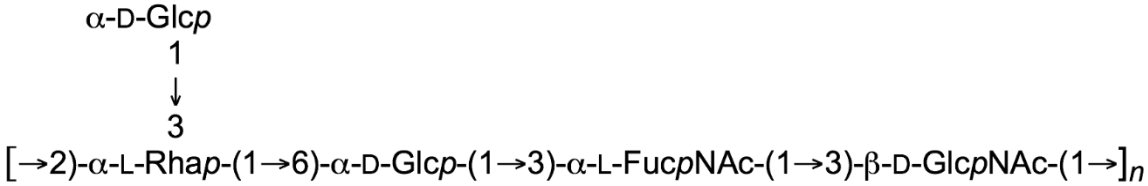
CLAIMS

What is claimed is:

1. A bioconjugate of an *E. coli* glucosylated O4 antigen polysaccharide covalently linked to a carrier protein, wherein the *E. coli* glucosylated O4 antigen polysaccharide comprises the structure of Formula (O4-Glc+):



wherein n is an integer of 1 to 100, preferably 3 to 50, for example 5 to 40, for example 7 to 25, for example 10 to 20.

2. The bioconjugate of claim 1, wherein the *E. coli* glucosylated O4 antigen polysaccharide is covalently linked to an Asn residue in a glycosylation site comprising a glycosylation consensus sequence Asn-X-Ser(Thr), wherein X can be any amino acid except Pro (SEQ ID NO: 1), preferably a glycosylation consensus sequence Asp(Glu)-X-Asn-Z-Ser(Thr), wherein X and Z are independently selected from any amino acid except Pro (SEQ ID NO: 2), in the carrier protein.

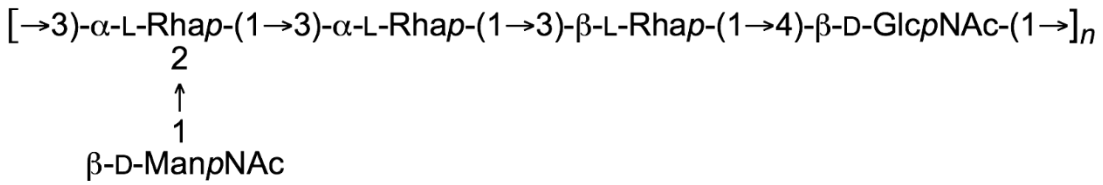
3. The bioconjugate of claim 1 or claim 2, wherein the carrier protein is selected from the group consisting of detoxified Exotoxin A of *P. aeruginosa* (EPA), *E. coli* flagellin (FliC), CRM197, maltose binding protein (MBP), Diphtheria toxoid, Tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A, clumping factor B, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* Sat protein, the passenger domain of *E. coli* Sat protein, *Streptococcus pneumoniae* Pneumolysin, Keyhole limpet hemocyanin (KLH), *P. aeruginosa* PcrV, outer membrane protein of *Neisseria meningitidis* (OMPC), and protein D from non-typeable *Haemophilus influenzae*, preferably wherein the carrier protein is detoxified exotoxin A of *Pseudomonas aeruginosa* (EPA), preferably comprising 1 to 20, preferably 1 to 10, preferably 2 to 4, glycosylation consensus sequences having SEQ ID NO: 1, the consensus sequences preferably having SEQ ID NO: 2, more preferably wherein the carrier protein comprises four glycosylation consensus sequences (EPA-4), more preferably wherein the carrier protein comprises SEQ ID NO: 3.

4. A composition comprising the bioconjugate of any one of claims 1-3.

5. The composition of claim 4, comprising at least one additional antigen polysaccharide covalently linked to a carrier protein.

