaOSP-CP1          | Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1          |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-CP1, Vi-CP1               | Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1     |
| Typhimurium-SseB, ParaOSP-SseB, Enteritidis-CP1, ParaOSP-CP1          | Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1, ParaOSP-CP1              |
| Typhimurium-SseB, ParaOSP-SseB, Vi-CP1, ParaOSP-CP1                   | Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1             |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB         | Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1             |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB    | Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB, Vi-CP1                      |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1 | Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1                 |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1 | Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1          |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1          | Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1, Vi-CP1                   |
| Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-CP1     | Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1              |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB             | Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1, Vi-CP1                   |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1          | Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1              |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1          | Typhimurium-IpaB, Vi-IpaB, Vi-CP1, ParaOSP-CP1                       |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-IpaB, Vi-CP1                   | Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1     |
| Typhimurium-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-CP1              | Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1              |
| Typhimurium-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1     |                                                                      |

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| <p> Typhimurium-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Vi-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, ParaOSP-IpaB, ParaOSP-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, Typhimurium-CP1, Enteritidis-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, Typhimurium-CP1, Vi-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, Typhimurium-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-CP1, Vi-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, Enteritidis-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Typhimurium-IpaB, Vi-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB<br/> Typhimurium-SseB, Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Vi-IpaB, Vi-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Vi-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, ParaOSP-IpaB, ParaOSP-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Typhimurium-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Enteritidis-IpaB, Vi-CP1, ParaOSP-CP1<br/> Typhimurium-SseB, Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1<br/> Typhimurium-SseB, Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1<br/> Typhimurium-SseB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1 </p> | <p> Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1<br/> Typhimurium-IpaB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1<br/> Typhimurium-IpaB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1<br/> Typhimurium-IpaB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1<br/> Typhimurium-IpaB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1<br/> Typhimurium-IpaB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1<br/> <br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, Typhimurium-CP1<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-CP1<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, Vi-CP1<br/> Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-CP1<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB, Vi-CP1<br/> Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-CP1<br/> Vi-SseB, ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB<br/> Vi-SseB, ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1<br/> Vi-SseB, ParaOSP-SseB, Vi-IpaB, Enteritidis-CP1<br/> Vi-SseB, ParaOSP-SseB, Vi-IpaB, Vi-CP1<br/> Vi-SseB, ParaOSP-SseB, Vi-IpaB, ParaOSP-CP1<br/> Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1<br/> Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB, Enteritidis-CP1<br/> Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB, Vi-CP1 </p> |
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| Typhimurium-SseB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1      | Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB, ParaOSP-CP1             |
| Typhimurium-SseB, Vi-IpaB, Typhimurium-CP1, Vi-CP1               | Vi-SseB, ParaOSP-SseB, Typhimurium-CP1, Enteritidis-CP1      |
| Typhimurium-SseB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1          | Vi-SseB, ParaOSP-SseB, Typhimurium-CP1, Vi-CP1               |
| Typhimurium-SseB, Vi-IpaB, Enteritidis-CP1, Vi-CP1               | Vi-SseB, ParaOSP-SseB, Typhimurium-CP1, ParaOSP-CP1          |
| Typhimurium-SseB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1          | Vi-SseB, ParaOSP-SseB, Enteritidis-CP1, Vi-CP1               |
| Typhimurium-SseB, Vi-IpaB, Vi-CP1, ParaOSP-CP1                   | Vi-SseB, ParaOSP-SseB, Enteritidis-CP1, ParaOSP-CP1          |
| Typhimurium-SseB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1 | Vi-SseB, ParaOSP-SseB, Vi-CP1, ParaOSP-CP1                   |
| Typhimurium-SseB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1          | Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB         |
| Typhimurium-SseB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1     | Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB    |
| Typhimurium-SseB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1          | Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1 |
| Typhimurium-SseB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1     | Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1 |
| Typhimurium-SseB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1              | Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1          |
| Typhimurium-SseB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1       | Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-CP1     |
| Typhimurium-SseB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1  | Vi-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB             |
| Typhimurium-SseB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1           | Vi-SseB, Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1          |
| Typhimurium-SseB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1           | Vi-SseB, Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1          |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, Typhimurium-IpaB        | Vi-SseB, Typhimurium-IpaB, Vi-IpaB, Vi-CP1                   |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, Enteritidis-IpaB        | Vi-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-CP1              |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, Vi-IpaB                 | Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1     |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, ParaOSP-IpaB            | Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1     |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, Typhimurium-CP1         | Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Vi-CP1              |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, Enteritidis-CP1         | Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB, ParaOSP-CP1         |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, Vi-CP1                  | Vi-SseB, Typhimurium-IpaB, Typhimurium-CP1, Enteritidis-CP1  |
| Enteritidis-SseB, Vi-SseB, ParaOSP-SseB, ParaOSP-CP1             | Vi-SseB, Typhimurium-IpaB, Typhimurium-CP1, Vi-CP1           |
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, Enteritidis-IpaB    | Vi-SseB, Typhimurium-IpaB, Typhimurium-CP1, ParaOSP-CP1      |
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, Vi-IpaB             | Vi-SseB, Typhimurium-IpaB, Enteritidis-CP1, Vi-CP1           |
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, ParaOSP-IpaB        | Vi-SseB, Typhimurium-IpaB, Enteritidis-CP1, ParaOSP-CP1      |
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, Typhimurium-CP1     | Vi-SseB, Typhimurium-IpaB, Vi-CP1, ParaOSP-CP1               |
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, Enteritidis-CP1     | Vi-SseB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-IpaB             |
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, Vi-CP1              |                                                              |

|                                                                    |                                                             |
|--------------------------------------------------------------------|-------------------------------------------------------------|
| Enteritidis-SseB, Vi-SseB, Typhimurium-IpaB, ParaOSP-CP1           | Vi-SseB, Enteritidis-IpaB, Vi-IpaB, Typhimurium-CP1         |
| Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB, Vi-IpaB               | Vi-SseB, Enteritidis-IpaB, Vi-IpaB, Enteritidis-CP1         |
| Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB          | Vi-SseB, Enteritidis-IpaB, Vi-IpaB, Vi-CP1                  |
| Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB, Typhimurium-CP1       | Vi-SseB, Enteritidis-IpaB, Vi-IpaB, ParaOSP-CP1             |
| Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB, Enteritidis-CP1       | Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Typhimurium-CP1    |
| Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB, Vi-CP1                | Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Enteritidis-CP1    |
| Enteritidis-SseB, Vi-SseB, Enteritidis-IpaB, ParaOSP-CP1           | Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB, Vi-CP1             |
| Enteritidis-SseB, Vi-SseB, Vi-IpaB, ParaOSP-IpaB                   | Vi-SseB, Enteritidis-IpaB, ParaOSP-IpaB, ParaOSP-CP1        |
| Enteritidis-SseB, Vi-SseB, Vi-IpaB, Typhimurium-CP1                | Vi-SseB, Enteritidis-IpaB, Typhimurium-CP1, Enteritidis-CP1 |
| Enteritidis-SseB, Vi-SseB, Vi-IpaB, Enteritidis-CP1                | Vi-SseB, Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1          |
| Enteritidis-SseB, Vi-SseB, Vi-IpaB, Vi-CP1                         | Vi-SseB, Enteritidis-IpaB, Typhimurium-CP1, ParaOSP-CP1     |
| Enteritidis-SseB, Vi-SseB, Vi-IpaB, ParaOSP-CP1                    | Vi-SseB, Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1          |
| Enteritidis-SseB, Vi-SseB, ParaOSP-IpaB, Typhimurium-CP1           | Vi-SseB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1     |
| Enteritidis-SseB, Vi-SseB, ParaOSP-IpaB, Enteritidis-CP1           | Vi-SseB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1     |
| Enteritidis-SseB, Vi-SseB, ParaOSP-IpaB, Vi-CP1                    | Vi-SseB, Enteritidis-IpaB, Vi-CP1, ParaOSP-CP1              |
| Enteritidis-SseB, Vi-SseB, ParaOSP-IpaB, ParaOSP-CP1               | Vi-SseB, Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1             |
| Enteritidis-SseB, Vi-SseB, Typhimurium-CP1, Enteritidis-CP1        | Vi-SseB, Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1             |
| Enteritidis-SseB, Vi-SseB, Typhimurium-CP1, Vi-CP1                 | Vi-SseB, Vi-IpaB, ParaOSP-IpaB, Vi-CP1                      |
| Enteritidis-SseB, Vi-SseB, Typhimurium-CP1, ParaOSP-CP1            | Vi-SseB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1                 |
| Enteritidis-SseB, Vi-SseB, Enteritidis-CP1, Vi-CP1                 | Vi-SseB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1          |
| Enteritidis-SseB, Vi-SseB, Enteritidis-CP1, ParaOSP-CP1            | Vi-SseB, Vi-IpaB, Typhimurium-CP1, Vi-CP1                   |
| Enteritidis-SseB, Vi-SseB, Vi-CP1, ParaOSP-CP1                     | Vi-SseB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1              |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-IpaB | Vi-SseB, Vi-IpaB, Enteritidis-CP1, Vi-CP1                   |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, Vi-IpaB          | Vi-SseB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1              |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-IpaB     | Vi-SseB, Vi-IpaB, Vi-CP1, ParaOSP-CP1                       |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, Typhimurium-CP1  | Vi-SseB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1     |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, Enteritidis-CP1  | Vi-SseB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1              |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, Vi-CP1           | Vi-SseB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1         |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-IpaB, ParaOSP-CP1      | Vi-SseB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1              |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB, Vi-IpaB          | Vi-SseB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1         |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-IpaB     | Vi-SseB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1      |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB, Typhimurium-CP1  | Vi-SseB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1               |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB, Enteritidis-CP1  | Vi-SseB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1               |

|                                                                       |                                                                       |
|-----------------------------------------------------------------------|-----------------------------------------------------------------------|
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB, Vi-CP1              | Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1      |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-IpaB, ParaOSP-CP1         | Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1               |
| Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB, ParaOSP-IpaB                 | Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1          |
| Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB, Typhimurium-CP1              | Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1               |
| Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB, Enteritidis-CP1              | Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1          |
| Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB, Vi-CP1                       | Enteritidis-SseB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1                   |
| Enteritidis-SseB, ParaOSP-SseB, Vi-IpaB, ParaOSP-CP1                  | Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1            |
| Enteritidis-SseB, ParaOSP-SseB, ParaOSP-IpaB, Typhimurium-CP1         | Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1       |
| Enteritidis-SseB, ParaOSP-SseB, ParaOSP-IpaB, Enteritidis-CP1         | Enteritidis-SseB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1                |
| Enteritidis-SseB, ParaOSP-SseB, ParaOSP-IpaB, Vi-CP1                  | Enteritidis-SseB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1                |
| Enteritidis-SseB, ParaOSP-SseB, ParaOSP-IpaB, ParaOSP-CP1             | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1 |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-CP1, Enteritidis-CP1      | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1 |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-CP1, Vi-CP1               | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1          |
| Enteritidis-SseB, ParaOSP-SseB, Typhimurium-CP1, ParaOSP-CP1          | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-CP1     |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-CP1, Vi-CP1               | Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB             |
| Enteritidis-SseB, ParaOSP-SseB, Enteritidis-CP1, ParaOSP-CP1          | Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1          |
| Enteritidis-SseB, ParaOSP-SseB, Vi-CP1, ParaOSP-CP1                   | Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1          |
| Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-IpaB         | Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, Vi-CP1                   |
| Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-IpaB    | Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-CP1              |
| Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Typhimurium-CP1 | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Typhimurium-CP1     |
| Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Enteritidis-CP1 | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Enteritidis-CP1     |
| Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, Vi-CP1          | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-IpaB, Vi-CP1              |
| Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-IpaB, ParaOSP-CP1     | Enteritidis-SseB, Typhimurium-IpaB, ParaOSP-IpaB, ParaOSP-CP1         |
| Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-IpaB             | Enteritidis-SseB, Typhimurium-IpaB, Typhimurium-CP1, Enteritidis-CP1  |
| Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, Typhimurium-CP1          | Enteritidis-SseB, Typhimurium-IpaB, Typhimurium-CP1, Vi-CP1           |
| Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, Enteritidis-CP1          | Enteritidis-SseB, Typhimurium-IpaB, Typhimurium-CP1, ParaOSP-CP1      |
| Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, Vi-CP1                   | Enteritidis-SseB, Typhimurium-IpaB, Enteritidis-CP1, Vi-CP1           |
| Enteritidis-SseB, Typhimurium-IpaB, Vi-IpaB, ParaOSP-CP1              |                                                                       |

