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(54) Title: VACCINE COMPOSITIONS COMPRISING SACCHARIDE ANTIGENS

(57) Abstract: The present invention pertains to vaccine compositions comprising an immune stimulating complex which is associated with a carrier protein A, saccharide antigens attached to a carrier protein B, and an aluminium containing adjuvant. The immune stimulating complex comprises saponins and sterols and is capable of binding a nucleic acid. The immune stimulating complex may further comprise a lipophilic moiety. The vaccine compositions are capable of inducing an immune response to the saccharide antigen and/or carrier protein A. The present invention furthermore relates to uses of such vaccines in treatment of infections, preferably in the prophylactic treatment of infections.



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Vaccine compositions comprising saccharide antigens

Background

5 Prokaryotes are surrounded by cell walls usually comprising saccharide moieties. In addition, many prokaryotes are encapsulated by polysaccharide.

Thus, immunological responses against a prokaryote may be specific to particular saccharide moieties displayed on the prokaryote surface, for example to the
10 polysaccharide capsule of a given prokaryote. Pathogenic bacteria commonly produce a thick mucous-like layer of polysaccharides. Examples of polysaccharide encapsulated bacteria includes *Haemophilus influenza type B*, *Streptococcus pneumoniae*, *Neisseria meningitides*, *Group B streptococcus* and *Salmonella typhi*.

15 Vaccines protecting against bacterial infections may in some instances comprise saccharide antigens. However, one major drawback of the current vaccine is the low efficiency, in particular vaccines comprising free saccharide antigen in general are non efficient in young children below the age of 2. In order to circumvent this problem vaccines have been prepared wherein saccharide antigens are covalently linked to a
20 protein carrier. Children below the age of 2 also respond to these kinds of vaccines (see for example Lees et al., 1996, Vaccine, 14(3):190.198). However, the efficiency of these vaccines is also low requiring large amounts of saccharide antigen to be administered.

25 Saccharide antigens are in general T-independent antigens and cannot be processed or presented on MHC molecules to interact with T-cells. They can however stimulate the immune system through an alternate mechanism.

Furthermore, there can be issues with repeat administration of multivalent vaccine
30 comprising polysaccharide-protein conjugates. For example, it has been reported that a *Haemophilus influenzae type b* polysaccharide (PRP) vaccine using tetanus toxoid (TT) as the protein carrier was tested in a dosage-range with simultaneous immunization with (free) TT and a pneumococcal polysaccharide-TT conjugate vaccine following a standard infant schedule. As the dosage of the pneumococcal vaccine was increased,
35 the immune response to the PRP polysaccharide portion of the Hib conjugate vaccine

was decreased, indicating immune interference of the polysaccharide (Dagan et al , Infect immun (1998), 66- 2093-2098). Accordingly, the number of different antigens which may be comprised within a given vaccine is limited.

5 Examples of currently used vaccines comprising saccharide antigen includes:

Prevenar (Wyeth) comprises capsule polysaccharides from 7 different pneumococcal serotypes covalently bound to CRM₁₉₇ protein (2 to 4 µg polysaccharide from each serotype per dosage). The vaccine may be given also to young children, is however, only effective against pneumococcus of the 7 serotypes comprised therein.

10

Pneumax (Sanofi Pasteur) comprises 23 different pneumococcal serotypes (25 µg polysaccharide from each serotype per dosage). The vaccine is not useful for children below the age of 2. In addition, the vaccine is only useful against pneumococcus of one of the specific 23 serotypes.

15

NeisVac-C (Baxter) comprises *Neisseria meningitides* serogroup C. The vaccine may be given to infants older than 2 months, however it is only effective against serogroup C.

20

ISCOMs are structurally well defined complexes of saponin and cholesterol. In the classical ISCOMs the major ISCOM constituents are quillaja saponins and cholesterol. The procedure for preparation of ISCOM comprises solubilization of amphipathic polypeptides in preferably nonionic detergents, addition of Quillaja saponins, cholesterol, and possibly also phosphatidylcholine. In the presence of amphipathic proteins, ISCOM particles are formed on removal of the detergent.

25

Morein (see e.g. US 4,578,269) was the first to describe that ISCOMs not only formed a very characteristic structural complex, but also possessed significant immunogenic properties when amphipathic antigens were inserted into this complex by hydrophobic interaction. Conventional ISCOMs (immunostimulating complexes) have since been used for vaccine formulations and combine a multimeric presentation of an antigen with an adjuvant functionality.

30

WO 02/080981 describes a particular form of ISCOMs, herein after also referred to as Posintro. Posintros are complexes of saponin and cholesterol comprising a contacting group capable of binding e.g. proteins or nucleic acids.

- 5 WO2004/030696 describes vaccine compositions for transdermal delivery. The compositions may comprise an immunogen, an occlusion vehicle and a delivery system in the form of a Posintro or an ISCOM.

10 Summary of invention

As outlined above, the current vaccines comprising saccharide antigen are in need of improvement, in particular in relation to efficiency. Thus, there is a need for more efficient vaccine compositions, wherein said vaccines may comprise only very small
15 amounts of saccharide antigen.

Accordingly, the present invention in one aspect provides a vaccine composition comprising

- 20 i) an immune stimulating complex comprising
- a. at least one saponin; and
 - b. at least one sterol; and
 - c. at least one contacting group capable of binding a nucleic acid, wherein
25 said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, said complex being associated with at least one carrier protein (herein designated carrier protein A) and
- ii) at least one saccharide antigen attached to a carrier protein (herein designated carrier protein B); and
- 30 iii) at least one aluminium containing adjuvant.

Preferably, the saccharide antigen attached to carrier protein B is adhered to said aluminium containing adjuvant. Optionally the immune stimulating complex further comprises a lipophilic moiety.

