AMENDED CLAIMS

1. Antigenic multivalent molecular construct consisting of a basic unit comprising a helper-T dependent carrier protein covalently bound to a minimum of three carbohydrate structures of different serological specificity, wherein each carbohydrate structure comprises at least one of the repeating basic epitopes consisting of a minimum of five to twelve monosaccharide residues, characterized in that at least one mole or fraction thereof of protein carrier carries at least one mole or fraction thereof of each of the at least three different type-specific carbohydrate antigens.

2. Antigenic multivalent molecular construct according to claim 1, wherein said carbohydrate structures are oligosaccharides or polysaccharides.

3. Antigenic multivalent molecular construct according to anyone of claims 1-2, wherein said repeating basic epitope consists of a minimum of eight to twelve monosaccharide residues.

4. Antigenic multivalent molecular construct according to anyone of the claims 1-3, wherein the helper-T dependent carrier protein is selected between the group of natural diphtheria mutants protein CRM197, diphtheria toxoid, and tetanus toxoid, Protein D from *Haemophilus influenzae*, Pneumococcal surface proteins, Pneumococcal toxin, and their variants or derivatives, such as tetanus toxoid chemically derivatized by adipic acid di-hydrazide spacer.

5. Antigenic multivalent molecular construct according to anyone of the preceding claims 1-4, wherein said carried carbohydrate antigens of different serological specificity are selected among those of *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *Candida albicans*, *Mycobacterium bovis* or a combination thereof.

6. Antigenic multivalent molecular construct according to claim 5, wherein the carried carbohydrate antigens are at least three capsular polysaccharides selected among type 1, 2, 3, 4, 5, 6A, 6B,6C,6D,7F,8,9N, 9V, 10A, 11A, 11B, 11C, 11F, 12F, 14,15A,15B,15C, 17F, 18C, 19A, 19F, 20, 22F,23A, 23F, 33F, 35B of *Streptococcus pneumoniae* and/or among polysaccharides of group A, C, W135 and Y of *Neisseria meningitidis* and/or polysaccharide of type b of *Haemophilus influenzae*, and/or K polysaccharide antigens of *Klebsiella pneumoniae* and/or polysaccharide antigens of *Staphylococcus aureus* and/or polysaccharide antigens of *Mycobacterium tuberculosis* and/or polysaccharide antigens of *Salmonella typhi* and/or *polysaccharide antigens* of *Escherichia coli* and/or polysaccharide antigens of *Vibrio cholerae* and/or polysaccharide antigens of *Candida albicans* and/or *Mycobacterium bovis*, or a combination thereof.

7. Antigenic multivalent molecular construct according to claim 6, wherein said construct comprises the carrier protein CRM197 and three capsular polysaccharides of *Streptococcus pneumoniae* selected among the triad of 3,6A,7F, 4,5,9V; 1,6B,14;

18C,19A,23F; 6C,19F,22F; 12F,15B,33F; 5,9V,19F; 1,14,19A; 22F,23F,33F; 4,6B,18C.

8. Antigenic multivalent molecular construct according to claim 6, wherein said construct comprises the carrier protein CRM197 and three capsular polysaccharides of *Streptococcus pneumoniae* selected among the triad of CRM197-3,6A,7F; CRM197-5,9V,19F; CRM197-1,14,19A; CRM197-22F,23F,33F; CRM197-4,6B,18C.

9. Antigenic multivalent molecular construct according to anyone of the preceding claims in monomeric or polymeric form.

10. Antigenic multivalent molecular construct according to anyone of the preceding claims for use in a vaccine for the protection of a subject from the infections due to at least one pathogen selected among *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio cholerae, Mycobacterium tuberculosis, Mycobacterium bovis* and *Candida albicans.*

11. A vaccine formulation comprising at least one antigenic multivalent molecular construct according to anyone of claims 1-9 in a physiologically acceptable vehicle, optionally together with an adjuvant or excipients pharmaceutically acceptable.