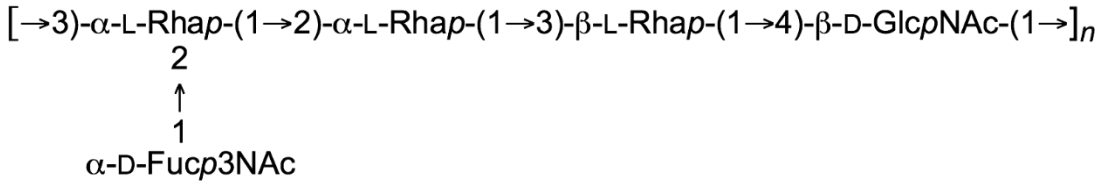
6. The composition of claim 5, wherein the at least one additional antigen polysaccharide is selected from the group consisting of *E. coli* O1A antigen polysaccharide, *E. coli* O2 antigen polysaccharide, *E. coli* O6A antigen polysaccharide, *E. coli* O8 antigen polysaccharide, *E. coli* O15 antigen polysaccharide, *E. coli* O16 antigen polysaccharide, *E. coli* O18A antigen polysaccharide, *E. coli* O25B antigen polysaccharide, and *E. coli* O75 antigen polysaccharide, preferably wherein

(i) the *E. coli* O1A antigen polysaccharide comprises the structure of Formula (O1A):



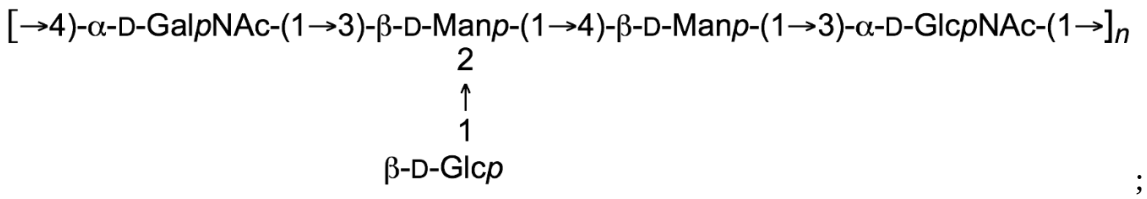
;

(ii) the *E. coli* O2 antigen polysaccharide comprises the structure of Formula (O2):



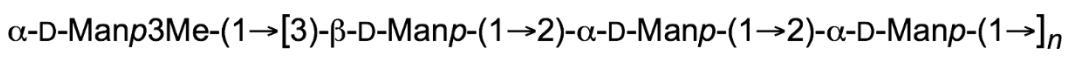
;

(iii) the *E. coli* O6A antigen polysaccharide comprises the structure of Formula (O6A):



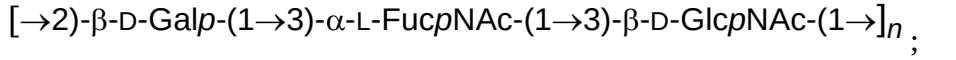
;

(iv) the *E. coli* O8 antigen polysaccharide comprises the structure of Formula (O8):



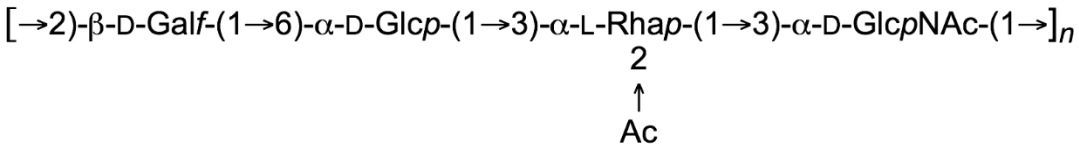
;

(v) the *E. coli* O15 antigen polysaccharide comprises the structure of Formula (O15):



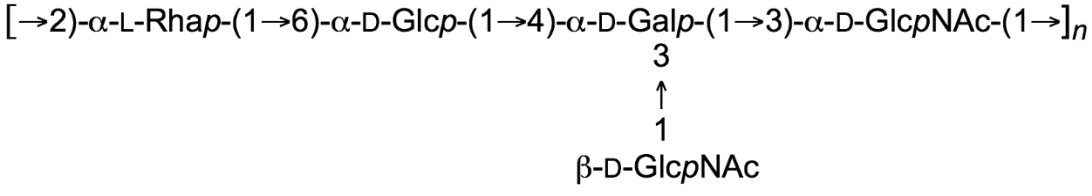
;

(vi) the *E. coli* O16 antigen polysaccharide comprises the structure of Formula (O16):