35

In a further aspect the present invention provides a vaccine composition comprising

- i) an immune stimulating complex comprising
 - a. at least one saponin; and
 - b. at least one sterol; and
 - 5 c. at least one contacting group capable of binding a nucleic acid, wherein said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, andsaid complex being associated with at least one carrier protein (herein designated carrier protein A) and
 - 10 ii) at least one saccharide antigen attached to a carrier protein (herein designated carrier protein B); and
 - iii) at least one aluminium containing adjuvant,
- said composition being capable of inducing an immune response to said
- 15 carrier protein A and said at least one saccharide antigen.

Optionally the immune stimulating complex comprises a lipophilic moiety.

The invention furthermore relates to uses of such vaccines in treatment of infections,

20 preferably in the prophylactic treatment of infections.

Description of drawings

25 Figure 1. NMRI mice were immunized subcutaneous with Prevenar high dose or low dose with the addition of PosintroTM-HBsAg. The mean values of anti-19F IgG titer +/- standard deviation from each group are shown (n=2-3).

Figure 2. NMRI mice were immunized subcutaneous with Prevenar high dose or low

30 dose with the addition of PosintroTM-HBsAg. The mean values of anti-23F IgG titer +/- standard deviation from each group are shown (n=2-3).

Figure 3. NMRI mice were immunized subcutaneous with Prevenar high dose or low dose with the addition of PosintroTM-HBsAg. The mean values of Max OD +/- standard deviation from each group are shown (n=2-3).

5 Figure 4. NMRI mice were immunized subcutaneous with Prevenar high dose or low dose with the addition of PosintroTM-HBsAg. The mean values of Max OD +/- standard deviation from each group are shown (n=2-3).

10 Figure 5 illustrates preferred sterols comprising a contacting group which may be contained in the immune stimulating complexes according to the invention. The listed compounds are ChoTB and ChoSC as described by Leventis, R. et.al. (1989) Biochim Biophys Acta 1023, 124; DC-Chol and TC-Chol as described by Avanti Lipids and further characterised herein; Lipid 67 (also known as GL-67), as described by Lee, E.R. et.al. (1996) Human Gene Therapy 7,1701; BGTC is an acronym for 3-beta[N',N'-
15 diguanidioethyl-aminoethane)carbamoyl]cholesterol; and BGSC is an acronym for 3-beta[4N-(1N,8N-diguanidino spermidine)-carbamoyl]cholesterol.

Figure 6. Mice were immunized with HBsAg combined with different adjuvant systems. Twelve days after the second immunization cells from the draining lymph nodes were
20 cultured in vitro with HBsAg or medium only as control. After 5 days incubation in vitro the BrdU proliferation assay was performed. The black bars show proliferation with the addition of HBsAg and the white bars show background proliferation without addition of HBsAg. The mean OD values from three individual animals $\pm$ SD are shown.

25 Figure 7.

Mice were immunized with HBsAg combined with different adjuvant systems. Twelve days after the second immunization cells from the draining lymph nodes were cultured in vitro with HBsAg or medium only as control.
30 After 5 days incubation in vitro the cells were harvested and analyzed on a flow cytometer. The total amount of proliferating cells were gated and counted. The HBsAg induced number of cells was obtained by subtracting the number of positive cells incubated with HBsAg with the number of positive cells incubated with medium only. HBsAg induced proliferating cell number with mean values $\pm$ SD from 5-6 individual
35 animals in each group except PBS control group constituted of 3 animals taken from

two individual experiments are shown. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS.

Figure 8. HBsAg induced CD4+ and CD8+ T-cells.

- 5 Mice were immunized with HBsAg combined with different adjuvant systems. Twelve days after the second immunization cells from the draining lymph nodes were cultured in vitro with HBsAg or medium only as control.
- After 5 days incubation in vitro the cells were harvested and analyzed on a flow cytometer. The total amount of proliferating cells were gated and counted. The HBsAg induced number of either **a.** antigen specific CD4+ T-cells or **b.** antigen specific CD8+ T-cells was obtained by subtracting the number of positive cells incubated with HBsAg with the number of positive cells incubated with medium only.
- 10 HBsAg induced CD4+ or CD8+ T-cell numbers with mean values $\pm$ SD from 5-6 individual animals in each group except the PBS control group constituted of 3 animals.
- 15 Induced cell numbers from two individual experiments are shown. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS; the Posintro group gave a p-value of 0.0535, in the CD4+ graph.

Figure 9. HBsAg induced cellular responses measured by DTH-assay.

- 20 Mice were immunized twice and one week after the last immunization all mice were immunized s.c. in both back footpads. Before injection the footpad thickness was measured. In one footpad PBS was injected and in the other footpad HBsAg. 24 hours later the footpad thickness was measured. The mean increase in mm of the footpad $\pm$ SD, from 4 individual animals in each group, was recorded for each adjuvant.

25

Figure 10. ELISPOT for anti-Hepatitis B secreting B-cells.

- Mice were immunized with HBsAg combined with different adjuvant systems. Twelve days after the second immunization cells from the spleen were cultured with HBsAg coated filter plates for detection of anti-HBsAg producing B-cells. HBsAg induced antibody secreting B-cells/ million spleen cells from 5-6 individual animals in each group except the PBS control group constituted of 3 animals. Data from two individual experiments are shown. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS.
- 30

- 35 Figure 11. HBsAg induced total IgG titers in guinea pigs.

Guinea pigs were immunized twice with HBsAg with different adjuvant systems. 4 weeks after the last immunization serum was extracted and total IgG titer was measured. Mean values $\pm$ standard deviation from 10 individual animals/group are shown. Hepatitis B specific IgG antibody titers were calculated from interpolation of the linear part of the titration curves as the dilution of serum at 50% saturation at a log10 scale for the dilution. All values were correlated to an internal standard. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving Twinrix.

