

We Claim:

1. An immunogenic composition comprising:
 - (i) a diphtheria toxoid, (D);
 - (ii) a tetanus toxoid, (T);
 - 5 (iii) an inactivated whole cell pertussis antigen (wP);
 - (iv) a hepatitis B virus surface antigen, (HBsAg);
 - (v) a *Haemophilus influenzae* type b (Hib) capsular saccharide conjugated to a carrier protein;
 - (vi) an inactivated polio virus antigen, (IPV);
 - 10 (vii) an adjuvant
 - (viii) a preservative; and
 - (ix) a dilution medium or buffer

wherein the composition is a fully liquid combination vaccine, showing improved immunogenicity, reduced reactogenicity and improved stability at 2-8°C and room

15 temperature.
2. The immunogenic composition as claimed in claim 1, wherein the composition further comprises one or more antigen selected from group of *Haemophilus influenzae* (a, c, d, e, f serotypes and the unencapsulated strains), Hepatitis (A, C, D, E, F and G strains), rotavirus, *Neisseria meningitidis* A antigen(s), *Neisseria meningitidis* C antigen(s), *Neisseria meningitidis* W-135 antigen(s), *Neisseria meningitidis* Y antigen(s), *Neisseria meningitidis* X antigen(s), *Streptococcus Pneumoniae* antigen(s), *Neisseria meningitidis* B antigen(s), *Staphylococcus aureus* antigen(s), Anthrax, BCG, Human papilloma virus, *Salmonella typhi* antigen(s), acellular pertussis antigen, modified adenylate cyclase, Malaria Antigen (RTS,S), Measles, Mumps, Rubella, Flavivirus Antigen, Dengue, Zika, Ebola, Chikungunya, Japanese encephalitis, and Diarrheal antigens.
- 25 3. The immunogenic composition as claimed in claim 2, wherein the acellular pertussis antigen comprises one or more antigens selected from modified
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adenylate cyclase, Pertussis toxoid (PT), Filamentous hemagglutinin (FHA), Pertactin (P69 or PRN) or Fimbrial proteins (FIM 1, 2 and 3).

4. The immunogenic composition as claimed in claim 1, wherein the composition comprises one or more adjuvant selected from the group of aluminium salt (Al^{3+}) such as aluminium hydroxide ($\text{Al}(\text{OH})_3$) or aluminium phosphate (AlPO_4), alum, calcium phosphate, MPLA, 3D-MPL, QS21, a CpG-containing oligodeoxynucleotide adjuvant, liposome, or oil-in-water emulsion.
5. The immunogenic composition as claimed in claim 4, wherein the composition comprises aluminium phosphate (AlPO_4) as adjuvant.
6. The immunogenic composition as claimed in any one of claims 1 to 5, wherein the composition comprises total aluminum content (Al^{3+}) in an amount of 0.1 mg per 0.5 ml to 0.6 mg per 0.5 ml.
7. The immunogenic composition as claimed in claim 1, wherein the inactivated whole cell pertussis antigen (wP) is of *Bordetella pertussis* strains 134, 509, 25525 and 6229.
8. The immunogenic composition as claimed in claim 1, wherein the IPV antigens are Salk strains selected from the group of Mahoney type 1, MEF Type 2 or Saukett type 3 or Sabin strains selected from the group of Type 1, Type 2 or Type 3.
9. The immunogenic composition as claimed in claim 8, wherein the IPV antigens are adsorbed onto aluminium salt having Al^{3+} concentration between 0.1 mg per 0.5 ml to 1.25 mg per 0.5 ml and percent adsorption of at least 70 %.
10. The immunogenic composition as claimed in claim 9, wherein the IPV antigens are adsorbed onto aluminium salt having Al^{3+} concentration between 0.1 mg per 0.5 ml to 0.6 mg per 0.5 ml and percent adsorption of at least 90 %.