[illegible]

|                                                                  |                                                                 |
|------------------------------------------------------------------|-----------------------------------------------------------------|
| Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1             | Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1         |
| Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1      | Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1    |
| Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1, Vi-CP1               | Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1         |
| Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1          | Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1    |
| Enteritidis-SseB, Vi-IpaB, Enteritidis-CP1, Vi-CP1               | Enteritidis-SseB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1             |
| Enteritidis-SseB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1          | Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1      |
| Enteritidis-SseB, Vi-IpaB, Vi-CP1, ParaOSP-CP1                   | Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1 |
| Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, Enteritidis-CP1 | Enteritidis-SseB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1          |
| Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, Vi-CP1          | Enteritidis-SseB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1          |
| Enteritidis-SseB, ParaOSP-IpaB, Typhimurium-CP1, ParaOSP-CP1     |                                                                 |
| Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1, Vi-CP1          |                                                                 |
| Enteritidis-SseB, ParaOSP-IpaB, Enteritidis-CP1, ParaOSP-CP1     |                                                                 |
| Enteritidis-SseB, ParaOSP-IpaB, Vi-CP1, ParaOSP-CP1              |                                                                 |
| Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1, Vi-CP1       |                                                                 |
| Enteritidis-SseB, Typhimurium-CP1, Enteritidis-CP1, ParaOSP-CP1  |                                                                 |
| Enteritidis-SseB, Typhimurium-CP1, Vi-CP1, ParaOSP-CP1           |                                                                 |
| Enteritidis-SseB, Enteritidis-CP1, Vi-CP1, ParaOSP-CP1           |                                                                 |
| Enteritidis-SseB, Enteritidis-IpaB, Typhimurium-CP1, Vi-CP1      |                                                                 |
| Enteritidis-SseB, Enteritidis-IpaB, Typhimurium-CP1, ParaOSP-CP1 |                                                                 |
| Enteritidis-SseB, Enteritidis-IpaB, Enteritidis-CP1, Vi-CP1      |                                                                 |
| Enteritidis-SseB, Enteritidis-IpaB, Enteritidis-CP1, ParaOSP-CP1 |                                                                 |
| Enteritidis-SseB, Enteritidis-IpaB, Vi-CP1, ParaOSP-CP1          |                                                                 |
| Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB, Typhimurium-CP1         |                                                                 |
| Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB, Enteritidis-CP1         |                                                                 |
| Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB, Vi-CP1                  |                                                                 |
| Enteritidis-SseB, Vi-IpaB, ParaOSP-IpaB, ParaOSP-CP1             |                                                                 |
| Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1, Enteritidis-CP1      |                                                                 |
| Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1, Vi-CP1               |                                                                 |
| Enteritidis-SseB, Vi-IpaB, Typhimurium-CP1, ParaOSP-CP1          |                                                                 |
| Enteritidis-SseB, Vi-IpaB, Enteritidis-CP1, Vi-CP1               |                                                                 |

|                                                         |  |
|---------------------------------------------------------|--|
| Enteritidis-SseB, Vi-IpaB, Enteritidis-CP1, ParaOSP-CP1 |  |
| Enteritidis-SseB, Vi-IpaB, Vi-CP1, ParaOSP-CP1          |  |

**[00228]** 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 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.25 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 1 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 1.5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 2 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 2.5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 3 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 3.5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 4 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 4.5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 5.5 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 6 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 7 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 8 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 9 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 10 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 11 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 12 pg. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is more than 12 pg, *e.g.*, 13 pg, 14 pg, 15 pg, 16 pg, 17 pg, 18 pg, 19 pg, 20 pg, 21 pg, 22 pg, 23 pg, 24 pg, 25 pg, or more.

**[00229]** 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 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 pg to about 6 pg. 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 pg to about 12 pg. 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 pg to about 20 pg. 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 pg to about 40 pg.

[00230] 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) 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) 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.

[00231] In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 0.20 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 0.40 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 1 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic complex is about 2 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 3 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 4 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 5 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 6 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 7 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 8 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 9 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 10 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 11 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 12 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 14 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 16 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 18 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 20 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 21 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 22 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 23 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 24 pg. In some embodiments, the combined weight of polysaccharides and

polypeptides in the vaccine contributed by each immunogenic composition is about 25 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 30 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 40 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 50 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 60 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 70 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 80 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 90 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 100 pg. In some embodiments, the combined weight of polysaccharides and polypeptides in the vaccine contributed by each immunogenic composition is about 110 pg.

[00232] 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 pg to about 110 pg.

#### ***Conjugated Immunogenic Complexes; Immunogenic and Vaccine Compositions Comprising Same***

[00233] 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 or O-specific polysaccharide of *S. enterica*. In some embodiments, one or more polypeptides of conjugated immunogenic complex comprise an antigenic polypeptide of *S. enterica*, *S. pneumoniae*, or *Shigella flexneri*. 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 at least one of: Rhavi-SseB, Rhavi-IpaB, or the CPI fusion protein.

#### ***Uses of Immunogenic and Vaccine Compositions***

[00234] 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.

**[00235]** 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.

**[00236]** In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces an immune response against *S. enterica*. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces an immune response against one or more serotypes of *S. enterica*. 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 *S. enterica*, 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 *S. enterica*, 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 *S. enterica*, 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. enterica*. 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 *S. enterica*, 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 *S. enterica*, 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 *S. enterica*, 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).

**[00237]** 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<sub>h</sub>1, T<sub>h</sub>2, or T<sub>h</sub>17 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.

**[00238]** In some embodiments, the immune response is to the polysaccharide. In some embodiments, the immune response is to the antigenic polypeptide (also referred to as a carrier protein), e.g., to any one or more of antigenic polypeptides SseB, IpaB, SP1500 or SP785 in the immunogenic composition. In some embodiments, there is an immune response is to the polysaccharide and to the antigenic polypeptide (also referred to as a carrier protein), e.g., to any one or more of antigenic polypeptides SseB, IpaB, SP1500 or SP785 in the immunogenic composition.

**[00239]** 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.

**[00240]** 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.

**[00241]** The *S. enterica* immunogenic compositions and vaccines described herein may be used for prophylactic and/or therapeutic treatment of *S. enterica*. Accordingly, this application provides a method for immunizing a subject suffering from or susceptible to *S. enterica* infection, comprising administering an immunologically effective amount of any of 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. enterica*.

**[00242]** 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. enterica*, 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 *S. enterica*, 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 *S. enterica*, 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 *S. enterica*, 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. enterica* in a noncolonized or uninfected subject. In another aspect, the method may reduce the duration of colonization in a subject who is already colonized.

**[00243]** In therapeutic embodiments, the vaccine may be administered to a subject suffering from *S. enterica* infection, in an amount sufficient to treat the subject. Treating the subject, in this case, refers to reducing *S. enterica* 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 embodiments, the vaccine reduces transmissibility of *S. enterica* from the vaccinated subject. In certain embodiments, the reductions described above are at least 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.

**[00244]** In therapeutic embodiments, the vaccine is administered to a subject postinfection. 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.

**[00245]** 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. enterica*, do not become colonized by *S. enterica*, 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<sub>h</sub>1 or T<sub>h</sub>17 cells.

**[00246]** In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against *S. enterica*. 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. enterica*. In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, induces an immune response against all serotypes of *S. enterica* 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. enterica*. In some embodiments, an immunogenic complex described herein, upon administration to a subject, induces a protective immune response against all serotypes of *S. enterica* comprised in such immunogenic composition or vaccine.

**[00247]** 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<sup>+</sup> T cell response, including T<sub>h</sub>1, T<sub>h</sub>2, or T<sub>h</sub>17 response, or a CD8<sup>+</sup> T cell response, or a CD4<sup>+</sup> and CD8<sup>+</sup> T cell response, or CD4<sup>+</sup>/CD8<sup>-</sup> 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.

**[00248]** 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.

**[00249]** 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.

[00250] In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein treats or prevents infection by *S. enterica*. 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. enterica*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein reduces the severity of infection by *S. enterica*. In some embodiments, upon administration to a subject, an immunogenic composition or vaccine described herein inhibits transmission of *S. enterica* from the subject to another subject.

[00251] In some embodiments, an immunogenic composition or vaccine described herein, upon administration to a subject, elicits immunogenicity against one or more of *Salmonella enterica* serotypes Typhimurium, Enteritidis, Typhi, and Paratyphi.

#### ***Antibody Compositions***

[00252] 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-idiotypic 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***

[00253] 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.

[00254] 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 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.

[00255] 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.

**[00256]** 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).

**[00257]** 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.

**[00258]** 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.

**[00259]** 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.

[00260] In some embodiments, a vaccine is a liquid. In some embodiments the liquid is a reconstituted lyophilate. 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.

[00261] 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).

[00262] 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.

[00263] 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.

[00264] Depot injectable formulations may also be prepared by entrapping the product in liposomes or microemulsions, which are compatible with body tissues.

[00265] 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).

[00266] 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 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.

**[00267]** *Adjuvants*

**[00268]** 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.

**[00269]** 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. Nonlimiting 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).

**[00270]** Adjuvants may also be classified by the response they induce. In some embodiments, the adjuvant induces the generation, proliferation, or activation of T<sub>H</sub>1 cells or T<sub>H</sub>2 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 cells. These categories are not mutually exclusive; in some cases, an adjuvant activates more than one type of cell.

**[00271]** In certain embodiments, the adjuvant induces the generation, proliferation, or activation of T<sub>H</sub>17 cells. The adjuvant may promote the CD4<sup>+</sup> or CD8<sup>+</sup> T cells to secrete IL-17. In some embodiments, an adjuvant that induces the generation, proliferation, or activation of T<sub>H</sub>17 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<sub>H</sub>17 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<sub>H</sub>17 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 derivatives 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 AE)P-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).

[00272] 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.

[00273] 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).

[00274] 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.

[00275] 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.

[00276] 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 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).

[00277] 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.

[00278] 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.

[00279] 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, levofloxacin, gatifloxacin.

[00280] 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.

[00281] *Additional Components and Excipients*

[00282] In addition to the antigens and the adjuvants described above, a vaccine formulation or immunogenic composition may include one or more additional components. In some embodiments, the vaccine formulation comprises aluminum phosphate (referred to herein as alum phosphate, or AP). In some embodiments, a vaccine formulation comprising *S. Paratyphi*-MAPS aluminum phosphate (referred to herein as alum phosphate, or AP). In some embodiments, the amount of alum phosphate is determined

by one of ordinary skill in the art. In some embodiments, the amount of alum phosphate is 250µg per 500µl injection (25µg polysaccharide). In some embodiments, a vaccine formulation or immunogenic composition comprises 250µg of alum phosphate per 500µl injection. In some embodiments, the alum phosphate is in a buffer comprising 20mM Histadine, pH 6, 150 mM NaCl, 0.02% tween 80.

[00283] 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.

[00284] 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.

[00285] 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 (TYTOXAPOT). A surfactant can be ionic or non-ionic.

[00286] 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.

[00287] 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***

[00288] 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 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.

**[00289]** 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.

**[00290]** 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.

**[00291]** 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).

**[00292]** 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 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.

**[00293]** 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.

**[00294]** 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.

**[00295]** *Combination Prophylaxis or Combination Therapy*

**[00296]** In some embodiments, an immunogenic complex, immunogenic composition, vaccine, or pharmaceutical composition disclosed herein may be administered in combination with another agent.