10 Figure 12. HBsAg induced total IgG titers in mice.

Mice were immunized twice with HBsAg with different adjuvant systems. 12 days after the last immunization serum was extracted and total IgG titer was measured. Mean values $\pm$ SD from 6 individual animals in each group are shown. Hepatitis B specific IgG antibody titers were calculated from interpolation of the linear part of the titration curves as the dilution of serum at 50% saturation at a log10 scale for the dilution. All values were correlated to an internal standard. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS.

Figure 13. HBsAg induced total IgG1 titers in mice.

20 Mice were immunized twice with HBsAg with different adjuvant systems. 12 days after the last immunization serum was extracted and total IgG1 titer was measured. Mean values $\pm$ SD from 6 individual animals in each group are shown. Hepatitis B specific IgG1 antibody titers were calculated from interpolation of the linear part of the titration curves as the dilution of serum at 50% saturation at a log10 scale for the dilution. All values were correlated to an internal standard. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS.

Figure 14. HBsAg induced total IgG2a titers in mice.

30 Mice were immunized twice with HBsAg with different adjuvant systems. 12 days after the last immunization serum was extracted and total IgG2a titer was measured. Mean values $\pm$ SD from 6 individual animals in each group are shown. Hepatitis B specific IgG2a antibody titers were calculated from interpolation of the linear part of the titration curves as the dilution of serum at 50% saturation at a log10 scale for the dilution. All

values were correlated to an internal standard. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS.

5 Figure 15. Mice were immunized with HBsAg combined with different adjuvant systems. 12 days after the second immunization cells from the draining lymph nodes were cultured in vitro with HBsAg or medium only as control. After 5 days incubation supernatant was taken and analyzed on the IFN-gamma contents using the Luminex detection system. Data from 3-6 individual animals/group and two individual experiments is shown. Statistical analysis, shown in the graph, was done by comparing
10 different groups with the group receiving HBsAg+PBS, the Posintro group gave a p-value of 0.0566.

Figure 16. Mice were immunized with HBsAg combined with different adjuvant systems. 12 days after the second immunization cells from the draining lymph nodes
15 were cultured in vitro with HBsAg or medium only as control. After 5 days incubation supernatant was taken and analyzed on the IL-3 contents using the Luminex detection system. Data from 3-6 individual animals/group and two individual experiments is shown. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS., the Posintro group gave a p-value of
20 0.0566.

Figure 17. Mice were immunized with HBsAg combined with different adjuvant systems. 12 days after the second immunization cells from the draining lymph nodes were cultured in vitro with HBsAg or medium only as control. After 5 days incubation
25 supernatant was taken and analyzed on IL-5 contents using the Luminex detection system. Data from 3-6 individual animals/group and two individual experiments is shown. Statistical analysis, shown in the graph, was done by comparing different groups with the group receiving HBsAg+PBS, the Posintro group gave a p-value of
30 0.0566.

Figure 18. NMRI mice were immunized parenteral with Prevenar[®] together with HBsAg combined with Posintro[™] or the commercial Hepatitis vaccines Twinrix[®] (with AI as adjuvant) or Fendrix[®] (with AI and MPL as adjuvant). Anti-Hepatitis B IgG titer was measured three weeks after second immunization in serum. Mean values+/- standard
35 deviation from each group are shown (n=4).

Figure 19. NMRI mice were immunized parenteral with NeisVac-C[®] together with HBsAg incorporated in Posintro[™]. Anti-C total IgG titer was measured two weeks after second immunization in serum. Mean values +/- standard deviation from each group are shown (n=14).

Figure 20. NMRI mice were immunized parenteral with NeisVac-C[®] together with HBsAg incorporated in Posintro[™]. Anti-C IgG1 (Th2) titer was measured two weeks after second immunization in serum. Mean values +/- standard deviation from each group are shown (n=14).

Figure 21. NMRI mice were immunized parenteral with NeisVac-C[®] together with HBsAg incorporated in Posintro[™]. Anti-C IgG2a (Th1) titer was measured two weeks after second immunization in serum. Mean values +/- standard deviation from each group are shown (n=14).

Detailed description of invention

Immune stimulating complex

The vaccine compositions according to the present invention comprise an immune stimulating complex. The immune stimulating complex according to the invention comprises

- a. at least one saponin and
- b. at least one sterol and
- c. optionally a lipophilic moiety

Preferably, said complex is an ISCOM. The immune stimulating complexes according to the present invention may in one preferred embodiment adopt a micro-particle structure in the form of a cage-like matrix similar to, or preferably identical to, that known as an ISCOM. An ISCOM adopts a cage-like structure with icosahedral symmetry. More preferably the immune stimulating complexes of the invention have a cage-like structure with icosahedral symmetry having a diameter of 30 to 40 nanometers and are composed of 12 nanometer ring-like subunits. Beside ISCOM

structures, the interaction between sterols and saponins have been reported to result in a variety of different structural entities, including entities such as e.g. lattices, honeycombs, rods, and amorphous particles, all of which structural entities may be adopted by the immune stimulating complexes according to the present invention.

5 However, other structures and matrix formations although less preferred may also be adopted by the immune stimulating complexes of the present invention. Any of the below mentioned immune stimulating complexes may adopt any of the aforementioned structures.