12. A vaccine formulation according to claim 11, wherein the dose of each type or group-specific carried carbohydrate antigen ranges between 0.1 to 10 µg, preferably being 1.0 µg.

13. A vaccine formulation according to claim 11 or 12, wherein said adjuvant is chosen between a mineral adjuvant selected from aluminium phosphate, aluminium

hydroxide; an organic adjuvant selected from squalene-based adjuvants such as MF59, QF 21, Addavax and a biological adjuvant selected from monophosphoryl-lipid A and trehalose dicorynomycolate.

14. A vaccine formulation according to anyone of the claims 11-13, wherein the amount of adjuvant ranges between 0.1-1 mg/dose, preferably being 0.5 mg/dose.

15. A vaccine formulation according to anyone of the claim 11-14, said formulation being suitable for the administration by subcutaneous, intramuscular, intracutaneous or transcutaneous route.

16. A broad-spectrum polyvalent vaccine formulation according to anyone of the preceding claims 11-15, for use in medical human or veterinary field for the protection of a subject from the infections due to at least one pathogen selected among *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, M. tuberculosis, Salmonella typhi, Escherichia coli, Vibrio cholerae, C. albicans* and *M. bovis,* or a combination thereof.

17. A broad-spectrum polyvalent vaccine formulation for use in medical human field according to claim 16, wherein said subject belongs to the paediatric population.

18. A broad-spectrum polyvalent vaccine formulation for use in medical human field according to claim 16-17, for the prevention of Invasive Pneumococcal Diseases due to *Streptococcus pneumoniae*, comprising one or more antigenic multivalent molecular constructs selected among CRM197-3,6A,7F; CRM197-4,5,9V; CRM197-1,6B,14; CRM197-18C,19A,23F; CRM197-6C,19F,22F; CRM197-

12F,15B,33F or among CRM197-3,6A,7F; CRM197-5,9V,19F; CRM197-1,14,19A; CRM197-22F,23F,33F; CRM197-4,6B,18C.

19. A broad spectrum vaccine formulation for use in medical human field according to claims 16-17, for the prevention of the infections due to *Neisseria meningitidis* comprising an antigenic multivalent molecular construct containing the group-specific carbohydrate structures A, C, W135 and Y.

20. A broad spectrum vaccine formulation for use in medical human or veterinary field according to claims 16-19, for the prevention of the systemic and pulmonary infections due to more than one infectious agent selected between/among *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, M. tuberculosis,* comprising more than one antigenic multivalent molecular construct containing said agent-specific carbohydrate structures.

21. A broad spectrum vaccine formulation for use in medical human or veterinary field according to claims 16-19, for the prevention of the intestinal infections due to more than one infectious agent selected between/among *Salmonella typhi, Escherichia coli*, and *Vibrio cholerae*, comprising more than one antigenic multivalent molecular construct containing said agent-specific carbohydrate structures.

22. A broad spectrum vaccine formulation for use in medical human field according to claims 16-19, for the prevention of the systemic and genitourinary infections due to *Candida albicans* and *Escherichia Coli* comprising more than one antigenic multivalent molecular construct containing said agent-specific carbohydrate structures.

23. Vaccine formulation according to any one of claims 11-22 for the separate, simultaneous or sequential administration with an Endotoxoid of gram negative bacteria, said Endotoxoid being selected in the group consisting of Endotoxoid of *N. meningitidis* Group B, *E. coli*, *S. typhi*, *V. cholerae*, *S. enteritidis*, *B. pertussis*.

