CLAIMS

1. A process for producing, on an industrial scale, capsular polysaccharide of *Haemophilus influenzae* type b (PRP) intended for vaccine purposes, according to which a strain of *Haemophilus influenzae* type b (Hib) is cultured in a culture medium, and the culture supernatant is harvested and treated in order to extract the capsular polysaccharide therefrom, said culture medium comprising at least:
   - one source of carbon,
   - protoporphyrin,
   - salts,
   - amino acids,
   - NAD or NADH,
   - vitamins,
   - pH-regulating means,

   characterized in that said culture medium is chemically defined, and comprises at least zinc.

2. The process as claimed in the preceding claim, characterized in that said pH-regulating means consist of buffer salts.

3. The process as claimed in either of the preceding claims, characterized in that said source of carbon may be multiple and is chosen from: glucose, fructose, galactose, glycerol, xylose, ribose, fucose, sialic acid and lactate.

4. The process as claimed in one of the preceding claims, characterized in that said protoporphyrin is synthetic protoporphyrin IX.

5. The process as claimed in one of the preceding claims, characterized in that said salts are chosen from potassium, magnesium, sodium, calcium, iron, zinc, cobalt and manganese salts.

6. The process as claimed in the preceding claim, characterized in that said salts are chosen from: K_2HPO_4; KH_2PO_4; $MgSO_4$. 7 H_2O; Na_2HPO_4.12 H_2O; NaH_2PO_4. 2 H_2O; $CaCl_2$. 2 H_2O; $FeSO_4$. 7 H_2O; $ZnSO_4$. 7 H_2O; $CoCl_2$. 6 H_2O; $MnSO_4$. H_2O.

7. The process as claimed in one of the preceding claims, characterized in that said amino acids are chosen from:
    - arginine,
    - alanine,
    - at least one among: asparagine, glutamine, aspartic acid, glutamic acid,
    - lysine,
    - histidine,
    - tryptophan,
    - valine,
    - isoleucine,
    - leucine,
    - tyrosine,
    - phenylalanine,
    - cystine or equivalent.

8. The process as claimed in the preceding claim, characterized in that said culture medium comprises at least arginine, alanine, histidine, tryptophan, tyrosine, phenylalanine, cystine, aspartic acid and glutamic acid.

9. The process as claimed in one of the preceding claims, characterized in that the cystine is replaced with glutathione or cysteine.

10. The process as claimed in one of the preceding claims, characterized in that said vitamins are chosen from: thiamine, pantothenate, uracil, hypoxanthine, biotin, riboflavin and pyridoxine.

11. The process as claimed in one of the preceding claims, characterized in that the arginine and the uracil are replaced with citrulline.

12. The process as claimed in one of the preceding claims, characterized in that it also comprises a step of conjugating the capsular polysaccharide obtained to a carrier protein.

13. The process as claimed in the preceding claim, characterized in that the carrier protein is tetanus toxoid.

14. The use of capsular polysaccharides obtained as claimed in any one of the preceding claims, for preparing a vaccine composition.

15. A process for preparing a vaccine composition, according to which:

- a) an antigen against *Haemophilus influenzae* type b (Hib), consisting of the capsular polysaccharide (PRP), is prepared, on an industrial scale, by culturing a strain of Hib in a chemically defined medium of which the nature and the amount of each of the constituents is perfectly defined and which comprises at least:
  - one source of carbon,
  - protoporphyrin,
  - salts,
  - amino acids,
  - NAD or NADH,
  - vitamins,
  - zinc,
  - and also pH-regulating means,
- b) the culture supernatant is harvested and treated in order to extract the purified capsular polysaccharide therefrom,
- c) the capsular polysaccharide obtained in step b) is conjugated to a carrier protein,
- d) the conjugate obtained in step c) is combined with at least one or more antigens intended for vaccination against one or more of the following infections: diphtheria, tetanus, polio, hepatitis B, chicken pox, mumps, rubella, infections caused by *Neisseria meningitidis* or *Streptococcus pneumoniae*, infections caused by rotavirus, in order to obtain a vaccine

combination which allows simultaneous immunization against several diseases.

16. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with diphtheria, tetanus and hepatitis B antigens.

17. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with diphtheria toxoid, tetanus toxoid, and 2 acellular *Bordetella pertussis* antigens (toxoid and filamentous hemagglutinin).

18. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with diphtheria toxoid, tetanus toxoid, and 3 acellular *Bordetella pertussis* antigens (toxoid, pertactin and filamentous hemagglutinin).

19. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with diphtheria toxoid, tetanus toxoid, and 5 acellular *Bordetella pertussis* antigens (toxoid, pertactin, agglutinogens and filamentous hemagglutinin).

20. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with diphtheria toxoid, tetanus toxoid, 2, 3 or 5 acellular *Bordetella pertussis* antigens and also inactivated type 1, 2 and 3 polioviruses.

21. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with diphtheria toxoid, tetanus toxoid, hepatitis B, 2, 3 or 5 acellular *Bordetella pertussis* antigens and also inactivated type 1, 2 and 3 polioviruses.

22. The process for preparing a vaccine composition as claimed in claim 15, characterized in that the conjugate obtained in step c) is combined with the whole *Bordetella pertussis* bacterium.