10 The molar ratio between saponins and sterols in the immune stimulating complexes according to the present invention is preferably from less than 1000:1 to preferably more than 1:1000. Preferred ratios are about 100:1, for example about 80:1, such as about 60:1, for example about 50:1, such as about 40:1, for example about 30:1, such as about 25:1, for example about 20:1, such as about 18:1, for example about 16:1,
15 such as about 14:1, for example about 12:1, such as about 10:1, for example about 9:1, such as about 8:1, for example about 7:1, such as about 6:1, for example about 5:1, such as about 4:1, for example about 3:1, such as about 2:1, for example about 1.9:1, such as about 1.8:1, for example about 1.7:1, such as about 1.6:1, for example about 1.5:1, such as about 1.4:1, for example about 1.3:1, such as about 1.2:1, for
20 example about 1.1:1, such as about 1:1, for example about 1:1.1, such as about 1:1.2, for example about 1:1.3, such as about 1:1.4, for example about 1:1.5, such as about 1:1.6, for example about 1:1.7, such as about 1:1.8, for example about 1:1.9, such as about 1:2.0, for example about 1:2.5, such as about 1:3, for example about 1:3.5, for example about 1:4, such as about 1:4.5, for example about 1:5, for example about
25 1:5.5, such as about 1:6, for example about 1:7, such as about 1:10, for example about 1:20, such as about 1:40, for example about 1:60, such as about 1:80, for example about 1:100.

In a preferred embodiment the immune stimulating complex is a complex comprising
30 i) at least one saponin and
ii) at least one sterol and
iii) at least one contacting group capable of binding a nucleic acid,

wherein said contacting group may be covalently attached to said at least one saponin
35 or to said at least one sterol or to said at least one lipophilic moiety. The contacting

group may be any group which is capable of contacting and binding a nucleic acid via electrostatic or hydrophobic interactions.

The immune stimulating complex is thus preferably capable of binding a nucleic acid. In general however, the immune stimulating complex is also capable of binding other molecules or complexes. In particular, the immune stimulating complex is in general capable of binding proteins, such as carrier protein A. The immune stimulating complex may preferably also be capable of binding saccharides, such as mono-, oligo-, or poly-saccharides.

In a more preferred embodiment said immunostimulating complex is a Posintro. The terms "Posintro" and "PosintroTM" as used herein cover an immune stimulating complex comprising

- i) at least one saponin and
- ii) at least one sterol and
- iii) at least one cationic moiety

wherein said cationic moiety may be covalently attached to said at least one saponin or to said at least one sterol or to said at least one lipophilic moiety.

Optionally the immune stimulating complex comprises a lipophilic moiety.

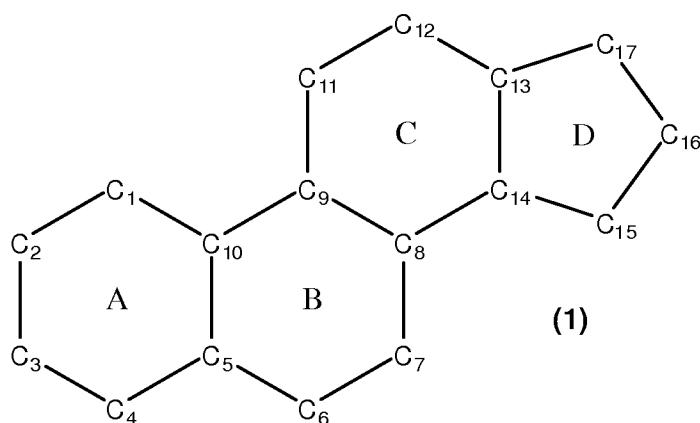
The Posintro of the present invention is capable of binding DNA and is in general further also capable of binding other molecules or complexes. The Posintro may in particular be capable of binding proteins, such as carrier protein A. The Posintro may also be capable of binding saccharides, such as mono-, oligo-, or poly-saccharides.

In particular, the immune stimulating complex may be any of the complexes comprising saponin and sterol described in US2003118635, for example the immune stimulating complex may be a Posintro, which is a common name for the saponin and sterol containing complexes containing cationic moiety described in US2003118635, and in a most preferred embodiment of the present invention, the immunostimulating complex is any complex as described and claimed in US2003118635. US2003118635 is incorporated herein in its entirety by reference.

The sterol of the complex may be any sterol capable of forming a complex with a saponin, for example the sterol may any of the sterols described in US2003118635, thus the sterol may be a first sterol or a second sterol as described in US2003118635. The saponin may be any saponin capable of forming a complex with a sterol, for example the saponin may be any of the saponins described in US2003118635, thus the saponin may for example be a first saponins or a second saponin as described in US2003118635.

Sterols, such as first and second type sterols and saponins, such as first and second type saponins according to the present invention may be as described in US2003118635, see e.g. pages 6-8, pages 25-27 and in particular for saponins pages 33-55, and in particular for sterols pages 55-60 of the original document as filed.

Thus the sterol may be any sterol comprising the characteristic skeleton structure of gonane as depicted below. Said gonane may be substituted independently at any of the carbons of the gonane skeleton with any suitable substituent (see below). All sterols of the invention comprises the characteristic molecular structure composed of 17 carbon atoms arranged in four rings conventionally denoted by the letters A, B, C, and D and bonded to hydrogen atoms and suitable substituents.



This skeleton structure (1) is named gonane when all C atoms are fully substituted with hydrogen atoms, i.e. one hydrogen atom bond to each of the carbon atoms no. 5, 8, 9, 10, 13, and 14 and two hydrogen atoms bound to each of the remaining carbon atoms no. 1, 2, 3, 4, 6, 7, 11, 12, 15, 16, and 17. This structure is often referred to as the steroid nucleus. This skeleton structure may be modified in a large number of ways by