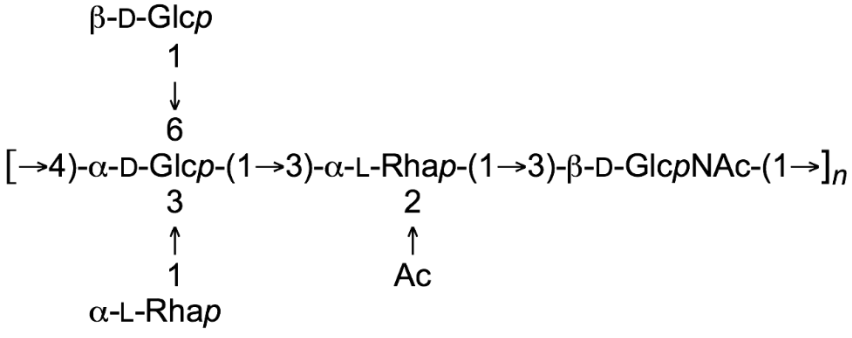
;

(vii) the *E. coli* O18A antigen polysaccharide comprises the structure of Formula (O18A):



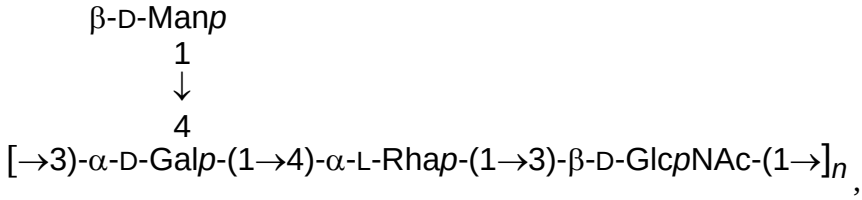
;

(viii) the *E. coli* O25B antigen polysaccharide comprises the structure of Formula (O25B):



; and

(ix) the *E. coli* O75 antigen polysaccharide comprises the structure of Formula (O75):



,

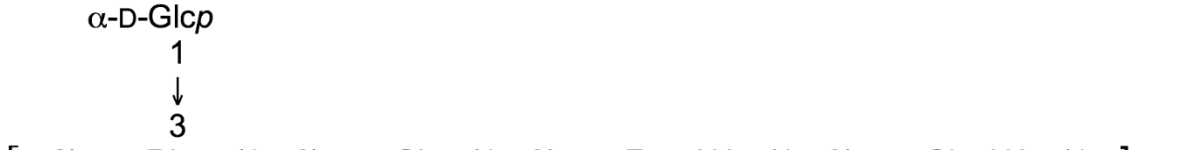
wherein each n is independently an integer of 1 to 100, preferably of 3 to 50, e.g. 5 to 40, e.g. 7 to 25, e.g. 10 to 20.

7. The composition of claim 6, wherein each carrier protein is EPA comprising SEQ ID NO: 3.

8. The composition of any one of claims 6 or 7, comprising at least the *E. coli* O1A, O2, glucosylated O4, O6A, O8, O15, O16, O18A, O25B, and O75 antigen polysaccharides each covalently linked to a carrier protein.

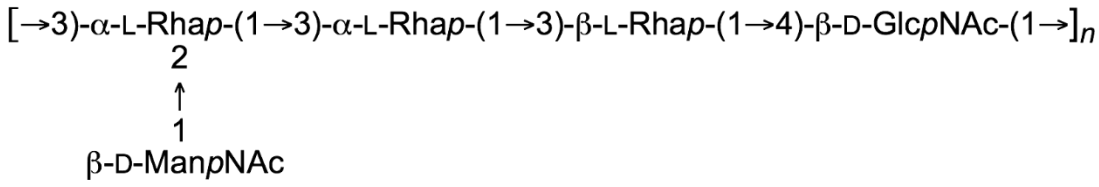
9. A composition comprising:

(i) a bioconjugate of an *E. coli* glucosylated O4 antigen polysaccharide covalently linked to a detoxified Exotoxin A of P. aeruginosa (EPA-4 carrier protein) comprising SEQ ID NO: 3, wherein the *E. coli* glucosylated O4 antigen polysaccharide comprises the structure of Formula (O4-Glc+):