**[00297]** *Dosing*

**[00298]** 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. Such additional immunization doses can be referred to as boosters. In some embodiments, a booster (or second or subsequent) immunization dose is administered 2 weeks, or 3 weeks, or about 1 month, or about 2 months, or about 6 months or about 1 year after the preceding dose (where the preceding dose can be initial dose or a second or third dose, or booster dose).

[00299] 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.

[00300] 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.

[00301] 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.

[00302] 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.

[00303] 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 allow one to develop alterations to the sample immunization schedules without undue experimentation.

[00304] 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.

**[00305] *Assays for Determining Immune Response***

[00306] 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.

**[00307]** 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).

**[00308]** 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).

**[00309]** 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.

**[00310]** 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). 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.

**[00311]** 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 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.

**[00312]** 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.

**[00313]** 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, 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.

**[00314]** 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 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.

**[00315]** 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.

[00316] 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 assessed in enzyme linked immunospot (ELISpot) assays directed against specific pathogen polysaccharides or polypeptides.

[00317] 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.

[00318] The RIA method detects specific antibodies through incubation of sera with radio- labeled polysaccharides or polypeptides in suspension (e.g., Schiffman et al, 1980). The antigenantibody complexes are then precipitated with ammonium sulfate and the radiolabeled pellets assayed for counts per minute (cpm).

[00319] In the ELISA detection method, specific antibodies from the sera of vaccinated subjects are quantitated by incubation with polysaccharides or polypeptides (either serotypespecific 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).

**[00320]** 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 >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).

**[00321]** 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).

**[00322]** 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-CSF; IL-1 $\alpha$ ; IL-1  $\beta$ ; 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- $\alpha$ ; IFN- $\beta$ ; IFN- $\gamma$ ; MIP-1 $\alpha$ ; MIP-1 $\beta$ ; TGF- $\beta$ ; TNF $\alpha$ , or TNF- $\beta$ . In a non-limiting example, efficacy of vaccination may be determined by assaying IL-17 levels (particularly IL-17 A) by stimulating T cells from a subject after vaccination. The IL-17 levels may be compared to IL-17 levels in 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.

**[00323]** 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<sub>H</sub>17 cell activation, where increased T<sub>H</sub>17 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 nonlimiting example, one may measure T<sub>H</sub>1 cell activation,

where increased T<sub>H</sub>1 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.

[00324] Alternatively, one may follow epidemiological markers such as incidence of, severity of, or duration of pneumococcal infection in vaccinated individuals compared to unvaccinated individuals.

[00325] 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 *Salmonella*. 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 animals. One can then measure the reduction in colonization or the reduction in lethality in vaccinated animals.

[00326] 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**

[00327] 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.

[00328] 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 (1-

cyano-4- dimethylaminopyridinium tetrafluoroborate), and EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride).

[00329] 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 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.

[00330] 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 (1-cyano-4- dimethylaminopyridinium tetrafluoroborate); EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride); sodium cyanoborohydride; cyanogen bromide; and ammonium bicarbonate/iodoacetic acid, for linking the co-factor to the polymer.

[00331] 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.

#### **Certain Definitions**

[00332] In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”; (iii) the terms “comprising” and “including” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) the terms “about” and “approximately” may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (v) where ranges are provided, endpoints are included.

[00333] **About:** The term “about”, when used herein in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by “about” in that context. For example, in some embodiments, the term “about” may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[00334] **Administration:** As used herein, the term “administration” typically refers to the administration of a composition to a subject or system to achieve delivery of an agent that is, or is included in, the composition. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be ocular, oral, parenteral, topical, etc. In some particular embodiments, administration may be bronchial (*e.g.*, by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis, intradermal, interdermal, transdermal, etc.), enteral, intra-arterial, intradermal, intragastrical, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (*e.g.*, intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (*e.g.*, by

intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, administration may involve dosing that is intermittent (*e.g.*, a plurality of doses separated in time) and/or periodic (*e.g.*, individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (*e.g.*, perfusion) for at least a selected period of time.

**[00335] Agent:** In general, the term “agent”, as used herein, may be used to refer to a compound or entity of any chemical class including, for example, a polypeptide, nucleic acid, saccharide, lipid, small molecule, metal, or combination or complex thereof. In appropriate circumstances, as will be clear from context to those skilled in the art, the term may be utilized to refer to an entity that is or comprises a cell or organism, or a fraction, extract, or component thereof. Alternatively or additionally, as context will make clear, the term may be used to refer to a natural product in that it is found in and/or is obtained from nature. In some instances, again as will be clear from context, the term may be used to refer to one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. In some embodiments, an agent may be utilized in isolated or pure form; in some embodiments, an agent may be utilized in crude form. In some embodiments, potential agents may be provided as collections or libraries, for example that may be screened to identify or characterize active agents within them. In some cases, the term “agent” may refer to a compound or entity that is or comprises a polymer; in some cases, the term may refer to a compound or entity that comprises one or more polymeric moieties. In some embodiments, the term “agent” may refer to a compound or entity that is not a polymer and/or is substantially free of any polymer and/or of one or more particular polymeric moieties. In some embodiments, the term may refer to a compound or entity that lacks or is substantially free of any polymeric moiety.

**[00336] Amino acid:** In its broadest sense, the term “amino acid”, as used herein, refers to any compound and/or substance that can be incorporated into a polypeptide chain, *e.g.*, through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure  $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$ . In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Non-standard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (*e.g.*, of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some embodiments, such

modification may, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some embodiments, the term “amino acid” may be used to refer to a free amino acid; in some embodiments it may be used to refer to an amino acid residue of a polypeptide.

**[00337] Antibody:** As used herein, the term “antibody” refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150 kDa tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kDa each) and two identical light chain polypeptides (about 25 kDa each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)– an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y’s stem). A short region, known as the “switch”, connects the heavy chain variable and constant regions. The “hinge” connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another “switch”. Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an “immunoglobulin fold” formed from two beta sheets (*e.g.*, 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as “complement determining regions” (CDR1, CDR2, and CDR3) and four somewhat invariant “framework” regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present invention include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. For purposes of the present

invention, in some embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an “antibody”, whether such polypeptide is naturally produced (*e.g.*, generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc., as is known in the art. Moreover, the term “antibody” as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, in some embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi- specific antibodies (*e.g.*, Zybodies<sup>®</sup>, etc.); antibody fragments such as Fab fragments, Fab’ fragments, F(ab’)2 fragments, Fd’ fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (*e.g.*, shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (*e.g.*, Probodies<sup>®</sup>); Small Modular ImmunoPharmaceuticals (“SMIPs<sup>TM</sup>”); single chain or Tandem diabodies (TandAb<sup>®</sup>); VHHs; Anticalins<sup>®</sup>; Nanobodies<sup>®</sup> minibodies; BiTE<sup>®</sup>s; ankyrin repeat proteins or DARPINS<sup>®</sup>; Avimers<sup>®</sup>; DARTs; TCR-like antibodies; Adnectins<sup>®</sup>; Affilins<sup>®</sup>; Trans-bodies<sup>®</sup>; Affibodies<sup>®</sup>; TrimerX<sup>®</sup>; MicroProteins; Fynomers<sup>®</sup>; Centyrins<sup>®</sup>; and KALBITOR<sup>®</sup>s. In some embodiments, an antibody may lack a covalent modification (*e.g.*, attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (*e.g.*, attachment of a glycan, a payload [*e.g.*, a detectable moiety, a therapeutic moiety, a catalytic moiety, etc.], or other pendant group [*e.g.*, poly-ethylene glycol, etc.]).

**[00338] Antigen:** The term “antigen”, as used herein, refers to (i) an agent that induces an immune response; and/or (ii) an agent that binds to a T cell receptor (*e.g.*, when presented by an MHC molecule) or to an antibody. In some embodiments, an antigen induces a humoral response (*e.g.*, including production of antigen-specific antibodies); in some embodiments, an antigen induces a cellular response (*e.g.*, involving T cells whose receptors specifically interact with the antigen). In some embodiments, an antigen induces a humoral response and a cellular response. In some embodiments, an antigen binds to an antibody and may or may not induce a particular physiological response in an organism. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, a polymer (in some embodiments other than a biologic polymer (*e.g.*, other than a nucleic acid or amino acid polymer)), etc. In some embodiments, an antigen is or comprises a polypeptide. In some embodiments, an antigen is or comprises a polysaccharide. Those of ordinary skill in the art will appreciate that, in general, an antigen may be provided in isolated or pure form, or alternatively may be provided in crude form (*e.g.*, together with other materials, for example in

an extract such as a cellular extract or other relatively crude preparation of an antigen-containing source). In some embodiments, antigens utilized in accordance with the present invention are provided in a crude form. In some embodiments, an antigen is a recombinant antigen. In some embodiments, an antigen is a polypeptide or a polysaccharide that, upon administration to a subject, induces a specific and/or clinically relevant immune response to such polypeptide or polysaccharide. In some embodiments, an antigen is selected to induce a specific and/or clinically relevant immune response to such polypeptide or polysaccharide.

**[00339] Associated with:** Two entities are “associated” with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. In some embodiments, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another. In some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of affinity interactions, electrostatic interactions, hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

**[00340] Binding:** It will be understood that the term “binding”, as used herein, typically refers to a non-covalent association between or among two or more entities. “Direct” binding involves physical contact between entities or moieties; indirect binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can typically be assessed in any of a variety of contexts – including where interacting entities or moieties are studied in isolation or in the context of more complex systems (*e.g.*, while covalently or otherwise associated with a carrier entity and/or in a biological system or cell).

**[00341] Carrier protein:** As used herein, the term “carrier protein” refers to a protein or peptide that is coupled, complexed, or otherwise associated with a hapten (*e.g.*, a small peptide or lipid) or less immunogenic antigen (*e.g.*, a polysaccharide) and that induces or improves an immune response to such a coupled, or complexed, or otherwise associated hapten (*e.g.*, a small peptide or lipid) or less immunogenic antigen (*e.g.*, a polysaccharide). In some embodiments, such an immune response is or comprises a response to a hapten or less immunogenic antigen that is coupled, complexed, or otherwise associated with such a carrier protein. In some embodiments, such an immune response is or comprises a response to both a carrier protein and a hapten or less immunogenic antigen that is coupled, complexed, or otherwise associated with such a carrier protein. In some embodiments, no significant immune response to a carrier protein itself occurs. In some embodiments, immune response to a carrier protein may be detected; in some such embodiments, immune response to such a carrier protein is strong. In some embodiments, a carrier protein is coupled, complexed, or otherwise associated with one or more other molecules.

**[00342] Colonization:** As used herein, the term “colonization” generally refers to the ability of a microbe to grow at a target site or surface. For example, the term “colonization” refers to the ability of a microbe (*e.g.*, a bacterium) to grow at an anatomical site (*e.g.*, a mucosal membrane, gastrointestinal tract, injury site, organ, *etc.*) of a host.

**[00343] Combination therapy:** As used herein, the term “combination therapy” refers to those situations in which a subject is exposed to two or more therapeutic regimens (*e.g.*, two or more therapeutic agents). In some embodiments, the two or more regimens may be administered simultaneously; in some embodiments, such regimens may be administered sequentially (*e.g.*, all “doses” of a first regimen are administered prior to administration of any doses of a second regimen); in some embodiments, such agents are administered in overlapping dosing regimens. In some embodiments, “administration” of combination therapy may involve administration of one or more agent(s) or modality(ies) to a subject receiving the other agent(s) or modality(ies) in the combination. For clarity, combination therapy does not require that individual agents be administered together in a single composition (or even necessarily at the same time), although in some embodiments, two or more agents, or active moieties thereof, may be administered together in a combination composition, or even in a combination compound (*e.g.*, as part of a single chemical complex or covalent entity).