replacing the hydrogen atoms of the structure with suitable substituents or optionally additional bonds between carbon atoms of the gonane skeleton, thereby generating e.g. double bonds of the C=C type. Substituents or additional bonds may be introduced at any of the 17 carbon atoms in the structure (1), and more than one substituent or additional bond may be introduced at a single carbon atom, in particular at the carbon atoms no. 1, 2, 3, 4, 6, 7, 11, 12, 15, 16, and 17. Suitable substituents may for example be hydroxyl (-OH); un-branched alkyls, such as methyl, ethyl, propyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl or longer alkyls; branched alkyls; branched or un-branched acyclic alkenes; branched or un-branched partly cyclic or entirely cyclic alkenes; alkynes; or sulphur, for instance in the form of a thiol-group (-SH), for example the substituents are selected from the group consisting of (-OH), C₁₋₁₀ linear or branched alkyl, sulphur, amides and any of the aforementioned substituted with an amino group, which may be primary, secondary, tertiary or quaternary, preferably quaternary. In particular carbon atom no. 3 may preferably be substituted with a thiol group (-SH), such as is the case in e.g. thiocholesterol, or more preferably carbon atom no. 3 may be substituted with a hydroxyl group (-OH), such as is the case in e.g. cholesterol and lanosterol. In particular, the C-17 carbon may be substituted with an alkyl chain, such as a C₁₋₁₀ linear or branched alkyl. Furthermore, any stereocenters, such as a chiral carbon atom, of the optionally substituted structure (1) may be of any suitable configuration. The skilled artisan will know how to isolate steroids from plants and animals, and optionally prepare derivatives by chemical and/or enzymatic treatment of natural steroids. The skilled artisan will also know how to synthesize synthetic steroids from simpler compounds, or natural precursors or parts thereof, and optionally prepare derivatives of such compounds by chemical and/or enzymatic treatment of steroids thus obtained.

In preferred embodiments of the present invention at least one sterol of the complex is selected from the group of sterols consisting of cholesterol, lanosterols, lumisterols, stigmasterols, sitosterols, mycosterols, ergosterols, and thiocholesterols. Preferably, at least one sterol is cholesterol.

It is furthermore preferred that the complex comprises at least two different sterols, wherein the first sterol preferably is any of the above-mentioned sterols. The second sterol preferably is covalently linked to a cationic moiety. The cationic moiety may be any moiety comprising a positive electrical charge capable of attracting a negative

electrical charged moiety at pH=7.0. Examples of cationic moieties includes amine salts and quaternary ammonium salts. Preferred examples of such compounds are illustrated in Figure 5. More preferably the second sterol is, comprises, or essentially consists of 3 β -[N-(Dimethylaminoethane)-carbamoyl]cholesterol (DC-cholesterol) and/or N-(trimethylammonioethane)-carbamoylcholesterol (TC-cholesterol).

Accordingly, one preferred sterol according to the invention is a sterol, and any cationic derivative thereof, in particular cationic derivatives obtained by linking a cationic moiety (such as any of the cationic moieties described herein above) to e.g. an OH-group of the sterol, including an OH-group located at position 3 of the steroid skeleton, including the OH-group of cholesterol located at position 3 (C3, or 3-OH). Consequently, preferred sterols according to the present invention are cholesterol (CAS (Chemical Abstract) accession no. 57-88-5) and any cationic derivative thereof, in particular cationic derivatives obtained by linking a cationic moiety, to the OH-group located at position 3 of the steroid skeleton (C3, or 3-OH).

Saponins according to the present invention are compounds comprising a saccharide part linked by means of a glycosidic bond to one of i) a triterpene aglycone part, ii) a steroid aglycone part, and iii) a steroid alkaloid aglycone part. Saponin as used herein denotes either a substantially purified saponin, or one or more saponins comprised in a crude composition or a composition obtained by predetermined purification means. Preferably, the saponin is a substantially purified saponin. The saponins pertaining to the present invention may be naturally occurring or synthetic, or they may be made by chemical synthesis, or enzymatic synthesis involving one or more enzyme catalysed steps, either in vitro or in vivo.

It is preferred that at least one saponin is selected from the group consisting of saponins comprising a triterpene glycoside, saponins comprising a steroid glycoside, and saponins comprising a steroid alkaloid glycoside. More preferably, the saponin comprises or essentially consists of a triterpene glycosid comprising an aglycone compound and a saccharide compound. In a preferred embodiment the saponin comprises or essentially consists of a triterpene glycosid isolated from a Quillaja plant species. More preferably, the saponin is isolated from a Quillaja saponaria species. Even more preferably the saponin is isolated from Quillaja saponaria Molina or Quillaja saponaria Officinalis. Most preferably, the saponin is Quillajabark Araloside A (Quil A).

Preferably, said Quil A comprises saponins such as QA-7, QA-17, QA-18 and QA-21 also denoted QS-7, QS-17, QS-18 and QS-21, respectively. Yet more preferably the saponin comprises or even essentially consists of QS-21. By "essentially consists of" is meant that no other saponin is detectable in the complex.

5

The immune stimulating complexes according to the invention may optionally comprise a lipophilic moiety. The lipophilic moiety may be any compound with a LogP value, which is higher than 1, preferably higher than 2, for example higher than 3. Preferred lipophilic moieties are a naturally-occurring, synthetic or semi-synthetic (modified

10 natural) compounds which is generally amphipathic. Lipids typically comprise a hydrophilic component and a hydrophobic component. The phrase semi-synthetic (modified natural) denotes a natural compound that has been chemically modified in some fashion.

15 The lipophilic moieties may serve the purpose of facilitating complex formation while at the same time acting as a "docking" group for contacting groups, such as cationic moieties. The lipophilic moiety may be an amphipathic compound whose molecules contain both polar and non-polar domains. Surfactants are examples of amphipathic compounds. Surfactants typically possess a non-polar portion that is often an alkyl, aryl

20 or terpene structure. In addition, a surfactant possesses a polar portion that can be anionic, cationic, amphoteric or non-ionic. Examples of anionic groups are carboxylate, phosphate, sulfonate and sulfate. Examples of cationic domains are amine salts and quaternary ammonium salts. Amphoteric surfactants possess both an anionic and a cationic domain. Non-ionic domains are typically derivatives of a fatty acid carboxy

25 group and include saccharide and polyoxyethylene derivatives.