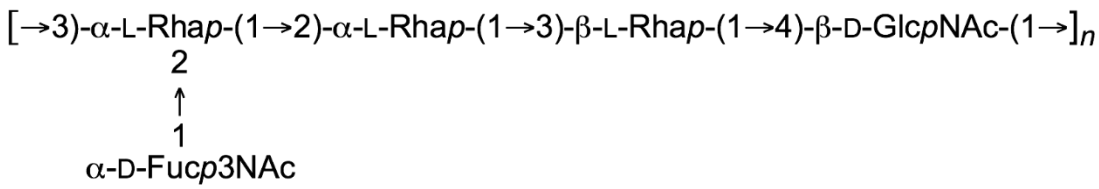
;

(ii) a bioconjugate of an *E. coli* O1A antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O1A antigen polysaccharide comprises the structure of Formula (O1A):



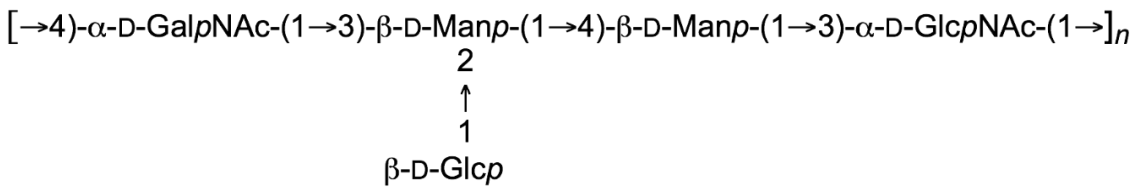
;

(iii) a bioconjugate of an *E. coli* O2 antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O2 antigen polysaccharide comprises the structure of Formula (O2):



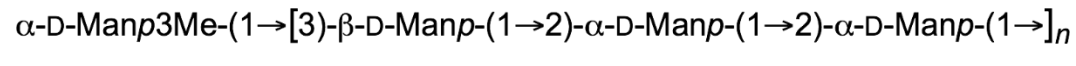
;

(iv) a bioconjugate of an *E. coli* O6A antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O6A antigen polysaccharide comprises the structure of Formula (O6A):



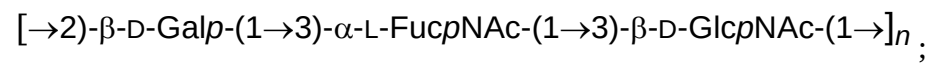
;

(v) a bioconjugate of an *E. coli* O8 antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O8 antigen polysaccharide comprises the structure of Formula (O8):



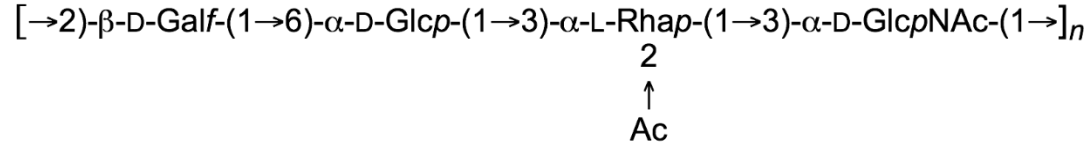
;

(vi) a bioconjugate of an *E. coli* O15 antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O15 antigen polysaccharide comprises the structure of Formula (O15):



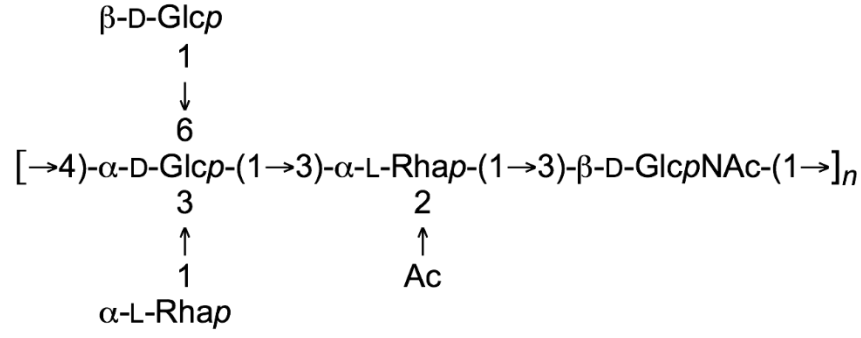
;

(vii) a bioconjugate of an *E. coli* O16 antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O16 antigen polysaccharide comprises the structure of Formula (O16):