11. The immunogenic composition as claimed in claim 1, wherein the D antigen are adsorbed onto aluminium salt having percentage adsorption of at least 50%.
12. The immunogenic composition as claimed in claim 1, wherein the T antigen are
5 adsorbed onto aluminium salt having percentage adsorption of at least 40%.
13. The immunogenic composition as claimed in claim 1, wherein the HBsAg antigen are adsorbed onto aluminium salt having percentage adsorption of at least 70%.
- 10 14. The immunogenic composition as claimed in claim 1, wherein the Hib antigen is a Hib polyribosylribitol phosphate (PRP) polysaccharide conjugated to a carrier protein using a cyanylation conjugation chemistry or reductive amination conjugation chemistry, wherein said cyanylation reagent is selected from Cyanogen Bromide, 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP), 1-cyano-4-pyrrolidinopyridinium tetrafluoroborate (CPPT), 1-cyanoimidazole
15 namely (1-CI), 1-cyanobenzotriazole (1-CBT) or 2-cyanopyridazine-3(2H)one (2-CPO); and carrier protein is selected from a group comprising of CRM197, Diphtheria toxoid, Neisseria meningitidis outer membrane complex, fragment C of tetanus toxoid, pertussis toxoid, protein D of *H. influenzae*, *E. coli* LT, *E. coli* ST, and exotoxin A from *Pseudomonas aeruginosa*, outer membrane complex c
20 (OMPC), porins, transferrin binding proteins, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal surface adhesin A (PsaA), pneumococcal PhtD, pneumococcal surface proteins BVH-3 and BVH-11, protective antigen (PA) of *Bacillus anthracis* and detoxified edema factor (EF) and lethal factor (LF) of *Bacillus anthracis*, ovalbumin, keyhole limpet hemocyanin (KLH), human serum albumin, bovine serum albumin (BSA) and purified protein derivative of tuberculin (PPD), synthetic peptides, heat shock proteins, pertussis proteins, cytokines, lymphokines, hormones, growth factors, artificial proteins comprising multiple
25 human CD4⁺ T cell epitopes from various pathogen-derived antigens such as N 19, iron-uptake proteins, toxin A or B from *C. difficile* and *S. agalactiae* proteins with or without linker.
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15. The immunogenic composition as claimed in claim 1, wherein the Hib antigen is not substantially adsorbed on to any adjuvant.
- 5 16. The immunogenic composition as claimed in claim 1, wherein the composition comprises preservative selected from the group of 2-phenoxyethanol, Benzethonium chloride (Phemerol), Phenol, Thiomersal, Formaldehyde, methyl and propyl parabens, or benzyl alcohol or a combination thereof.
- 10 17. The immunogenic composition as claimed in claim 16, wherein the composition comprises 2-phenoxyethanol as preservative.
18. The immunogenic composition as claimed in claim 1, wherein the composition is free of preservative.
- 15 19. The immunogenic composition as claimed in claim 1, wherein the composition comprises buffer selected from the group of sodium chloride, phosphate buffer saline.
- 20 20. The immunogenic composition as claimed in claim 19, wherein the composition comprises sodium chloride as dilution medium or buffer.
- 25 21. The immunogenic composition as claimed in claim 1, wherein the composition further comprises a pharmaceutically acceptable transporter, excipient, binder, carrier, isotonic agent, emulsifier or humectant.
22. The immunogenic composition as claimed in claim 20, wherein the composition comprises pharmaceutically acceptable excipient selected from the group of sugars and polyols, surfactants, polymers, salts, aminoacids, pH modifiers.
- 30 23. The immunogenic composition as claimed in any one of the claims 1-22, wherein the composition comprises D antigen in an amount of about 10 Lf per 0.5 ml to 25

5 Lf per 0.5 ml; T antigen in an amount of about 2 Lf per 0.5 ml to 10 Lf per 0.5 ml; wP antigen in an amount of about 12 IOU per 0.5 ml to 16 IOU per 0.5 ml; HBsAg in an amount of about 7 µg per 0.5 ml to 15 µg per 0.5 ml; Hib antigen in an amount of about 7 µg per 0.5 ml to 13 µg per 0.5 ml; IPV antigen Salk type 1 in an amount of about 1 - 50 DU, Salk Type 2 in an amount of about 1 - 20 DU or Salk type 3 in an amount of about 1 - 50 DU, respectively, per 0.5 ml; aluminium phosphate in an amount of about 0.1 mg per 0.5 ml to 2.5 mg per 0.5 ml; 2-Phenoxyethanol in an amount of about 1 mg per 0.5 ml to 6 mg per 0.5 ml; sodium chloride in an amount of about 0.5% to 1.5%.