A lipophilic moiety can also comprise two or more compounds possessing non-polar domains, wherein each of the compounds has been bonded to a linking group, which, in turn, is covalently attached to a component of the immune stimulating complex,

30 including any saponin component and/or any sterol component comprised in said complex, including any residues of said sterol component and any residues of said saponin component, including any aglycone part and/or any saccharide part.

One group of preferred lipophilic moieties is phospholipids such as

35 phosphatidylcholine, phosphatidylethanolamine, triglycerides, fatty acids, and

hydrophobic amino acids residues including membrane spanning hydrophobic amino acid segments.

5 In one embodiment the immune stimulating complexes to be used with the present invention comprises i) a saponin derived from a Quil A fraction as described by WO 92/06710, which is incorporated herein by reference, ii) cholesterol and iii) phosphatidylcholine or phosphatidylethanolamine.

10 Examples of lipophilic moieties capable of being used in connection with the present invention are lipids other than sterols, for example fats or fat resembling substances such as e.g. triglycerides or mixed triglycerides containing fatty acids with up to 50 carbon acids such as saturated fatty acids having for example 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 and 30 carbon atoms e.g. butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid; 15 unsaturated fatty acids with up to 30 carbon atoms, such as hexadecene acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid; hydroxy-fatty acids such as 9,10-dihydroxy stearic acid; unsaturated hydroxy fatty acids such as castor oil; branched fatty acids such as glycerol ethers; waxes i.e. esters between higher fatty acids and monohydric alcohols; phospholipides such as derivatives of glycerol phosphates such as derivatives of phosphatidic acids i.e. lecithin, cephalin, inositol phosphatides, 20 spingosine derivatives with 14, 15, 16, 17, 18, 19 and 20 carbon atoms; glycolipids; isoprenoids; sulpholipids; and carotenoids.

25 Additional examples of lipophilic moieties capable of forming part of the complexes according to the present invention are lipids covalently linked to a cationic moiety. Examples of such lipids are phosphatidyl ethanolamine, phosphatidyl choline, glycerol-3-ethylphosphatidyl choline and fatty acyl esters thereof, di- and trimethyl ammonium propane, di- and tri-ethylammonium propane and fatty acyl esters thereof.

30 Suitable lipophilic moieties which may be comprised in the complexes of the invention may also be any of the lipophilic moieties described on pp. 66-74 of WO 02/080981. In the case where the complexes according to the present invention do form ISCOMS, or ISCOM-like structures, such ISCOMS or structures may be prepared e.g. essentially 35 as described in US2003118635, see e.g. pages 4-6 (of the original document as filed)

and example 2. ISCOM-like complexes may also be prepared essentially as described in US Patent 5679354, such as in examples 1 to 5.

It is preferred that the Posintro has a zeta-potential which is not too negative.

5 Accordingly, the Posintro preferably has a zeta-potential which is less negative or more positive than about -50 mV, such as a zeta-potential of about -45 mV, for example a zeta-potential of about -40 mV, such as a zeta-potential of about -37 mV, for example a zeta-potential of about -35 mV, such as a zeta-potential of about -32 mV, for example a zeta-potential of about -30 mV, such as a zeta-potential of about -29 mV, for example a
10 zeta-potential of about -28 mV, such as a zeta-potential of about -27 mV, for example a zeta-potential of about -26 mV, such as a zeta-potential of about -25 mV, for example a zeta-potential of about -20 mV, such as a zeta-potential of about -15 mV, for example a zeta-potential of about -10 mV, such as a zeta-potential of about -5 mV, for example a zeta-potential of about 0 mV, such as a zeta-potential of about 5 mV, for example a
15 zeta-potential of about 10 mV, such as a zeta-potential of about 15 mV, for example a zeta-potential of about 20 mV, and preferably a zeta-potential of less than 50 mV.

Preferred Posintro to be used with the present invention may thus have a zeta-potential of from about -50 mV to about -40 mV, such as from about -40 mV to about -35 mV,
20 for example of from about -35 mV to about -30 mV, such as from about -30 mV to about -25 mV, for example of from about -25 mV to about -20 mV, such as from about -20 mV to about -15 mV, for example of from about -15 mV to about -10 mV, such as from about -10 mV to about -5 mV, for example of from about -5 mV to about 0 mV, such as from about 0 mV to about 5 mV, for example of from about 5 mV to about 10
25 mV, such as from about 10 mV to about 15 mV, for example of from about 15 mV to about 20 mV, such as from about 20 mV to about 30 mV, for example of from about 30 mV to about 40 mV, such as from about 40 mV to about 50 mV.

Using as a standard a reference complex comprising i) any given saponin(s) of interest
30 and ii) cholesterol, Posintros to be used with the present invention comprise i) any given saponin(s) of interest, ii) cholesterol, and iii) any given second sterol of interest, preferably a cholesterol derivative as listed in Fig. 5 herein, and preferably said Posintros have a zeta-potential which is at least about 5 mV less negative or more positive than said reference complexes, such as a zeta-potential which is at least about
35 10 mV less negative or more positive than said reference complexes, for example a

zeta-potential which is at least about 15 mV less negative or more positive than said reference complexes, such as a zeta-potential which is at least about 20 mV less negative or more positive than said reference complexes, for example a zeta-potential which is at least about 25 mV less negative or more positive than said reference complexes, such as a zeta-potential which is at least about 30 mV less negative or more positive than said reference complexes, for example a zeta-potential which is at least about 35 mV less negative or more positive than said reference complexes, such as a zeta-potential which is at least about 40 mV less negative or more positive than said reference complexes, for example a zeta-potential which is at least about 45 mV less negative or more positive than said reference complexes, such as a zeta-potential which is at least about 50 mV less negative or more positive than said reference complexes.

The values of the zeta-potentials are preferably determined as described in Example 4 of WO 02/080981.