**[00344] Derivative:** As used herein, the term “derivative”, or grammatical equivalents thereof, refers to a structural analogue of a reference substance. That is, a “derivative” is a substance that shows significant structural similarity with the reference substance, for example sharing a core or consensus structure, but also differs in certain discrete ways. Such a substance would be said to be “derived from” said reference substance. In some embodiments, a derivative is a substance that can be generated from the reference substance by chemical manipulation. In some embodiments, a derivative is a substance that can be generated through performance of a synthetic process substantially similar to (*e.g.*, sharing a plurality of steps with) one that generates the reference substance.

**[00345] Domain:** The term “domain” as used herein refers to a section or portion of an entity. In some embodiments, a “domain” is associated with a particular structural and/or functional feature of the entity so that, when the domain is physically separated from the rest of its parent entity, it substantially or entirely retains the particular structural and/or functional feature. Alternatively or additionally, a domain may be or include a portion of an entity that, when separated from that (parent) entity and linked with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features that characterized it in the parent entity. In some embodiments, a domain is a section or portion of a molecule (*e.g.*, a small molecule, carbohydrate, lipid, nucleic acid, or polypeptide). In some embodiments, a domain is a section of a polypeptide; in some such embodiments, a domain is characterized by a particular structural element (*e.g.*, a particular amino acid sequence or sequence motif,  $\alpha$ -helix character,  $\beta$ -sheet character, coiled-coil character, random coil character, *etc.*), and/or by a particular functional feature (*e.g.*, binding activity, enzymatic activity, folding activity, signaling activity, *etc.*).

**[00346] Dosage form or unit dosage form:** Those skilled in the art will appreciate that the term “dosage form” may be used to refer to a physically discrete unit of an active agent (*e.g.*, a therapeutic or diagnostic agent) for administration to a subject. Typically, each such unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (*i.e.*, with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or agent administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

**[00347] Dosing regimen:** Those skilled in the art will appreciate that the term “dosing regimen” may be used to refer to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, individual doses are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (*i.e.*, is a therapeutic dosing regimen).

**[00348] Fragment:** A “fragment” of a material or entity as described herein has a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment includes a discrete portion of the whole which discrete portion shares one or more functional characteristics found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a fragment of a polymer, *e.g.*, a polypeptide or polysaccharide, comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (*e.g.*, residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the monomeric units (*e.g.*, residues) found in the whole

polymer. The whole material or entity may in some embodiments be referred to as the “parent” of the whole.

**[00349] Homology:** As used herein, the term “homology” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% similar (*e.g.*, containing residues with related chemical properties at corresponding positions). For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as similar to one another as “hydrophobic” or “hydrophilic” amino acids, and/or as having “polar” or “non-polar” side chains. Substitution of one amino acid for another of the same type may often be considered a “homologous” substitution.

**[00350] Identity:** As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “substantially identical” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In some embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (*e.g.*, nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller, 1989, which has been incorporated into the ALIGN program (version 2.0). In some exemplary embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

**[00351] Improve, increase, inhibit or reduce:** As used herein, the terms “improve”, “increase”, “inhibit”, “reduce”, or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may be or comprise a measurement in a particular system (*e.g.*, in a single subject) under otherwise comparable conditions absent presence of (*e.g.*, prior to and/or after) a particular agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement may be or comprise a measurement in comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment.

**[00352] Immunologically effective amount or immunologically effective dose:** As used herein, “immunologically effective amount” or “immunologically effective dose” refers to an amount of an antigenic or immunogenic substance, *e.g.*, an antigen, immunogen, immunogenic complex, immunogenic composition, vaccine, or pharmaceutical composition, which when administered to a subject, either in a single dose or as part of a series of doses, that is sufficient to enhance a subject’s own immune response against a subsequent exposure to a pathogen. In some embodiments, the pathogen is *Salmonella enterica*. In some embodiments, the immune response is against one or more different serotypes of *S. enterica*. In some embodiments, the immune response is against two or more different serotypes of *S. enterica*. In some embodiments, the immune response is against four or more different serotypes of *S. enterica*. An immunologically effective amount may vary based on the subject to be treated, the species of the subject, the degree of immune response desired to induce, etc. In some embodiments, an immunologically effective amount is sufficient for treatment or protection of a subject having or at risk of having disease. In some embodiments, an immunologically effective amount refers to a non-toxic but sufficient amount that can be an amount to treat, attenuate, or prevent infection and/or disease (*e.g.*, bacterial infection, pneumococcal infection, bacterial colonization, pneumococcal colonization, complications associated with bacterial infection, complications associated with pneumococcal infection, etc.) in any subject. In some embodiments, an immunologically effective amount is sufficient to induce an immunoprotective response upon administration to a subject.

**[00353] Immunoprotective response or protective response:** As used herein, “immunoprotective response” or “protective response” refers to an immune response that mediates antigen or immunogen-induced immunological memory. In some embodiments, an immunoprotective response is induced by the administration of a substance, *e.g.*, an antigen, immunogen, immunogenic complex, immunogenic composition, vaccine, or pharmaceutical composition to a subject. In some embodiments, immunoprotection involves one or more of active immune surveillance, a more rapid and effective response upon immune activation as compared to a response observed in a naïve subject, efficient clearance of the activating agent or pathogen, followed by rapid resolution of inflammation. In some embodiments, an immunoprotective response is an adaptive immune response. In some embodiments, an

immunoprotective response is sufficient to protect an immunized subject from productive infection by a particular pathogen or pathogens to which a vaccine is directed (*e.g.*, *S. enterica* infection).

**[00354] Immunization:** As used herein, “immunization”, or grammatical equivalents thereof, refers to a process of inducing an immune response to an infectious organism or agent in a subject (“active immunization”), or alternatively, providing immune system components against an infectious organism or agent to a subject (“passive immunization”). In some embodiments, immunization involves the administration of one or more antigens, immunogens, immunogenic complexes, vaccines, immune molecules such as antibodies, immune sera, immune cells such as T cells or B cells, or pharmaceutical compositions to a subject. In some embodiments, immunization is performed by administering an immunologically effective amount of a substance, *e.g.*, an antigen, immunogen, immunogenic complex, immunogenic composition, vaccine, immune molecule such as an antibody, immune serum, immune cell such as a T cell or B cell, or pharmaceutical composition to a subject. In some embodiments, immunization results in an immunoprotective response in the subject. In some embodiments, active immunization is performed by administering to a subject an antigenic or immunogenic substance, *e.g.*, an antigen, immunogen, immunogenic complex, vaccine, or pharmaceutical composition. In some embodiments, passive immunization is performed by administering to a subject an immune system component, *e.g.*, an immune molecule such as an antibody, immune serum, or immune cell such as a T cell or B cell.

**[00355] Isolated:** As used herein, the term “isolated”, or grammatical equivalents thereof, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered “isolated” or even “pure”, after having been combined with certain other components such as, for example, one or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polysaccharide that occurs in nature is considered to be “isolated” when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with

components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide or polysaccharide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an "isolated" polypeptide or polysaccharide. Alternatively or additionally, in some embodiments, a polypeptide or polysaccharide that has been subjected to one or more purification techniques may be considered to be an "isolated" polypeptide or polysaccharide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

**[00356] Linker:** As used herein, the term "linker" is used to refer to an entity that connects two or more elements to form a multi-element agent. For example, those of ordinary skill in the art appreciate that a polypeptide whose structure includes two or more functional or organizational domains often includes a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide comprising a linker element has an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker (L). In some embodiments, a polypeptide linker is at least 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, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide. A variety of different linker elements that can appropriately be used when engineering polypeptides (*e.g.*, fusion polypeptides) are known in the art (Holliger et al, 1993; Poljak, 1994).

**[00357] Pharmaceutical composition:** As used herein, the term "pharmaceutical composition" refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, a pharmaceutical composition may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

**[00358] Pharmaceutically acceptable:** As used herein, the term "pharmaceutically acceptable" applied to the carrier, diluent, or excipient used to formulate a composition as disclosed herein means that the

carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

**[00359] Plurality:** As used herein, the term “plurality” includes at least 2 or more, including, e.g., at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or more.

**[00360] Polysaccharide:** The term “polysaccharide” as used herein refers to a polymeric carbohydrate molecule composed of long chains of monosaccharide units bound together by glycosidic, phosphodiester, or other linkages, and on hydrolysis give the constituent monosaccharides or oligosaccharides. Polysaccharides range in structure from linear to highly branched. Examples include storage polysaccharides such as starch and glycogen, structural polysaccharides such as cellulose and chitin and microbial polysaccharides, and antigenic polysaccharides found in microorganisms including, but not limited to, capsular polysaccharides (CPS), O polysaccharides (OPS), core O polysaccharides (COPS), and lipopolysaccharides (LPS).

**[00361] Polypeptide:** The term “polypeptide”, as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids, e.g., linked to each other by peptide bonds. Those of ordinary skill in the art will appreciate that the term “polypeptide” is intended to be sufficiently general as to encompass not only polypeptides having a complete sequence recited herein, but also to encompass polypeptides that represent functional fragments (*i.e.*, fragments retaining at least one activity) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%, 60%, 70%, or 80%, and further usually including at least one region of much higher identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99% in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another polypeptide of the same class, is encompassed within the relevant term “polypeptide” as used herein. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, e.g., terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof.

**[00362] Prevention:** The term “prevent” or “prevention”, as used herein in connection with a disease, disorder, and/or medical condition, refers to reducing the risk of developing the disease, disorder and/or condition, and/or a delay of onset, and/or reduction in frequency and/or severity of one or more characteristics or symptoms of a particular disease, disorder or condition. In some embodiments, prevention is assessed on a population basis such that an agent is considered to “prevent” a particular disease, disorder or condition if a statistically significant decrease in the development, frequency, and/or intensity of one or more symptoms of the disease, disorder or condition is observed in a population susceptible to the disease, disorder, or condition. In some embodiments, prevention may be considered complete when onset of a disease, disorder or condition has been delayed for a predefined period of time.

**[00363] Protein:** As used herein, the term “protein” encompasses a polypeptide. Proteins may include moieties other than amino acids (*e.g.*, may be glycoproteins, proteoglycans, etc.) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a “protein” can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. Polypeptides may contain l-amino acids, d-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

**[00364] Recombinant:** As used herein, the term “recombinant” is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell; polypeptides isolated from a recombinant, combinatorial human polypeptide library; polypeptides isolated from an animal (*e.g.*, a mouse, rabbit, sheep, fish, etc.) that is transgenic for or otherwise has been manipulated to express a gene or genes, or gene components that encode and/or direct expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof; and/or polypeptides prepared, expressed, created or isolated by any other means that involves splicing or ligating selected nucleic acid sequence elements to one another, chemically synthesizing selected sequence elements, and/or otherwise generating a nucleic acid that encodes and/or directs expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements results from mutagenesis (*e.g.*, *in vivo* or *in vitro*) of a known sequence element, *e.g.*, from a natural or synthetic source such as, for example, in the germline of a source organism of interest (*e.g.*, of a human, a mouse, etc.).

**[00365] Reference:** As used herein, the term “reference” describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, subject, population, sample, sequence or value of interest is compared with a reference or control agent, animal, subject, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is

determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

**[00366] Response:** As used herein, a “response” to treatment may refer to any beneficial alteration in a subject’s condition that occurs as a result of or correlates with treatment. Such alteration may include stabilization of the condition (*e.g.*, prevention of deterioration that would have taken place in the absence of the treatment), amelioration of symptoms of the condition, and/or improvement in the prospects for cure of the condition, etc. It may refer to a subject’s response or to a tumor’s response. Subject or tumor response may be measured according to a wide variety of criteria, including clinical criteria and objective criteria. Techniques for assessing response include, but are not limited to, clinical examination, positron emission tomography, chest X-ray CT scan, MRI, ultrasound, endoscopy, laparoscopy, presence or level of biomarkers in a sample obtained from a subject, cytology, and/or histology. The exact response criteria can be selected in any appropriate manner, provided that when comparing groups of subjects and/or tumors, the groups to be compared are assessed based on the same or comparable criteria for determining response rate. One of ordinary skill in the art will be able to select appropriate criteria.