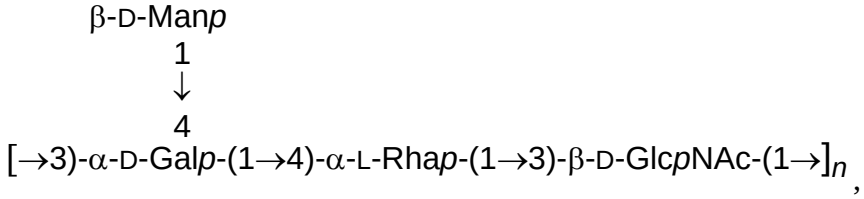
;

(viii) a bioconjugate of an *E. coli* O25B antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O25B antigen polysaccharide comprises the structure of Formula (O25B):



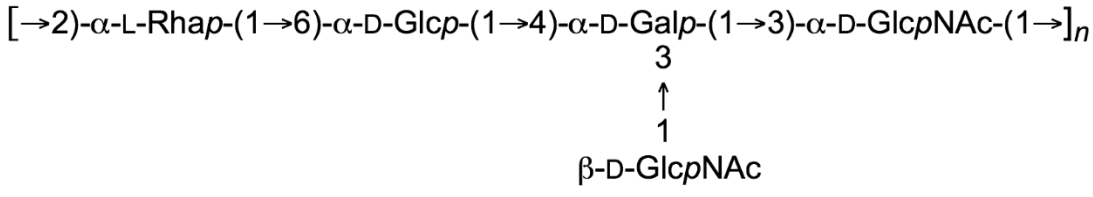
; and

(ix) a bioconjugate of an *E. coli* O75 antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O75 antigen polysaccharide comprises the structure of Formula (O75):



wherein each n is independently an integer of 1 to 100, preferably 3 to 50, e.g. 5 to 40, e.g. 7 to 25, e.g. 10 to 20, the composition preferably further comprising:

(x) a bioconjugate of an *E. coli* O18A antigen polysaccharide covalently linked to an EPA-4 carrier protein, wherein the *E. coli* O18A antigen polysaccharide comprises the structure of Formula (O18A):

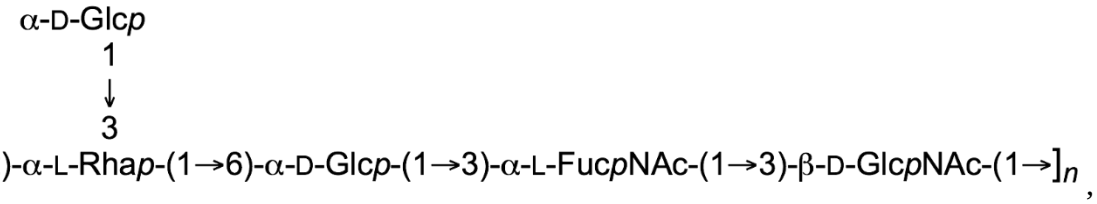


, and n is an integer of 1 to 100, preferably 3 to 50, e.g. 5 to 40, e.g. 7 to 25, e.g. 10 to 20.

10. The bioconjugate of any one of claims 1-3, or the composition of any one of claims 4-9, for use in inducing antibodies against an *E. coli* glucosylated O4 antigen in a subject.

11. The bioconjugate of any one of claims 1-3, or the composition of any one of claims 4-9, for use in vaccinating a subject against *E. coli*, in particular extra-intestinal pathogenic *E. coli* (ExPEC).

12. A recombinant prokaryotic host cell for producing a bioconjugate of an *E. coli* glucosylated O4 antigen polysaccharide covalently linked to a carrier protein, wherein the glucosylated O4 antigen polysaccharide comprises the structure of Formula (O4-Glc+):



wherein n is an integer of 1 to 100, preferably of 3 to 50, e.g. of 5 to 40,

the host cell comprising:

(i) a nucleotide sequence of an *rfb* gene cluster for the *E. coli* O4 antigen polysaccharide;

(ii) a nucleotide sequence encoding a glucosyl transferase having at least 80% sequence identity to SEQ ID NO: 4, wherein the glucosyl transferase is capable of modifying the *E. coli* O4 antigen polysaccharide to produce the *E. coli* glucosylated O4 antigen polysaccharide;