In embodiments of the invention, wherein the aluminium adjuvant comprises at least one anionic group, in particular, when the adjuvant is $\text{Al}(\text{OH})_3$ it is preferred that the immune stimulating complex is a Posintro. In this embodiment the Posintro may be any of the Posintros described above.

Carrier protein

The vaccine compositions according to the present invention in general comprises at least two carrier proteins, herein designated carrier protein A and carrier protein B, respectively. It is also comprised within the invention that the vaccine composition may comprise more than two carrier proteins, such as more than one kind of carrier protein A and more than one kind of carrier protein B. Preferably, however, the vaccine composition comprises only one kind of carrier protein A and one kind of carrier protein B.

In one embodiment the vaccine compositions according to the present invention comprises only carrier protein A.

Carrier protein A is associated with the immune stimulating complex according to the invention, whereas carrier protein B is associated with at least one saccharide antigen. In general, carrier protein A is associated with the immune stimulating complex by means of hydrophobic or electrostatic interaction, a combination of these or covalently bound. In general, carrier protein A is not covalently linked to any saccharide antigen.

In general carrier protein B is associated with at least one saccharide antigen, preferably by means of a covalent bond. Frequently, carrier protein B will be covalently linked to at least one saccharide antigen via a linker. It is furthermore preferred that carrier protein B is not directly associated with the immune stimulating complex.

Carrier protein B can thus be linked to the saccharide antigen by means of a linker. The linker may for example be a labile linker such as e.g. a linker of the type disclosed in WO 97/49425. The linker may also be the type of linker disclosed in Shafer et al., 2000, Vaccine 18:1273-1281 or Lees et al., 1996, Vaccine 14(3):190-198, such as a hexanediamine or adipic dihydrazide.

It is possible that carrier protein A and carrier protein B are similar proteins. Thus carrier protein A and carrier protein B may be derived from the same protein, they may have similar sequences or even identical sequences. It is however preferred that carrier protein A is different to carrier protein B.

The function of a carrier protein can for example be to increase the molecular weight of the immunogenic determinant or the antigenic determinant in order to increase the activity or immunogenicity of the determinant, to confer stability to the determinant, to increase the biological activity of the determinant, or to increase its serum half-life. In preferred embodiments of the invention an immune response may be induced by carrier protein A.

Carrier protein A and carrier protein B may independently be any suitable carrier protein known to the skilled person. Thus carrier protein A and carrier protein B may independently be selected from the group consisting of bacterially derived proteins, virally derived proteins, lipopeptides, members of the polyhistidine triad family of proteins and fusions thereof, keyhole limpet hemocyanin, PPD (purified protein derivative of Tuberculin), pneumococcal pneumolysin (PLY), including detoxified PLY

for example dPLY-GMBS or dPLY-formol, OMPC (meningococcal outer membrane protein - usually extracted from *N. meningitidis* serogroup), PorB (from *N. meningitidis*), PD (*Haemophilus influenzae* protein D), heat shock protein, *Plasmodium falciparum* pfg27, lactate dehydrogenase peptide, gp120 of HIV, pertussis proteins, cytokines, lymphokines, artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens, such as N19 protein, pneumococcal surface protein, iron uptake proteins, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin, ovalbumin, immunoglobulins and hormones, such as insulin and immune stimulating peptides such as P2 and/ or P30 from Tetanus Toxin or the synthetic PADRE epitope (Pan DR Epitope). For example carrier protein A and carrier protein B may independently be selected from the carrier proteins described in WO2007/071711, in particular from the carrier proteins described therein on p. 8, l. 18 to p. 11, l. 28 or from the proteins mentioned in US patent application 2006/0051361, sections 43-47 or in WO2005/105141 p. 5, l. 18-36.

Bacterially derived proteins may be any protein, which is naturally produced by a bacterium or fragments and variants thereof, wherein fragments comprises at least 10, such as at least 20, for example at least 30 consecutive amino acids of the naturally produced bacterial protein and wherein variants are proteins sharing at least 70%, such as at least 80%, for example at least 90%, such as at least 95% sequence identity with a naturally produced bacterial protein. Bacterially derived proteins are preferably bacterial toxins or variants of bacterial toxins, for example such variants which are not or are less toxic to mammals, such as human beings. Variants of bacterial toxins, which are inactivated either by genetic mutation, chemical treatment or by conjugation are also referred to as toxoids herein. Bacterial toxins may for example be tetanus toxin and/or diphtheria toxin and/or *Pseudomonas aeruginosa* exotoxin A or toxin A or B of *C. difficile*. Variants of bacterial toxins useful as carrier proteins within the scope of the present invention includes bacterial toxoids, such as tetanus toxoid, fragment C of tetanus toxoid, diphtheria toxoid (such as diphtheria toxoid as described in US patent 4,496,538, for example in Example III), pertussis toxoid, bacterial cytolysins or pneumolysin. Mutations of pneumolysin (Ply) have been described which lower the toxicity of pneumolysin (WO 90/06951, WO 99/03884). Useful variants of diphtheria toxin which lower its toxicity are known and include for example CRM197 and other mutants described in US 4,709,017, US 5,843,711, US 5,601,827, and US 5,917,017, as well as CRM176, CRM228, CRM 45 (Uchida et al J. Biol. Chem. 218; 3838-3844,

1973); CRM 9, CRM 45, CRM102, CRM 103 and CRM107 and other mutations described by Nicholls and Youle in Genetically Engineered Toxins, Ed: Frankel, Maecel Dekker Inc, 1992. CRM197 (SEQ ID NO:2) is a form of the diphtheria toxin with little or no toxicity. The CRM197 protein has the same molecular weight as the diphtheria toxin but differs from it by a single base change in the structural gene. This leads to a glycine to glutamine change of amino acid at position 52 which makes fragment A unable to bind NAD and therefore non-toxic (Pappenheimer 1977, Ann Rev, Biochem. 46; 69-94, Rappuoli Applied and Environmental Microbiology Sept 1983 p560-564).