**[00367] Risk:** As will be understood from context, “risk” of a disease, disorder, and/or condition refers to a likelihood that a particular subject will develop the disease, disorder, and/or condition. In some embodiments, risk is expressed as a percentage. In some embodiments, risk is from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 up to 100%. In some embodiments, risk is expressed as a risk relative to a risk associated with a reference sample or group of reference samples. In some embodiments, a reference sample or group of reference samples have a known risk of a disease, disorder, condition and/or event. In some embodiments a reference sample or group of reference samples are from subjects comparable to a particular subject. In some embodiments, relative risk is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

**[00368] Serotype:** As used herein, the term “serotype”, also referred to as a serovar, refers to a distinct variation within a species of bacteria or virus or among immune cells of different subjects. These microorganisms, viruses, or cells are classified together based on their cell surface antigens, allowing the epidemiologic classification of organisms to the sub-species level. A group of serovars with common antigens may be referred to as a serogroup or sometimes serocomplex.

**[00369] Subject:** As used herein, the term “subject” refers an organism, typically a mammal (*e.g.*, a human, in some embodiments including prenatal human forms). In some embodiments, a subject is suffering from a relevant disease, disorder or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or

condition. In some embodiments, a subject is a patient. In some embodiments, a subject is a subject to whom diagnosis and/or therapy is and/or has been administered.

**[00370] Susceptible to:** A subject who is “susceptible to” a disease, disorder, or condition is at risk for developing the disease, disorder, or condition. In some embodiments, a subject who is susceptible to a disease, disorder, or condition does not display any symptoms of the disease, disorder, or condition. In some embodiments, a subject who is susceptible to a disease, disorder, or condition has not been diagnosed with the disease, disorder, and/or condition. In some embodiments, a subject who is susceptible to a disease, disorder, or condition is a subject who has been exposed to conditions associated with development of the disease, disorder, or condition. In some embodiments, a risk of developing a disease, disorder, and/or condition is a population-based risk (*e.g.*, family members of subjects suffering from the disease, disorder, or condition).

**[00371] Symptoms are reduced:** As used herein, “symptoms are reduced” when one or more symptoms of a particular disease, disorder or condition is reduced in magnitude (*e.g.*, intensity, severity, etc.) and/or frequency, *e.g.*, to a statistically and/or clinically significant or relevant level. For purposes of clarity, a delay in the onset of a particular symptom is considered one form of reducing the frequency of that symptom.

**[00372] Treatment:** As used herein, the term “treatment” (also “treat” or “treating”) refers to any administration of a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

**[00373] Vaccination:** As used herein, the term “vaccination” refers to the administration of a composition intended to generate an immune response, for example to a disease-causing agent. For the purposes of the present invention, vaccination can be administered before, during, and/or after exposure to a disease-causing agent, and in some embodiments, before, during, and/or shortly after exposure to the agent. In some embodiments, vaccination includes multiple administrations, appropriately spaced in time, of a vaccinating composition. In some embodiments, vaccination initiates immunization.

**[00374]** *In some embodiments, the present invention may be defined in any of the following numbered paragraphs:*

1. A vaccine comprising an immunogenic complex, wherein the immunogenic complex comprises:
  - (a) a biotinylated polysaccharide antigen; and

- (b) a fusion protein comprising:
- ii. a biotin-binding moiety; and
  - iii. at least one polypeptide antigen;

wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica*, and further 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 paragraph 1, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica* selected from Vi polysaccharide of Typhi, and O-specific polysaccharide of Typhimurium, Enteritidis, and Paratyphi.
3. The vaccine of paragraph 1 or paragraph 2, wherein the at least one polypeptide antigen of the fusion protein is or comprises a polypeptide antigen from *Salmonella*, *Shigella*, or *Streptococcus pneumoniae*.
4. The vaccine of any of paragraphs 1-3, wherein the at least one polypeptide antigen of the fusion protein comprises a *Salmonella* SseB polypeptide or antigenic fragment thereof.
5. The vaccine of any of paragraphs 1-3, wherein the at least one polypeptide antigen of the fusion protein comprises a *Shigella* IpaB polypeptide or antigenic fragment thereof.
6. The vaccine of any of paragraphs 1-3, wherein the at least one polypeptide antigen of the fusion protein comprises a *Streptococcus pneumoniae* SP1500 polypeptide or antigenic fragment thereof; a *Streptococcus pneumoniae* SP0785 polypeptide or antigenic fragment thereof, or both.
7. The vaccine of any one of the preceding paragraphs, wherein the at least one polypeptide antigen of the fusion protein 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: 4.
8. The vaccine of any one of the preceding paragraphs, wherein the at least one polypeptide antigen of the fusion protein 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: 5.
9. The vaccine of any one of the preceding paragraphs, wherein the at least one polypeptide antigen of the fusion protein 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 either SEQ ID NO: 8 or SEQ ID NO: 9, or a combination of SEQ ID NO: 8 and SEQ ID NO: 9.
10. The vaccine of any one of the preceding paragraphs, wherein the biotin-binding moiety is 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: 3 or a biotin-binding fragment thereof.

11. An immunogenic composition (e.g., a vaccine) comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:  
a plurality of biotinylated polysaccharide antigens comprising polysaccharide antigens of one or more *Salmonella enterica* serotypes; and  
a plurality of fusion proteins, each fusion protein comprising:  
a biotin-binding moiety; and  
a polypeptide antigen,  
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. The immunogenic composition of paragraph 11, wherein the different species each comprise a distinct polysaccharide antigen of one or more *Salmonella enterica* serotypes and/or a distinct polypeptide antigen.
13. The immunogenic composition of paragraph 11 or 12, wherein the one or more *Salmonella enterica* serotypes is or comprises *S. Typhimurium*, *S. Enteritidis*, *S. Typhi* Vi, *S. Paratyphi* A, or combinations thereof.
14. The immunogenic composition of any one of paragraphs 11-13, wherein the polypeptide antigen is or comprises a polypeptide antigen from *Salmonella*, *Shigella*, and/or *Streptococcus pneumoniae*.
15. The immunogenic composition of paragraph 14, wherein the polypeptide antigen is or comprises: an SseB polypeptide antigen of *Salmonella*, an IpaB polypeptide antigen of *Shigella*, and/or a polypeptide antigen comprising an SP1500 polypeptide and/or an SP0785 polypeptide, of *S. pneumoniae*.
16. The immunogenic composition of any one of paragraphs 11-15, comprising at least two different species of immunogenic complexes, wherein the different species comprise:  
a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and  
a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein,  
wherein the first fusion protein and the second fusion protein each independently comprise:  
a biotin-binding moiety; and  
a polypeptide antigen,  
wherein each of the first and second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.
17. The immunogenic composition of paragraph 16, wherein the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*.

18. The immunogenic composition of paragraph 17, wherein the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof.
19. The immunogenic composition of any one of paragraphs 11-15, comprising at least two different species of immunogenic complexes, wherein the different species comprise:  
a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi* Vi non-covalently complexed with a first fusion protein; and  
a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi* A non-covalently complexed with a second fusion protein,  
wherein the first fusion protein and the second fusion protein each independently comprise:  
a biotin-binding moiety; and  
a polypeptide antigen,  
wherein each of the first and second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.
20. The immunogenic composition of paragraph 19, wherein the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SP1500 polypeptide, an SP0785 polypeptide, or both, of *S. pneumoniae*, and/or the fusion protein is CP1.
21. The immunogenic composition of paragraph 20, wherein the SP1500 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; the SP0785 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; and the CP1 fusion protein 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, or an immunogenic fragment thereof.
22. The immunogenic composition of any one of paragraphs 11-15, comprising at least four different species of immunogenic complexes, wherein the different species comprise:  
a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and  
a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein,  
a third biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi* Vi non-covalently complexed with a third fusion protein; and

a fourth biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a fourth fusion protein,  
wherein the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein each independently comprise:  
a biotin-binding moiety; and  
a polypeptide antigen,  
wherein each of the first, second, third, and fourth biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

23. The immunogenic composition of paragraph 22, wherein the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*, and the polypeptide antigen of the third fusion protein and of the fourth fusion protein is or comprises an SP1500 polypeptide, an SP0785 polypeptide, or both, of *S. pneumoniae*, and/or the fusion protein is CP1.

24. The immunogenic composition of paragraph 23, wherein the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof; the SP1500 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; the SP0785 polypeptide antigen of *S. pneumoniae* 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: 9, or an immunogenic fragment thereof; and the CP1 fusion protein 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, or an immunogenic fragment thereof.

25. The immunogenic composition of any one of paragraphs 11-15, comprising at least four different species of immunogenic complexes, wherein the different species comprise:  
a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and  
a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein,  
a third biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi Vi* non-covalently complexed with a third fusion protein; and  
a fourth biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a fourth fusion protein,

wherein the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein each independently comprise:

a biotin-binding moiety; and

a polypeptide antigen,

wherein each of the first, second, third, and fourth biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

26. The immunogenic composition of paragraph 25, wherein the polypeptide antigen of the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*.

27. The immunogenic composition of paragraph 26, wherein the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof.

28. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

a biotin-binding moiety; and

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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, 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.

29. The vaccine of 28, wherein the 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: 4.

30. The vaccine of 28, wherein the 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.

31. The vaccine of 28, wherein the 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9.

32. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise: (a) a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein; (b) a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Paratyphi non-covalently complexed with a fusion protein; wherein each fusion protein comprises (i) a biotin-binding moiety; and (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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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.

33. The vaccine of 32, wherein the 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: 4.

34. The vaccine of 32, wherein the 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.

35. The vaccine of 32, wherein the 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9.

36. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:  
a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein;  
a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein;  
a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein;  
a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Paratyphi non-covalently complexed with a fusion protein;  
wherein each fusion protein comprises  
a biotin-binding moiety; and

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% SEQ ID NO: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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.

37. The vaccine of 36, wherein the 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: 4.

38. The vaccine of 36, wherein the 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.

39. The vaccine of 36, wherein the 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9.

40. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Paratyphi non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

a biotin-binding moiety;

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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, or an antigenic fragment thereof; and

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, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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.

41. The vaccine of paragraph 40, wherein the vaccine comprises a stoichiometrically equal ratio, by weight, of each of the polysaccharide antigens of the different species.

42. The vaccine of paragraph 40, wherein the vaccine comprises at least one of the polysaccharide antigens of the different species at a stoichiometrically different ratio, by weight.

43. The vaccine of any of paragraphs 28-42, wherein the vaccine comprises a stoichiometrically different ratio, by weight, of each of the polysaccharide antigens of the different species.

44. The vaccine of any one of the preceding paragraphs, wherein the biotin-binding moiety is 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: 3.

45. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Salmonella enterica* non-covalently associated with a fusion protein, wherein the fusion protein comprises a biotin-binding moiety and at least one polypeptide antigen.

46. The immunogenic complex of paragraph 45, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica* having a serotype selected from Typhimurium, Enteritidis, Typhi, and Paratyphi.

47. The immunogenic complex of either paragraph 45 or 46, wherein the fusion protein comprises SseB, IpaB, or an SP1500 polypeptide, an SP0785 polypeptide, or both.

48. The immunogenic complex of paragraph 47, wherein the fusion protein comprises:  
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: 4 or an antigenic fragment thereof;  
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; or  
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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, or an antigenic fragment thereof.