24. A conjugation process for preparing the antigenic multivalent molecular construct according to anyone of claims 1-9, which comprises the following steps:

a) chemical activation of the at least three antigenically different carbohydrate structures to mono-functionality or polyfunctionality by O-de-hydrogen uncoupling via oxidation and reductive amination forming imine reduced bonds with an alkyl diamine spacer, then derivatized to active esters;

b) simultaneous coupling, or a step-by-step coupling, of the at least three ester-derivative carbohydrate structures to the amino groups of the polyfunctional carrier protein through the formation of amide bonds;

wherein at least one mole or fraction thereof of protein carrier is reacted with at least one mole or fraction thereof of each of the at least three different type-specific carbohydrate antigens.

25. A conjugation process according to claim 24, wherein the carbohydrate structures are chemically activated in their corresponding diamine butyric acid derivatives and the active esters are succinimidyl esters.

26. A conjugation process according to anyone of the claims 24-25 wherein said polyfunctional carrier protein is CRM197.

27. A conjugation process according to anyone of the claim 23-26, wherein said at least three carbohydrate structures are selected among the following triads of polysaccharides from *Streptococcus pneumoniae* 3,6A,7F; 4,5,9V; 1,6B,14; 18C,19A,23F; 6C,19F,22F; 12F,15B,33F; 5,9V,19F; 1,14,19A; 22F,23F,33F; 4,6B,18C.

28. A conjugation process for preparing the antigenic multivalent molecular construct according to anyone of claims 1-9, which comprises the simultaneous coupling or the step-by-step coupling of the amino groups of the polyfunctional carrier protein with at least three antigenically different carbohydrate structures, via reductive amination forming imine-reduced bonds, such carbohydrate structures being previously activated to monofunctionality or polyfunctionality, with or without spacers, by O-de-hydrogen uncoupling via oxidation,
wherein at least one mole or fraction thereof of protein carrier is reacted with at least one mole or fraction thereof of each of the at least three different type-specific carbohydrate antigens.

29. Conjugation process according to anyone of the claims 24-28, wherein the carbohydrate structures of step a) comprise at least one of the repeating basic epitopes consisting of a minimum of five to twelve monosaccharide residues as assessed by molecular mass determination and NMR spectroscopy, said repeating basic epitopes being antigenically assessed by reactivity with type-specific or group-specific polyclonal or monoclonal antibodies through the determination of their respective MIC50 values in the inhibition of their homologous Polysaccharide-Antibody reference system.

30. Antigenic multivalent molecular construct obtainable through the conjugation process according to anyone of the claims 24-29.

CLAIMS

1. Antigenic multivalent molecular construct consisting of a basic unit comprising a helper-T dependent carrier protein covalently bound to a minimum of three carbohydrate structures of different serological specificity by a linker comprising an imine and an amide bond_, wherein each carbohydrate structure comprises at least one of the repeating basic epitopes consisting of a minimum of five to twelve monosaccharide residues, characterized in that at least one protein carrier carries at least one mole each of the at least three different type-specific carbohydrate antigens.

2. Antigenic multivalent molecular construct according to claim 1, wherein said carbohydrate structures are oligosaccharides or polysaccharides.

3. Antigenic multivalent molecular construct according to anyone of claims 1-2, wherein said repeating basic 20 epitope consists of a minimum of eight to twelve monosaccharide residues.

4. Antigenic multivalent molecular construct according to anyone of the claims 1-3, wherein the helper-T dependent carrier protein is selected between the group of natural diphtheria mutants protein CRM197, diphtheria toxoid, and tetanus toxoid, Protein D from *Haemophilus influenzae,* Pneumococcal surface proteins, Pneumococcal toxin, and their variants or derivatives, such as tetanus toxoid chemically derivatized by adipic acid di-hydrazide spacer.

5. Antigenic multivalent molecular construct according to anyone of the preceding claims 1-4, wherein said carried carbohydrate antigens of different serological specificity are selected among those of *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, Mycobacterium tuberculosis, Salmonella typhi, Escherichia coli, Vibrio cholerae, Candida albicans, Mycobacterium bovis* or a combination thereof.