(iii) nucleotide sequences encoding a translocase and a glycosyltransferase having at least 80% sequence identity to SEQ ID NOs: 7 and 8 respectively, wherein the translocase is capable of translocating bactoprenol-linked glucose and the glycosyltransferase is capable of glucosylating bactoprenol;

(iv) a nucleotide sequence encoding the carrier protein; and

(v) a nucleotide sequence encoding an oligosaccharyl transferase capable of covalently linking the *E. coli* glucosylated O4 antigen polysaccharide to the carrier protein to produce the bioconjugate.

13. The recombinant prokaryotic host cell of claim 12, wherein:

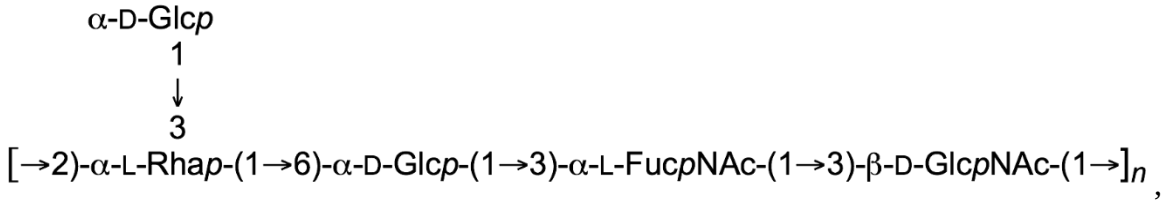
the glucosyl transferase has SEQ ID NO: 4;

the oligosaccharyl transferase comprises the amino acid sequence of SEQ ID NO: 6, preferably SEQ ID NO: 6 comprising the amino acid mutation N311V, more preferably SEQ ID NO:6 comprising the amino acid mutations Y77H and N311V; and

the carrier protein comprises at least one glycosylation site comprising a glycosylation consensus sequence having SEQ ID NO: 1, preferably having SEQ ID NO: 2, preferably wherein the carrier protein is a detoxified exotoxin A of *Pseudomonas aeruginosa* (EPA), more preferably wherein the EPA comprises 1-10, preferably 2-4, glycosylation sites each comprising a glycosylation consensus sequence having SEQ ID NO: 2, more preferably wherein the carrier protein is EPA with four glycosylation consensus sequences (EPA-4), more preferably wherein the carrier protein comprises SEQ ID NO: 3.

14. The recombinant prokaryotic host cell of any one of claims 12 or 13, which is an *E. coli* cell, e.g. an *E. coli* K-12 strain, such as strain W3110.

15. A method of producing a bioconjugate of an *E. coli* glucosylated O4 antigen polysaccharide covalently linked to a carrier protein, wherein the *E. coli* glucosylated O4 antigen polysaccharide comprises the structure of Formula (O4-Glc+):



wherein n is an integer of 1 to 100, preferably of 3 to 50, e.g. of 5 to 40, the method comprising culturing the recombinant prokaryotic host cell of any one of claims 12-14 under conditions for production of the bioconjugate.

~~16. The bioconjugate of an *E. coli* glucosylated O4 antigen polysaccharide covalently linked to a carrier protein produced by the method of claim 15.~~

~~17. A composition comprising the bioconjugate of claim 16.~~

~~18~~16. A composition according to claim 9, wherein the bioconjugate of the O25B antigen polysaccharide is present in the composition at a concentration that is about 1.5-6 times, e.g. about 2 to 4 times, higher than the concentration of any of the other bioconjugates.

~~19~~17. A composition according to claim ~~18~~16, wherein the bioconjugates of O1A:O2:glucosylated O4:O6A:O8:O15:O16:O18A:O25B:O75 are present in a ratio by weight of polysaccharide of 1:1:1:1:1:1:1:1:2:1, or 2:1:1:2:1:1:1:1:4:1.

~~20~~18. A composition according to any one of claims ~~18~~ 16 or ~~19~~17, wherein the concentration of the bioconjugate of the O25B antigen polysaccharide is 2 to 50 µg/mL, preferably 8 to 40 µg/mL, e.g. 16-32 µg/mL.