49. The immunogenic complex of any one of paragraphs 45-48, 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.

50. A vaccine comprising one or more immunogenic complexes of any one of paragraphs 45-49.

51. A pharmaceutical composition comprising the vaccine of any one of paragraphs 1-44 and 46, and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition comprising the immunogenic complex of any one of paragraphs 45-49, and a pharmaceutically acceptable carrier.
53. The pharmaceutical composition of paragraph 51 or 52, further comprising one or more adjuvants.
54. The pharmaceutical composition of paragraph 53, wherein the one or more adjuvants is or comprises a co-stimulation factor.
55. The pharmaceutical composition of paragraph 53 or 54, wherein the one or more adjuvants are selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphated aluminum hydroxide.
56. The pharmaceutical composition of any one of paragraphs 53-55, wherein the one or more adjuvants is or comprises aluminum phosphate.
57. The pharmaceutical composition of any one of paragraphs 51-56, wherein the pharmaceutical composition is formulated for injection.
58. The pharmaceutical composition of any one of paragraphs 51-57, wherein upon administration to a subject, the pharmaceutical composition induces an immune response.
59. The pharmaceutical composition of paragraph 58, 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.
60. 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 SseB, IpaB, SP0785 or SP1500; wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Salmonella enterica* serotypes selected from Typhimurium, Enteritidis, Typhi, and Paratyphi.
61. A method of immunizing a subject against *Salmonella enterica* infection and/or colonization comprising administering to the subject an immunologically effective amount of the vaccine of any one of paragraphs 1-44 and 46.
62. A method of immunizing a subject against *Salmonella enterica* infection and/or colonization comprising administering to the subject an immunologically effective amount of the immunogenic complex of any one of paragraphs 45-49.
63. A method of immunizing a subject against *Salmonella enterica* infection and/or colonization comprising administering to the subject an immunologically effective amount of the pharmaceutical composition of any one of paragraphs 51-58.
64. The method of any one of paragraphs 61-63, wherein the vaccine, immunogenic composition, or pharmaceutical composition induces an immune response.
65. The method of any one of paragraphs 61-64, wherein the immune response is to at least one polysaccharide antigen or at least one polypeptide of a fusion protein.

66. The method of any one of paragraphs 61-64, wherein the subject is immunized against *Salmonella enterica* infection and/or colonization with one dose of a vaccine.
67. The method of any one of paragraphs 61-64, wherein the subject is immunized against *Salmonella enterica* infection and/or colonization with two doses of a vaccine.
68. The method of any one of paragraphs 61-64, wherein the subject is immunized against *Salmonella enterica* infection and/or colonization with three doses of a vaccine.
69. A fusion protein comprising a rhizavidin protein and at least one peptide or polypeptide antigen, wherein the rhizavidin protein comprises amino acids of SEQ ID NO: 3, or 85% sequence identity to amino acids of SEQ ID NO: 3, and *Salmonella* peptide or polypeptide comprises a fragment of at least 20 amino acids of the SseB protein, or the *Shigella* peptide or polypeptide comprises a fragment of at least 20 amino acids of the IpaB protein.
70. The fusion protein of paragraph 69, wherein the SseB protein comprises at least SEQ ID NO: 4 or a protein of at least 20 amino acids that has at least 85% sequence identity to SEQ ID NO: 4.
71. The fusion protein of paragraph 69, wherein the IpaB protein comprises at least SEQ ID NO: 5 or a protein of at least 20 amino acids that has at least 85% sequence identity to SEQ ID NO: 5.
72. The fusion protein of paragraph 69, wherein the fusion protein comprises at least SEQ ID NO: 1.
73. The fusion protein of paragraph 69, wherein the fusion protein comprises at least SEQ ID NO: 2.

## EXAMPLES

### *Materials and Methods*

**[00375]** Aluminum phosphate (alum) was from Brenntag North America (2% Alhydrogel). Vi polysaccharide was obtained from Dr. Szu from NIH {Szu, 1989 #13}. Adipic acid dihydrazide (ADH), 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide Hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS), and 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) were purchased from Thermo Fisher. Restriction endonucleases and T7 shuffle express competent cells were purchased from New England Biolabs. Plasmid pETDuet was purchased from Novagen. All other reagents were obtained from Sigma.

### **[00376]** *Protein Purification*

**[00377]** DNA fragments encoding of His-SseB and SseA or His-IpaB and IpgC were synthesized and cloned into a pETDuet vector containing Rhavi by restriction enzyme digestion and ligation. Sequence-confirmed plasmids were transformed E. coli T7 shuffle express cells and transformants containing the relevant cloned proteins were grown to OD<sub>600</sub>=1 at 25°C, and protein expression was induced with 0.2 mM IPTG at 16°C overnight. Cells were spun down and pellets were resuspended in lysis buffer (20 mM Tris-HCl, 500 mM NaCl, pH8.0) and then lysed by sonication. Supernatant was loaded over a Ni-NTA column and SseA or IpgC was washed off column with 0.05% LDAO in lysis buffer; proteins Rhavi-

SseB and Rhavi-IpaB were eluted in imidazole buffer. Protein-containing elutions were combined, purified over a gel-filtration column for dimer fractions.

**[00378]** *Purification of OSP from S. Paratyphi, S. Typhimurium and S. Enteritidis*

**[00379]** OSPs were purified from *S. Paratyphi* 9150 (ATCC), clinical strains of *S. Typhimurium* and *S. Enteritidis* using a protocol established previously with modifications {Micoli, 2013}. Briefly, bacteria were resuspended in 2% acetic acid and boiled for 2 hours at 100°C. Supernatant was dialyzed against water extensively and then lyophilized. Resuspended OSP was treated with DNase and Proteinase K and then loaded on a gel-filtration column to separate the OSP with larger sizes. Final OSP was lyophilized and frozen in -80°C.

**[00380]** *Biotinylation of Vi and OSP*

**[00381]** Vi was biotinylated with Amine-PEG3-Biotin as described previously with minor modification {Zhang, 2013}. Briefly, Vi was resuspended to 5 mg/ml in buffer A (0.2 M MES, 150 mM NaCl, pH 5.8), EDC (100 mg/ml in buffer A) and NHS (100 mg/ml in Dimethylformamide) was added into the solution for 15 minutes at room temperature. Solution pH was adjusted to pH7.0 by adding 1M NaHCO<sub>3</sub> (pH 10). Amine-PEG3-Biotin (40 mg/ml in water) was added to a ratio of 1:1 (w:w). Reaction was stirred for another 2 hours at RT before adding glycine to 20 mM final concentration. OSP was biotinylated with CDAP using protocol as described previously {Lu, 2009} {Zhang, 2013}. Biotinylated OSP was dialyzed against saline extensively before being used for MAPS assembly. MAPS was assembled at a 3:1 (w:w) protein: polysaccharide ratio and purified with size exclusion columns. Protein concentration was determined by the BCA method (Pierce), and OSP concentration was determined using the Anthrone method {Roe, 1955}.

**[00382]** *Antigen preparations and immunization*

**[00383]** For immunization experiments with Vi and OSP MAPS, vaccines were mixed with aluminum phosphate (alum) at the indicated concentration in a 5 ml tube, which was then tumbled overnight at 4°C to allow for adsorption one day before immunization. Rabbit immunization experiments were carried out at Cocalico Biologicals Inc.

**[00384]** *Enzyme-linked immunosorbent assay (ELISA)*

**[00385]** IgG antibody titers against Vi and OSP were measured using the methods described previously {Konadu, 1996} {Lu, 2012}.

**[00386]** *Bacterial killing assays*

**[00387]** Salmonella Typhi killing assay was performed as described previously {Lu, 2012 #1013} {Gondwe, #51; Hale, 2006 #163; Gondwe, 2010 #51} with Salmonella typhimurium strains carrying an empty vector (Strain C5) or expressing Vi polysaccharide on the surface (Strain C5.507) {Lu, 2012 #1013}. Bactericidal assays for *S. Paratyphi*, *S. Typhimurium* and *S. Enteritidis* were carried out as described previously using ATCC 9150 strain or clinical strains {Boyd, 2014}.

**EXAMPLE 1. MAPS vaccine against *Salmonella enterica* serovars Typhimurium and Enteritidis**

[00388] *Salmonella enterica* serovars Typhimurium and Enteritidis are the most common serovars causing invasive nontyphoidal salmonellosis in low -income countries (LIC). O-specific polysaccharide (OSP) and pathogen-specific proteins are potential targets. Presented herein is a platform vaccine technology (Multiple Antigen Presenting System, or MAPS) to combine OSPs and pathogen-specific protein to confer broad protection (**FIG. 1**).

[00389] MAPS vaccines present a few advantages: ease of manufacture and tech transfer, and low cost of goods. Additionally, previous work using pneumococcal MAPS in older adults showed higher or similar functional PS antibody levels compared to currently available PCV13 or PPSV23 vaccines. MAPS vaccines could also confer additional protection from included pathogen-specific antigens.

*Selection of Carrier Proteins*

[00390] The carrier function of three proteins, SseB or IpaB fused to rhizavidin (Rhavi), or CP1, was compared. Next, the antibody titer and bactericidal activity was evaluated following immunization of rabbits with monovalent MAPS. The immunogenicity of bivalent and quadrivalent *Salmonella* MAPS in rabbits was also evaluated.

[00391] As used herein, “monovalent MAPS” refers to a vaccine containing one PS and one carrier protein. “Bivalent MAPS” refers to a mixture of two monovalent MAPS vaccines, also referred to as multivalent or 2V. “Quadrivalent MAPS”, also referred to as “4V” refers to a mixture of four monovalent MAPS vaccines.

[00392] Rabbits were immunized on days 0 and 21 with *S. Enteritidis*-MAPS with three carrier proteins: Rhavi-SseB, Rhavi-IpaB and CP1. *S. Typhi*-MAPS comprising the Vi polysaccharide and a polypeptide antigen selected (also referred to herein as carrier protein) from CP1, Rhavi-rEPA or Rhavi-CRM197 were also assessed and the IgG antibody levels to Vi assessed (see **FIG. 10**) as well as the duration of the response in vivo in immunized Guinea pigs (**FIG. 11**) and the inventors selected CP1, Rhavi-SseB and Rhavi-IpaB to proceed with further experiments.

[00393] Immunological memory using adoptive transfer of whole splenocytes from wild type mice immunized with Vi-DT conjugate or *S. Typhi*-MAPS comprising the Vi polysaccharide and Rhavi-rEPA antigen was assessed in immunocompromised mice that were immunized with Vi, DT or rEPA (**FIG. 12A**), and it was determined that immunocompromised mice receiving splenocytes from mice administered *S. Typhi*-MAPS comprising the Vi polysaccharide and rEPA antigen generated more Vi-memory B cells than mice that had received splenocytes from mice immunized with Vi-DT conjugate (**FIG. 12B**).

[00394] The inventors determined that the presence of aluminium phosphate (AP) significantly enhanced antibody response to *Paratyphi* OSP (referred to herein as “ParaOSP”) after immunization of *S. Paratyphi*-MAPS (**FIG. 13**).

*Bactericidal titers following immunization with MAPS*

[00395] As is shown in **FIG. 2A-2B**, sera from mice immunized with *S. Enteritidis*-MAPS was collected prior to first immunization (Pre), three weeks after the first immunization (P1), and three weeks after the second immunization (P2). Antibody against OSP (**FIG. 2A**) or the carrier protein (**FIG. 2B**) was measured by ELISA. Rhavi-SseB induced the highest OSP antibody. Rhavi-SseB had excellent carrier function and was immunogenic in a *Salmonella* Enteritidis MAPS.

[00396] Bactericidal activity was carried out using protocols described in the Materials and Methods, below. Titer was calculated from the sera dilution factor that kills 50% of the bacteria in the assay. As is shown in **FIG. 3**, Rhavi-SseB was the carrier associated with highest bactericidal titers. As is shown in **FIG. 4**, the antibody against *S. Enteritidis* OSP correlated well with bactericidal titers.