6. Antigenic multivalent molecular construct according to claim 5, wherein the carried carbohydrate antigens are at least three capsular polysaccharides selected among type 1, 2, 3, 4, 5, 6A, 6B,6C,6D,7F,8,9N, 9V, 10A, 11A, 11B, 11C, 11F, 12F, 14,15A,15B,15C, 17F, 18C, 19A, 19F, 20, 22F,23A, 23F, 33F, 35B of *Streptococcus pneumoniae* and/or among polysaccharides of group A, C, W135 and Y of *Neisseria meningitidis* and/or polysaccharide of type b of *Haemophilus influenzae,* and/or K polysaccharide antigens of

Klebsiella pneumoniae and/or polysaccharide antigens of *Staphylococcus aureus* and/or polysaccharide antigens of *Mycobacterium tuberculosis* and/or polysaccharide antigens of *Salmonella typhi* and/or *polysaccharide antigens* of *Escherichia coli* and/or polysaccharide antigens of *Vibrio cholerae* and/or polysaccharide antigens of *Candida albicans* and/or *Mycobacterium bovis,* or a combination thereof.

7. Antigenic multivalent molecular construct according to claim 6, wherein said construct comprises the carrier protein CRM197 and three capsular polysaccharides of *Streptococcus pneumoniae* selected among the triad of 3,6A,7F, 4,5,9V; 1,6B,14; 18C, 19A, 23F; 6C, 19F, 22F; 12F, 15B, 33F; 5, 9V, 19F;1,14,19A; 22F,23F,33F; 4,6B,18C.

8. Antigenic multivalent molecular construct according to claim 6, wherein said construct comprises the carrier protein CRM197 and three capsular polysaccharides of *Streptococcus pneumoniae* selected among the triad of CRM197-3,6A,7F; CRM197 5,9V,19F; CRM197-1,14,19A; CRM197-22F,23F,33F; CRM197-4,6B,18C.

9. Antigenic multivalent molecular construct according to anyone of the preceding claims in monomeric or polymeric form.

10. Antigenic multivalent molecular construct according to anyone of the preceding claims for use in a vaccine for the protection of a subject from the infections due to at least one pathogen selected among *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Candida albicans*.

11. A vaccine formulation comprising at least one antigenic multivalent molecular construct according to anyone of claims 1-9 in a physiologically acceptable vehicle, optionally together with an adjuvant or excipients pharmaceutically acceptable.

12. A vaccine formulation according to claim 11, wherein the dose of each type or group-specific carried carbohydrate antigen ranges between 0.1 to 10 µg, preferably being 1.0 µg.

13. A vaccine formulation according to claim 11 or 12, wherein said adjuvant is chosen between a mineral adjuvant selected from aluminum phosphate, aluminum hydroxide; an

organic adjuvant selected from squalene-based adjuvants such as MF59, QF 21, Addavax and a biological adjuvant selected from monophosphoryl-lipid A and trehalose dicorynomycolate.

14. A vaccine formulation according to anyone of the claims 11-13, wherein the amount of adjuvant ranges between 0.1-1 mg/dose, preferably being 0.5 mg/dose.

15. A vaccine formulation according to anyone of the claim 11-14, said formulation being suitable for the administration by subcutaneous, intramuscular, intracutaneous or transcutaneous route.

16. A broad-spectrum polyvalent vaccine formulation according to anyone of the preceding claims 11-15, for use in medical human or veterinary field for the protection of a subject from the infections due to at least one pathogen selected among *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *M. tuberculosis*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *C. albicans* and *M. bovis*, or a combination thereof.

17. A broad-spectrum polyvalent vaccine formulation for use in medical human field according to claim 16, wherein said subject belongs to the paediatric population.