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24. The immunogenic composition as claimed in any one of claims 1 to 23, wherein the composition comprises D antigen in an amount of about 10 Lf per 0.5 ml to 25 Lf per 0.5 ml; T antigen in an amount of about 2 Lf per 0.5 ml to 10 Lf per 0.5 ml; wP antigen in an amount of about 12 IOU per 0.5 ml to 16 IOU per 0.5 ml; HBsAg
15 in an amount of about 7 µg per 0.5 ml to 15 µg per 0.5 ml; Hib antigen in an amount of about 7 µg per 0.5 ml to 13 µg per 0.5 ml; IPV antigen Sabin type 1 in an amount of about 1 - 50 DU, Sabin Type 2 in an amount of about 1 - 20 DU or Sabin type 3 in an amount of about 1 - 50 DU, respectively, per 0.5 ml; aluminium phosphate in an amount of about 0.1 mg per 0.5 ml to 2.5 mg per 0.5 ml; 2-
20 Phenoxyethanol in an amount of about 1 mg per 0.5 ml to 6 mg per 0.5 ml; sodium chloride in an amount of about 0.5% to 1.5%.

25. The immunogenic composition as claimed in any one of claims 1 to 24, wherein the composition comprises D antigen in an amount of about 10 Lf per 0.5 ml; T
25 antigen in an amount of about 2 Lf per 0.5 ml; wP antigen in an amount of about 12 IOU per 0.5 ml; HBsAg in an amount of about 8 µg per 0.5 ml; Hib antigen in an amount of about 8 µg per 0.5 ml; IPV antigen Salk type 1 in an amount of about 40 DU, Salk Type 2 in an amount of about 8 DU or Salk type 3 in an amount of about 32 DU, respectively, per 0.5 ml; aluminium phosphate in an amount of about
30 1.25 mg per 0.5 ml; 2-Phenoxyethanol in an amount of about 2.5 mg per 0.5 ml; sodium chloride in an amount of about 0.9%.

26. The immunogenic composition as claimed in any one of claims 1 to 25, wherein the composition comprises D antigen in an amount of about 20 Lf per 0.5 ml; T antigen in an amount of about 4 Lf per 0.5 ml; wP antigen in an amount of about 14 IOU per 0.5 ml; HBsAg in an amount of about 15 µg per 0.5 ml; Hib antigen in an amount of about 10 µg per 0.5 ml; IPV antigen Salk type 1 in an amount of about 40 DU, Salk Type 2 in an amount of about 8 DU or Salk type 3 in an amount of about 32 DU, respectively, per 0.5 ml; aluminium phosphate in an amount of about 1.25 mg per 0.5 ml; 2-Phenoxyethanol in an amount of about 2.5 mg per 0.5 ml; sodium chloride in an amount of about 0.9%.
27. The immunogenic composition as claimed in any one of claims 1 to 26, wherein the composition comprises D antigen in an amount of about 25 Lf per 0.5 ml; T antigen in an amount of about 10 Lf per 0.5 ml; wP antigen in an amount of about 16 IOU per 0.5 ml; HBsAg in an amount of about 15 µg per 0.5 ml; Hib antigen in an amount of about 13 µg per 0.5 ml; IPV antigen Salk type 1 in an amount of about 40 DU, Salk Type 2 in an amount of about 8 DU or Salk type 3 in an amount of about 32 DU, respectively, per 0.5 ml; aluminium phosphate in an amount of about 1.25 mg per 0.5 ml; 2-Phenoxyethanol in an amount of about 2.5 mg per 0.5 ml; sodium chloride in an amount of about 0.9%.
28. The process of manufacturing a fully liquid combination vaccine composition showing improved immunogenicity, reduced reactogenicity and improved stability comprising
- (i) a diphtheria toxoid, (D);
 - (ii) a tetanus toxoid, (T);
 - (iii) an inactivated whole cell pertussis antigen, (wP)
 - (iv) a hepatitis B virus surface antigen, (HBsAg);
 - (v) a *Haemophilus influenzae* type b (Hib) capsular saccharide conjugated to a carrier protein; and
 - (vi) an inactivated polio virus antigen, (IPV);
- comprising the steps of:

- a). Addition of Normal saline in a blending vessel / container
b). Addition of Component - I comprising Diphtheria Toxoid
c). Addition of Component – II comprising Tetanus Toxoid
d). Addition of inactivated whole cell pertussis antigen
5 e). Addition of Component – III comprising Hepatitis B surface Antigen
f). Addition of Hib Antigen
g). Addition of IPV antigen
h). Addition of 2-Phenoxy Ethanol
i). Adjusting pH to 6.0 to 7.0 with Sodium Hydroxide / Sodium Carbonate
10 j). Adding normal saline to make up the volume.
29. The process as claimed in claim 28, wherein the preparation of the component I comprises the following steps:
a). Transfer of aluminum phosphate in the container / vessel
15 b). Addition of the Diphtheria Toxoid
c). pH adjustment to 4.5 to 5.5 with Acetic Acid / Sodium Hydroxide
d). stabilization
e). pH adjustment to 5.5 to 6.5 with Sodium Hydroxide / Sodium Carbonate
f). stabilization
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30. The process as claimed in claim 28, wherein the preparation of the component II comprises the following steps:
a). Transfer of aluminum phosphate in the container / Vessel
b). addition of the Tetanus Toxoid
25 c). pH adjustment to 4.5 to 5.5 with Acetic Acid / Sodium Hydroxide
d). stabilization
e). pH adjustment to 5.5 to 6.5 with Sodium Hydroxide / Sodium Carbonate
f). stabilization
- 30 31. The process as claimed in claim 28, wherein the preparation of the component III comprises the following steps:

- a). Transfer of aluminum phosphate in the container / Vessel
 - b). addition of the Hepatitis B surface Antigen
 - c). pH adjustment to 4.5 to 5.5 with Acetic Acid / Sodium Hydroxide
 - d). stabilization
 - 5 e). pH adjustment to 5.8 to 6.8 with Sodium Hydroxide / Sodium Carbonate
 - f). stabilization
32. The process as claimed in claim 28, wherein the preparation of the inactivated whole cell pertussis antigen comprises the following steps:
- 10 a). inactivation at 56°C for 10 – 15 minutes in presence of formaldehyde of *Bordetella pertussis* strains 134
 - b). inactivation at 56°C for 10 – 15 minutes in presence of formaldehyde of *Bordetella pertussis* strains 509
 - c). inactivation at 56°C for 10 – 15 minutes in presence of formaldehyde of *Bordetella pertussis* strains 25525 and 6229
 - 15 c). inactivation at 56°C for 10 – 15 minutes in presence of formaldehyde of *Bordetella pertussis* strains 6229
 - d). subsequently mixing inactivated *Bordetella pertussis* strains 134, 509, 25525 and 6229 in a ratio of 1:1:0.25:0.25.
 - 20 e). optionally adsorbed onto aluminium based adjuvant.
- wherein the process is devoid of thimerosal and inactivated whole cell pertussis antigen remains non-clumpy and homogeneous thereby leading to reduced reactogenicity and giving better potency for a longer duration.
- 25 33. The process as claimed in claim 28, wherein the preparation of the Hib antigen comprises the steps of:
- a). Fermentation of *Haemophilus influenzae* Type b
 - b). Inactivation at 37 °C for 2 hours in presence of 0.1% formaldehyde
 - c). Purification of Hib polyribosylribitol phosphate (PRP) polysaccharide

- d). Conjugation of purified product of step c to tetanus toxoid (TT) using a Cyanogen Bromide cyanylation conjugation chemistry in presence of adipic acid dihydrazide (ADH) linker
- e). purification of conjugate of step d
- 5 f). filtration of purified conjugate preferably through 0.22 μm filter
- wherein the percentage free PRP is no more than 5% in the total purified Hib bulk conjugate.
- 10 34. A fully liquid combination vaccine composition showing improved immunogenicity, reduced reactogenicity and improved stability substantially as herein described with reference to accompanying examples.
- 15 35. The process for manufacturing a fully liquid combination vaccine composition showing improved immunogenicity, reduced reactogenicity and improved stability substantially as herein described with reference to accompanying examples.