*Antibody and bactericidal titers of S. Typhimurium OSP-SseB MAPS*

[00397] As is shown in **FIG. 5**, Rhavi-SseB MAPS generated robust antibody titers and bactericidal activity against *S. Typhimurium*. Antibody against OSP (left) and SseB (middle) were measured by ELISA. Bactericidal assay (right) was performed as described in the Materials and Methods section. Different strains for *S. Typhimurium* (Q55, Q65, LT2, P104, S11 and S12) and *S. Enteritidis* strains (I15, S-1, D82, J73 and R27) were assessed to identify the strain for purification of the OSP for use in a *Salmonella*-MAPS immunogenic complex, and S12 and J73 were selected for *Typhimurium* and *S. Enteritidis*, respectively for further experiments (**FIG. 6**). A robust immune response was detected to *S. Typhimurium* S12 OSP (**FIG. 7A**) which correlated with the bactericidal titers (**FIG. 7B**) when the MAPS comprises any one of the antigens assessed; Rhavi-IpaB, Rhavi-SseB and CP1 after P1 or P2. Similarity antibody titer (AU) to S12 OSP or J73 OSP after immunization with monovalent *S. Typhimurium* OSP-SseB MAPS or monovalent *S. Enteritidis* OSP-SseB MAPS (**FIG. 8A**) correlated with bactericidal killing assay (**FIG. 8B**).

**EXAMPLE 2: Bivalent MAPS and Quadrivalent MAPS vaccines using polysaccharides from *Salmonella enterica* serovars *Typhimurium* and *Enteritidis*, *S. Typhi* Vi and *Paratyphi* OSP**

*Bivalent and Quadrivalent Salmonella MAPS Vaccines*

[00398] Example 2 shows results from exemplary quadrivalent *Salmonella*-MAPS vaccines. It is envisioned any number of combinations of *S. Typhimurium*, *S. Enteritidis*, *S. Typhi* and *S. Paratyphi* MAPS immunogenic complexes can be assembled or combined by a person of ordinary skill in the art to form bivalent (2V), multivalent, 3V, quadrivalent (4V) *Salmonella*-MAPS vaccines. Without being limited to theory, exemplary combinations for 2V, 3V and 4V *salmonella*-MAPS vaccines are disclosed in Tables 1A-1C herein. It is envisioned that other combinations can be compiled to generate *Salmonella*-MAPS vaccines that comprise between 1-50 species of immunogenic complexes.

[00399] The following bivalent (2V) MAPS vaccines were generated against *Salmonella* strains, and are exemplary 2V *salmonella*-MAPS vaccines, and assessed in further experiments.

[00400] Bivalent MAPS comprising: *S. Typhimurium*-SseB and *S. Enteritidis*-SseB

[00401] Bivalent MAPS comprising: *S. Typhi* Vi-CP1 and *S. Paratyphi* A-CP1

*Bivalent Salmonella MAPS generate robust PS antibody titers*

[00402] As is shown in FIG. 9A-9B, a bivalent *Typhimurium* (S12) and *Enteritidis* (J73) SseB MAPS (FIG. 9A) and a bivalent Vi and Paratyphi CP1 MAPS (FIG. 9B) generated robust antibody response. Antibody against OSP or Vi was measured by ELISA.

[00403] As shown in FIG. 14A-14B, a robust immune response to Vi polysaccharide or OSP (as detected by anti-OSP IgG) was detected with both monovalent *S. Typhi*-MAPS comprising the Vi polysaccharide or monovalent *S. Paratyphi*-MAPS comprising the OSP polysaccharide which was comparable to the bivalent MAPS vaccine comprising them both. The immune response to Vi polysaccharide or OSP was detected at doses as low as 0.02µg after 2 weeks after the second immunization (P2) of the bivalent vaccine comprising *S. Typhi*-Vi MAPS and *S. Paratyphi*-OSP MAPS, which was a comparable immune response at the same time point using a higher dose of 25µg (FIG. 15A-15D). The immune response from a dose of 1µg was similar to the dose of 25µg at all time examined, demonstrating that a dose from 0.02µg to 1µg, or ≤ 1 µg is sufficient to induce a robust immune response to the polysaccharide in the salmonella-MAPS immunogenic complex. The immune response persisted for longer than 10 weeks post the second immunization (P2) with the bivalent vaccine comprising *S. Typhi*-Vi MAPS and *S. Paratyphi*-OSP MAPS, as determined by the immune response to Vi polysaccharide or OSP (as detected by anti-OSP IgG) (FIG. 16A-16B). Moreover, the immune response was enhanced by a second (or booster) administration (P2) as detected by increased affinity index (AI) for Vi and ParaOPS (FIG. 17A-17B).

[00404] Both *S. Typhi*-Vi MAPS and *S. Paratyphi*-OSP MAPS demonstrated effective bactericidal activity, where Typhi killing was assessed with *S. Typhimurium* strain expressing Vi, and *S. Paratyphi* killing was assessed with bactericidal assays (FIG. 18A-18B).

**EXAMPLE 3. Quadrivalent MAPS vaccine against *Salmonella enterica* serovars with *Typhimurium* and *Enteritidis*, *S. Typhi* Vi and *Paratyphi* OSP**

[00405] Example 3 shows results from exemplary quadrivalent Salmonella-MAPS vaccines. It is envisioned any number of combinations of *S. Typhimurium*, *S. Enteritidis*, *S. Typhi* and *S. Paratyphi* MAPS immunogenic complexes can be assembled or combined by a person of ordinary skill in the art to form bivalent (2V), multivalent, 3V, quadrivalent (4V) Salmonella-MAPS vaccines. Without being limited to theory, exemplary combinations for 2V, 3V and 4V salmonella-MAPS vaccines are disclosed in Tables 1A-1C, respectively herein. It is envisioned that other combinations can be compiled to generate Salmonella-MAPS vaccines that comprise between 1-50 species of immunogenic complexes.

[00406] The following quadrivalent (4V) MAPS vaccines were generated against *Salmonella* strains, and are exemplary 4V salmonella-MAPS vaccines, and assessed in further experiments.

*Quadrivalent MAPS generate robust antibodies against PS*

[00407] Quadrivalent MAPS (“Salmonella-MAPS1”) comprising :

- i. *S. Typhimurium*-SseB

- ii. *S. Enteritidis*-SseB
- iii. *S. Typhi* Vi-CP1
- iv. *S. Paratyphi* A-CP1

[00408] Quadrivalent MAPS (“Salmonella-MAPS2”) comprising:

- i. *S. Typhimurium*-SseB
- ii. *S. Enteritidis*-SseB
- iii. *S. Typhi* Vi-SseB
- iv. *S. Paratyphi* A-SseB

[00409] As is shown in **FIG. 19A-19B**, quadrivalent salmonella-MAPS comprising Rhavi-SseB made with *Typhimurium* and *Enteritidis* OSP, or salmonella-MAPS comprising CP1 with Vi and Paratyphi OSP (**FIG. 17A**), and quadrivalent salmonella-MAPS comprising Rhavi-SseB carrier protein and Typhimurium and Enteritidis OSP, Vi or Paratyphi OSP (**FIG. 19B**) were very immunogenic. Antibody against each OSP or Vi was measured by ELISA.

[00410] The efficacy of the bivalent and quadrivalent was assessed *in vivo* in rabbits, and the bivalent Salmonella-MAPS comprising polysaccharides from both *S. Typhi* (comprising the Vi polysaccharide) and *S. Paratyphi* (comprising the OSP polysaccharide) and comprising the carrier protein Rhavi-SseB or CP1 induced a robust immune response after P1, which was enhanced after by a second immunization P2 (**FIG. 20A**), and similar increase in antibody titer (AI) was seen with the quadrivalent salmonella-MAPS with the Rhavi-SseB carrier protein comprising polysaccharides from: *S. Typhimurium* (S12), *S. Enteritidis* (J73), *S. Typhi* and *S. Paratyphi* (**FIG. 20B**). The bivalent (**FIG. 21A-21B**) and the quadrivalent salmonella MAPS (**FIG. 21C**) also were effective at killing bacteria of each serovars type.

[00411] Opsonophagocytic killing assays (OPA) or OPK titers demonstrated the effective killing of *Typhimurium* (S12) and *S. Enteritidis* (J73) with SseB antisera (**FIG. 22A-22B**) or OSP antisera, and that the addition of SseB antisera enhanced killing by the OSP antisera (**FIG. 23A-23B**).

[00412] A comparison of the effective killing of *S. Typhimurium* or *S. Paratyphi* after P2 administration of monovalent, bivalent and quadrivalent salmonella-MAPS shows that all MAPS complexes assessed were effective at reducing bacteria as compared to the control (**FIG. 24A-24B**). Similar robust immune response was seen with the monovalent, bivalent and quadrivalent salmonella-MAPS vaccines with *S. Typhi* and *S. Enteritidis* (**FIG. 25A-25B**).

[00413] The results presented herein demonstrate that (1) salmonella-MAPS made with polysaccharides from the Salmonella serovars selected from: *S. Typhimurium*, *S. Enteritidis*, *S. Typhi* or *S. Paratyphi* OSP generate robust antibody titers with high bactericidal killing activity against their respective serovars; (2) bivalent salmonella-MAPS and quadrivalent salmonella-MAPS show similar activity as monovalent salmonella-MAPS; and (3) SseB fused with rhizavidin, or IpaB fused with rhizavidin are excellent carrier proteins and are immunogenic.

## REFERENCES

All the references cited herein and throughout the application are incorporated by reference in their entirety.

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### EQUIVALENTS AND SCOPE

**[00414]** Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the embodiments described herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the appended claims.

**[00415]** Articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between two or more members of a group are considered satisfied if one, more than one, or all of the group members are present, unless indicated to the contrary or otherwise evident from the context. The disclosure of a group that includes “or” between two or more group members provides embodiments in which exactly one member of the group is present, embodiments in which more than one members of the group are present,

and embodiments in which all of the group members are present. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

**[00416]** It is to be understood that the disclosure encompasses all variations, combinations, and permutations in which one or more limitation, element, clause, or descriptive term, from one or more of the claims or from one or more relevant portion of the description, is introduced into another claim. For example, a claim that is dependent on another claim can be modified to include one or more of the limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of making or using the composition according to any of the methods of making or using disclosed herein or according to methods known in the art, if any, are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

**[00417]** Where elements are presented as lists, e.g., in Markush group format, it is to be understood that every possible subgroup of the elements is also disclosed, and that any element or subgroup of elements can be removed from the group. It is also noted that the term “comprising” is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where an embodiment, product, or method is referred to as comprising particular elements, features, or steps, embodiments, products, or methods that consist, or consist essentially of, such elements, features, or steps, are provided as well. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

**[00418]** Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in some embodiments, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. For purposes of brevity, the values in each range have not been individually spelled out herein, but it will be understood that each of these values is provided herein and may be specifically claimed or disclaimed. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

**[00419]** Where websites are provided, URL addresses are provided as non-browser-executable codes, with periods of the respective web address in parentheses. The actual web addresses do not contain the parentheses.

**[00420]** In addition, it is to be understood that any particular embodiment of the present disclosure may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. Any embodiment, element, feature,

application, or aspect of the compositions and/or methods of the disclosure, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

## CLAIMS

What is claimed is:

1. A vaccine comprising an immunogenic complex, wherein the immunogenic complex comprises:
  - (a) a biotinylated polysaccharide antigen; and
  - (b) a fusion protein comprising:
    - ii. a biotin-binding moiety; and
    - iii. at least one polypeptide antigen;

wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica*, and further 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 biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica* selected from Vi polysaccharide of Typhi, and O-specific polysaccharides of Typhimurium, Enteritidis, and Paratyphi.
3. The vaccine of claim 1 or claim 2, wherein the at least one polypeptide antigen of the fusion protein is or comprises a polypeptide antigen from *Salmonella*, *Shigella*, or *Streptococcus pneumoniae*.
4. The vaccine of any of claims 1-3, wherein the at least one polypeptide antigen of the fusion protein comprises a *Salmonella* SseB polypeptide or antigenic fragment thereof.
5. The vaccine of any of claims 1-3, wherein the at least one polypeptide antigen of the fusion protein comprises a *Shigella* IpaB polypeptide or antigenic fragment thereof.
6. The vaccine of any of claims 1-3, wherein the at least one polypeptide antigen of the fusion protein comprises a *Streptococcus pneumoniae* SP1500 polypeptide or antigenic fragment thereof; a *Streptococcus pneumoniae* SP0785 polypeptide or antigenic fragment thereof, or both.
7. The vaccine of any one of the preceding claims, wherein the at least one polypeptide antigen of the fusion protein 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: 4.
8. The vaccine of any one of the preceding claims, wherein the at least one polypeptide antigen of the fusion protein 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: 5.