18. A broad-spectrum polyvalent vaccine formulation for use in medical human field according to claim 16-17, for the prevention of Invasive Pneumococcal Diseases due to *Streptococcus pneumoniae,* comprising one or more antigenic multivalent molecular constructs selected among CRM197-3,6A,7F; CRM197-4,5,9V; CRM197-1,6B,14; CRM197-18C,19A,23F; CRM197-6C,19F,22F; CRM197-12F,15B,33F or among CRM197-3,6A,7F; CRM197-5,9V,19F; CRM197-1,14,19A; CRM197-22F,23F,33F; CRM197-4,6B,18C.

19. A broad spectrum vaccine formulation for use in medical human field according to claims 16-17, for the prevention of the infections due to *Neisseria meningitidis* comprising an antigenic multivalent molecular construct containing the group-specific carbohydrate structures A, C, W135 and Y.

20. A broad spectrum vaccine formulation for use in 10 medical human or veterinary field according to claims 16-19, for the prevention of the systemic and pulmonary infections due to more than one infectious agent selected between/among *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Staphylococcus*

aureus, *M. tuberculosis*, comprising more than one antigenic multivalent molecular construct containing said agent-specific carbohydrate structures.

21. A broad spectrum vaccine formulation for use in medical human or veterinary field according to claims 16-19, for the prevention of the intestinal infections due to more than one infectious agent selected between/among *Salmonella typhi, Escherichia coli,* and *Vibrio cholerae,* comprising more than one antigenic multivalent molecular construct containing said agent-specific carbohydrate structures.

22. A broad spectrum vaccine formulation for use in medical human field according to claims 16-19, for the prevention of the systemic and genitourinary infections due to *Candida albicans* and *Escherichia Coli* comprising more than one antigenic multivalent molecular construct containing said agent-specific carbohydrate structures.

23. Vaccine to any one of claims 16-22 for the separate, simultaneous or sequential administration with an Endotoxoid of gram negative bacteria, said Endotoxoid being selected in the group consisting of Endotoxoid of *N. meningitidis* Group B, *E. coli, S. typhi, V. cholerae, S. enteritidis, B. pertussis.*

24. A conjugation process for preparing the antigenic multivalent molecular construct according to anyone of 10 claims 1-9, which comprises the following steps:

a) chemical activation of the at least three antigenically different carbohydrate structures to mono-functionality or polyfunctionality by O-dehydrogen uncoupling via oxidation and reductive amination forming imine reduced bonds with an alkyl diamine spacer, then derivatized to active esters;

b) simultaneous coupling, or a step-by-step coupling, of the at least three ester-derivative carbohydrate structures to the amino groups of the polyfunctional carrier protein through the formation of amide bonds;

wherein at least one mole or fraction thereof of protein carrier is reacted with at least one mole or fraction thereof of each of the at least three different type-specific carbohydrate antigens.

25. A conjugation process according to claim 24, wherein the carbohydrate structures are chemically activated in their corresponding diamine butyric acid derivatives and the active esters are succinimidyl esters.

26. A conjugation process according to anyone of the claims 24-25 wherein said

polyfunctional carrier protein is CRM197.

27. A conjugation process according to anyone of the claims 24-26, wherein said at least three carbohydrate structures are selected among the following triads of polysaccharides from *Streptococcus pneumoniae* 3,6A,7F; 4,5,9V; 1, 6B, 14; 18C, 19A, 23F; 6C, 19F, 22F; 12F, 15B, 33F; 5,9V,19F; 1,14,19A; 22F,23F,33F; 4,6B,18C.

28. A conjugation process for preparing the antigenic multivalent molecular construct according to anyone of claims 1-9, which comprises the simultaneous coupling or the step-by-step coupling of the amino groups of the polyfunctional carrier protein with at least three antigenically different carbohydrate structures, via reductive amination forming imine-reduced bonds, such carbohydrate structures being previously activated to monofunctionality or polyfunctionality, with or without spacers, by 0-de-hydrogen uncoupling via oxidation, wherein at least one mole or fraction thereof of protein carrier is reacted with at least one mole or fraction thereof of each of the at least three 20 different type-specific carbohydrate antigens.