Listing of Claims:

1. A process for producing, on an industrial scale, capsular polysaccharide of *Haemophilus influenzae* type b (PRP) suitable for use in a vaccine, the process comprising culturing a strain of *Haemophilus influenzae* type b (Hib) in a culture medium, and harvesting and treating supernatant from the culture medium to extract the capsular polysaccharide therefrom, said culture medium comprising at least:
   - a source of carbon,
   - a protoporphyrin,
   - salts,
   - amino acids,
   - NAD or NADH,
   - vitamins,
   - pH-regulating means, and
   - zinc,

   wherein said culture medium is chemically defined.

2. The process as claimed in claim 1, wherein said pH-regulating means consist of buffer salts.

3. The process as claimed in claim 1, wherein said source of carbon is glucose, fructose, galactose, glycerol, xylose, ribose, fucose, sialic acid, lactate, or a combination thereof.

4. The process as claimed in claim 1, wherein said protoporphyrin is synthetic protoporphyrin IX.

5. The process as claimed in claim 1, wherein said salts are chosen from potassium, magnesium, sodium, calcium, iron, zinc, cobalt and manganese salts.

6. The process as claimed in claim 1, wherein said salts are chosen from: K_2HPO_4; KH_2PO_4; $MgSO_4$. 7 H_2O; Na_2HPO_4.12 H_2O; NaH_2PO_4. 2 H_2O; $CaCl_2$. 2 H_2O; $FeSO_4$. 7 H_2O; $ZnSO_4$. 7 H_2O; $CoCl_2$. 6 H_2O; and $MnSO_4$. H_2O.

7. The process as claimed in claim 1, wherein the composition comprises:

- arginine or citrulline,
- alanine,
- histidine,
- tryptophan,
- tyrosine,
- phenylalanine,
- at least one of cystine, cysteine, or glutathione, and
- at least one of asparagine, glutamine, aspartic acid, or glutamic acid.

8. The process as claimed in claim 1, wherein said culture medium comprises at least arginine, alanine, histidine, tryptophan, tyrosine, phenylalanine, cystine, aspartic acid and glutamic acid.

9. The process as claimed in claim 7, wherein the at least one of cysteine, cysteine, or glutathione is glutathione or cysteine.

10. The process as claimed in claim 1, wherein said vitamins are chosen from: thiamine, pantothenate, uracil, hypoxanthine, biotin, riboflavin and pyridoxine.

11. The process as claimed in claim 7, wherein the arginine or citrulline is citrulline.

12. The process as claimed in claim 1, further comprising conjugating the capsular polysaccharide obtained to a carrier protein.

13. The process as claimed in claim 12, wherein the carrier protein is tetanus toxoid.

14. A process for preparing a vaccine composition, the process comprising

a) preparing an antigen against *Haemophilus influenzae* type b (Hib) according to the method of claim 1,

b) conjugating the capsular polysaccharide obtained in a) to a carrier protein,

c) combining the conjugate obtained in b) with at least one or more antigens suitable for use in a pediatric vaccine.

15. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with diphtheria, tetanus and hepatitis B antigens.

16. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with diphtheria toxoid, tetanus toxoid, and acellular *Bordetella pertussis* antigens toxoid and filamentous hemagglutinin.

17. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with diphtheria toxoid, tetanus toxoid, and acellular *Bordetella pertussis* antigens toxoid, pertactin and filamentous hemagglutinin.

18. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with diphtheria toxoid, tetanus toxoid, and acellular *Bordetella pertussis* antigens toxoid, pertactin, agglutinogens and filamentous hemagglutinin.

19. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with diphtheria toxoid, tetanus toxoid, 2, 3 or 5 acellular *Bordetella pertussis* antigens and also inactivated type 1, 2 and 3 polioviruses.

20. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with diphtheria toxoid, tetanus toxoid, hepatitis B, 2, 3 or 5 acellular *Bordetella pertussis* antigens and also inactivated type 1, 2 and 3 polioviruses.

21. The process for preparing a vaccine composition as claimed in claim 15, wherein the conjugate obtained in b) is combined with the whole *Bordetella pertussis* bacterium.

22. The process as claimed in claim 7 wherein the culture medium further comprises one or more of lysine, valine, isoleucine, and leucine, and phenylalanine.

23. The process as claimed in claim 1 wherein the culture medium comprises at least thiamine, pantothenate, uracil or citrulline, hypoxanthine, biotin, riboflavin, and pyridoxine.

24. The process as claimed in claim 1, wherein said vitamins are chosen from thiamine, pantothenate, citrulline, hypoxanthine, biotin, riboflavin and pyridoxine.

25. The process according to claim 15 wherein the one or more antigens are selected from antigens suitable for use in vaccination against diphtheria, tetanus, polio, hepatitis B, chicken pox, mumps, rubella, infections caused by *Neisseria meningitidis* or *Streptococcus pneumoniae*, or infections caused by rotavirus.

26. The process according to claim 15, wherein the conjugate obtained in c) is combined with at least one or more antigens against diphtheria, tetanus, poliomyelitis, hepatitis B, infections caused by *Neisseria meningitidis* or by *Streptococcus pneumonia.*