9. The vaccine of any one of the preceding claims, wherein the at least one polypeptide antigen of the fusion protein 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 either SEQ ID NO: 8 or SEQ ID NO: 9, or a combination of SEQ ID NO: 8 and SEQ ID NO: 9.
10. The vaccine of any one of the preceding claims, wherein the biotin-binding moiety is 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: 3 or a biotin-binding fragment thereof.
11. An immunogenic composition (e.g., a vaccine) comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:
- a plurality of biotinylated polysaccharide antigens comprising polysaccharide antigens of one or more *Salmonella enterica* serotypes; and
  - a plurality of fusion proteins, each fusion protein comprising:
    - a biotin-binding moiety; and
    - a polypeptide antigen,
- 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. The immunogenic composition of claim 11, wherein the different species each comprise a distinct polysaccharide antigen of one or more *Salmonella enterica* serotypes and/or a distinct polypeptide antigen.
13. The immunogenic composition of claim 11 or 12, wherein the one or more *Salmonella enterica* serotypes is or comprises *S. Typhimurium*, *S. Enteritidis*, *S. Typhi* Vi, *S. Paratyphi* A, or combinations thereof.
14. The immunogenic composition of any one of claims 11-13, wherein the polypeptide antigen is or comprises a polypeptide antigen from *Salmonella*, *Shigella*, and/or *Streptococcus pneumoniae*.
15. The immunogenic composition of claim 14, wherein the polypeptide antigen is or comprises: an SseB polypeptide antigen of *Salmonella*, an IpaB polypeptide antigen of *Shigella*, and/or a polypeptide antigen comprising an SP1500 polypeptide and/or an SP0785 polypeptide, of *S. pneumoniae*.

16. The immunogenic composition of any one of claims 11-15, comprising at least two different species of immunogenic complexes, wherein the different species comprise:

a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and

a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein,

wherein the first fusion protein and the second fusion protein each independently comprise:

a biotin-binding moiety; and

a polypeptide antigen,

wherein each of the first and second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

17. The immunogenic composition of claim 16, wherein the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*.

18. The immunogenic composition of claim 17, wherein the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof.

19. The immunogenic composition of any one of claims 11-15, comprising at least two different species of immunogenic complexes, wherein the different species comprise:

a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi* Vi non-covalently complexed with a first fusion protein; and

a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a second fusion protein,

wherein the first fusion protein and the second fusion protein each independently comprise:

a biotin-binding moiety; and

a polypeptide antigen,

wherein each of the first and second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

20. The immunogenic composition of claim 19, wherein the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SP1500 polypeptide, an SP0785 polypeptide, or both, of *S. pneumoniae*, and/or the fusion protein is CP1.

21. The immunogenic composition of claim 20, wherein the SP1500 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; the SP0785 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; and the CP1 fusion protein 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, or an immunogenic fragment thereof.

22. The immunogenic composition of any one of claims 11-15, comprising at least four different species of immunogenic complexes, wherein the different species comprise:

a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and

a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein,

a third biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi* Vi non-covalently complexed with a third fusion protein; and

a fourth biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a fourth fusion protein,

wherein the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein each independently comprise:

a biotin-binding moiety; and

a polypeptide antigen,

wherein each of the first, second, third, and fourth biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

23. The immunogenic composition of claim 22, wherein the polypeptide antigen of the first fusion protein and of the second fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*, and the polypeptide antigen of the third fusion protein and of the fourth fusion protein is or comprises an SP1500 polypeptide, an SP0785 polypeptide, or both, of *S. pneumoniae*, and/or the fusion protein is CP1.

24. The immunogenic composition of claim 23, wherein the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof; the SP1500 polypeptide antigen of *S. pneumoniae* 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: 8, or an immunogenic fragment thereof; the SP0785 polypeptide antigen of *S. pneumoniae* 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: 9, or an immunogenic fragment thereof; and the CP1 fusion protein 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, or an immunogenic fragment thereof.

25. The immunogenic composition of any one of claims 11-15, comprising at least four different species of immunogenic complexes, wherein the different species comprise:

- a first biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhimurium* non-covalently complexed with a first fusion protein; and

- a second biotinylated polysaccharide antigen comprising a polysaccharide of *S. Enteritidis* non-covalently complexed with a second fusion protein,

- a third biotinylated polysaccharide antigen comprising a polysaccharide of *S. Typhi* Vi non-covalently complexed with a third fusion protein; and

- a fourth biotinylated polysaccharide antigen comprising a polysaccharide of *S. Paratyphi A* non-covalently complexed with a fourth fusion protein,

wherein the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein each independently comprise:

- a biotin-binding moiety; and

- a polypeptide antigen,

wherein each of the first, second, third, and fourth biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of the respective fusion protein to form an immunogenic complex.

26. The immunogenic composition of claim 25, wherein the polypeptide antigen of the first fusion protein, the second fusion protein, the third fusion protein, and the fourth fusion protein is or comprises an SseB polypeptide antigen of *Salmonella*.

27. The immunogenic composition of claim 26, wherein the SseB polypeptide antigen of *Salmonella* 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: 4 or an immunogenic fragment thereof.

28. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

a biotin-binding moiety; and

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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, 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.

29. The vaccine of claim 28, wherein the 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: 4.

30. The vaccine of claim 28, wherein the 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.

31. The vaccine of claim 28, wherein the 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9.

32. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Paratyphi non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

a biotin-binding moiety; and

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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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.

33. The vaccine of claim 32, wherein the 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: 4.

34. The vaccine of claim 32, wherein the 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.

35. The vaccine of claim 32, wherein the 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9.

36. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein;

a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Paratyphi non-covalently complexed with a fusion protein;

wherein each fusion protein comprises

a biotin-binding moiety; and

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% SEQ ID NO: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, 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.

37. The vaccine of claim 36, wherein the 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: 4.

38. The vaccine of claim 36, wherein the 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.

39. The vaccine of claim 36, wherein the 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 either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9.

40. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

- a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhimurium non-covalently complexed with a fusion protein;

- a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Enteritidis non-covalently complexed with a fusion protein;

- a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Typhi non-covalently complexed with a fusion protein;

- a biotinylated polysaccharide antigen of *Salmonella enterica* serotype Paratyphi non-covalently complexed with a fusion protein;

- wherein each fusion protein comprises

- a biotin-binding moiety;

- 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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, or an antigenic fragment thereof; and

- 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, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO: 9, 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.

41. The vaccine of claim 40, wherein the vaccine comprises a stoichiometrically equal ratio, by weight, of each of the polysaccharide antigens of the different species.

42. The vaccine of claim 40, wherein the vaccine comprises at least one of the polysaccharide antigens of the different species at a stoichiometrically different ratio, by weight.

43. The vaccine of any of claims 28-42, wherein the vaccine comprises a stoichiometrically different ratio, by weight, of each of the polysaccharide antigens of the different species.

44. The vaccine of any one of the preceding claims, wherein the biotin-binding moiety is 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: 3.

45. An immunogenic complex comprising a biotinylated polysaccharide antigen of *Salmonella enterica* non-covalently associated with a fusion protein, wherein the fusion protein comprises a biotin-binding moiety and at least one polypeptide antigen.

46. The immunogenic complex of claim 45, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Salmonella enterica* having a serotype selected from Typhimurium, Enteritidis, Typhi, and Paratyphi.

47. The immunogenic complex of either claim 45 or 46, wherein the fusion protein comprises SseB, IpaB, or an SP1500 polypeptide, an SP0785 polypeptide, or both.

48. The immunogenic complex of claim 47, wherein the fusion protein comprises:

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: 4 or an antigenic fragment thereof;

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; or

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: 4, SEQ ID NO: 5, or either SEQ ID NO: 8, SEQ ID NO: 9, or both SEQ ID NO: 8 and SEQ ID NO:9, or an antigenic fragment thereof.

49. The immunogenic complex of any one of claims 45-48, 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.

50. A vaccine comprising one or more immunogenic complexes of any one of claims 45-49.

51. A pharmaceutical composition comprising the vaccine of any one of claims 1-44 and 46, and a pharmaceutically acceptable carrier.

52. A pharmaceutical composition comprising the immunogenic complex of any one of claims 45-49, and a pharmaceutically acceptable carrier.

53. The pharmaceutical composition of claim 51 or 52, further comprising one or more adjuvants.

54. The pharmaceutical composition of claim 53, wherein the one or more adjuvants is or comprises a co-stimulation factor.

55. The pharmaceutical composition of claim 53 or 54, wherein the one or more adjuvants are selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphated aluminum hydroxide.

56. The pharmaceutical composition of any one of claims 53-55, wherein the one or more adjuvants is or comprises aluminum phosphate.

57. The pharmaceutical composition of any one of claims 51-56, wherein the pharmaceutical composition is formulated for injection.

58. The pharmaceutical composition of any one of claims 51-57, wherein upon administration to a subject, the pharmaceutical composition induces an immune response.

59. The pharmaceutical composition of claim 58, 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.

60. 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 SseB, IpaB, SP0785 or SP1500;

wherein the plurality of biotinylated polysaccharide antigens comprises polysaccharides of one or more *Salmonella enterica* serotypes selected from Typhimurium, Enteritidis, Typhi, and Paratyphi.

61. A method of immunizing a subject against *Salmonella enterica* infection and/or colonization comprising administering to the subject an immunologically effective amount of the vaccine of any one of claims 1-44 and 46.

62. A method of immunizing a subject against *Salmonella enterica* infection and/or colonization comprising administering to the subject an immunologically effective amount of the immunogenic complex of any one of claims 45-49.

63. A method of immunizing a subject against *Salmonella enterica* infection and/or colonization comprising administering to the subject an immunologically effective amount of the pharmaceutical composition of any one of claims 51-58.

64. The method of any one of claims 61-63, wherein the vaccine, immunogenic composition, or pharmaceutical composition induces an immune response.

65. The method of any one of claims 61-64, wherein the immune response is to at least one polysaccharide antigen or at least one polypeptide of a fusion protein.

66. The method of any one of claims 61-64, wherein the subject is immunized against *Salmonella enterica* infection and/or colonization with one dose of a vaccine.

67. The method of any one of claims 61-64, wherein the subject is immunized against *Salmonella enterica* infection and/or colonization with two doses of a vaccine.

68. The method of any one of claims 61-64, wherein the subject is immunized against *Salmonella enterica* infection and/or colonization with three doses of a vaccine.

69. A fusion protein comprising a rhizavidin protein and at least one peptide or polypeptide antigen, wherein the rhizavidin protein comprises amino acids of SEQ ID NO: 3, or 85% sequence identity to amino acids of SEQ ID NO: 3, and *Salmonella* peptide or polypeptide comprises a fragment of at least 20

amino acids of the SseB protein, or the *Shigella* peptide or polypeptide comprises a fragment of at least 20 amino acids of the IpaB protein.

70. The fusion protein of claim 69, wherein the SseB protein comprises at least SEQ ID NO: 4 or a protein of at least 20 amino acids that has at least 85% sequence identity to SEQ ID NO: 4.

71. The fusion protein of claim 69, wherein the IpaB protein comprises at least SEQ ID NO: 5 or a protein of at least 20 amino acids that has at least 85% sequence identity to SEQ ID NO: 5.

72. The fusion protein of claim 69, wherein the fusion protein comprises at least SEQ ID NO: 1.

73. The fusion protein of claim 69, wherein the fusion protein comprises at least SEQ ID NO: 2.