Virally derived protein may be any protein, which is naturally encoded by a viral genome or fragments and variants thereof, wherein fragments comprises at least 10, such as at least 20, for example at least 30 consecutive amino acids of the natural viral protein and wherein variants are proteins sharing at least 70%, such as at least 80%, for example at least 90%, such as at least 95% sequence identity with a natural viral protein. Preferred virally derived proteins are proteins derived from Hepatitis type B, Polio, Morbilli paramyxovirus, Parotis, Rubella, and human papillom virus. One example of a preferred virally derived protein is Hepatitis type B surface antigen (HBsAg) (SEQ ID NO: 1).

The polyhistidine triad family of proteins comprises PhtA, PhtB, PhtD or PhtE proteins that have an amino acid sequence sharing 80%, 85%, 90%, 95%, 98%, 99% or 100% identity with a sequence disclosed in WO 00/37105 or WO 00/39299 (e.g. with amino acid sequence 1-838 or 21-838 of SEQ ID NO 4 of WO 00/37105 for PhtD). A histidine triad motif is the portion of polypeptide that has the sequence HxxHxH where H is histidine and x is an amino acid other than histidine. In an embodiment carrier protein A and/or carrier protein B comprises one or more histidine triad motifs. 299).

As mentioned above carrier protein A and/or carrier protein B may be a lipopeptide, in particular carrier protein A may be a lipopeptide. The lipopeptide may be any lipopeptide, such as a synthetic lipopeptide, such as any of the synthetic lipopeptides described in US patent application 2007/0160631. Suitable lipopeptide preferably comprises at least one lysine moiety and a lipid moiety linked to the peptide via the N-terminal amino group or via the ϵ -amino group of lysine. Preferred synthetic lipopeptides includes lipopeptides of formula VI of US patent application 2007/016063

p. 8-9, sections 108-115. More preferred synthetic lipopeptides includes the lipopeptides disclosed in figures 1 and 2 of US patent application 2007/016063.

5 The carrier protein A and/or carrier protein B and in particular carrier protein A may be HBsAg or functional equivalents thereof. Functional equivalents of HBsAg are polypeptides sharing at least 70%, preferably at least 80%, more preferably at least 90%, even more preferably at least 95% sequence identity with HBsAg of SEQ ID NO: 1.

10 In a preferred embodiment carrier protein A and carrier protein B may independently be selected from the group consisting of tetanus toxoid, diphtheria toxoid, diphtheria toxin mutant CRM197, any of the above mentioned lipopeptides and HBsAg and any of the functional equivalents of HBsAg described above. Preferably, carrier protein A is HBsAg, any of the functional equivalents of HBsAg described above or any of the
15 above mentioned lipopeptides, more preferably HBsAg. Carrier protein B preferably is CRM197.

For immunization of humans, the protein carrier must be a physiologically acceptable carrier acceptable to humans and safe. However, tetanus toxoid and/or diphtheria toxoid
20 are suitable carriers in one embodiment of the invention.

Carrier protein A and carrier protein B may be prepared by any suitable means, for example they may be purified from a natural source by conventional techniques or they may be recombinantly produced. Methods for preparation of recombinant proteins are
25 well known to the skilled person and are for example described in Molecular Cloning: A Laboratory Manual (*Third Edition*) by Sambrook and Russell. Briefly, a nucleic acid is introduced into a desirable host cell, said nucleic acid comprising a first nucleic acid sequence encoding the carrier protein is operably linked to a second nucleic acid sequence directing expression of the first nucleic acid sequence in said host cell.
30 Carrier protein expressed by the host cell may then be purified by conventional means.

In embodiments of the invention wherein the carrier protein, such as carrier protein A is HBsAg, then said HBsAg may be purified from plasma or more preferably recombinantly manufactured.

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In embodiments of the invention wherein the carrier protein, such as carrier protein B is CRM197 then CRM197 is preferably recombinantly produced.

5 **Aluminium adjuvant**

The vaccine compositions according to the invention comprise one aluminium containing adjuvant.

10 An aluminium containing adjuvant according to the present invention is any compound capable of modifying an immune response to a particular antigen, preferably augmenting an immune response to a particular antigen when co-administered with said antigen to a mammal, wherein the compound comprises aluminium. Modification of the immune response means augmentation, intensification, or broadening the specificity of either or both antibody and cellular immune responses.

15 It is preferred that the aluminium containing adjuvant has a rigid structure, for example in the form of a grid or a crystal. More preferably, said grid structure has an overall negative charge.

20 It is furthermore preferred that the saccharide antigen attached to carrier protein A is capable of being adsorbed onto said aluminium containing adjuvant. It is also preferred that said immune stimulating complex is capable of being adsorbed onto said aluminium containing adjuvant. Thus, in a very preferred embodiment of the invention the vaccine composition comprises an aluminium containing adjuvant to which immune
25 stimulating complex and saccharide antigen/carrier protein B is adsorbed.

The aluminium containing adjuvant may comprise aluminium in any form such as aluminium in alum or aluminium in the form of any other salt. Thus, preferably the aluminium containing adjuvant comprises Al^{3+} ions.

30 The corresponding anion may be any suitable anion, for example sulphate, hydroxide, halogens or phosphate.

35 Thus the aluminium containing adjuvant may be selected from the group consisting of $\text{AlK}(\text{SO}_4)_2$, $\text{AlNa}(\text{SO}_4)_2$, $\text{AlNH}_4(\text{SO}_4)$, $\text{Al}(\text{PO}_4)$ and $\text{Al}(\text{OH})_3$.

In a preferred embodiment of the invention, the aluminium containing adjuvant is aluminium phosphate or aluminium hydroxide, such as $\text{Al}(\text{PO}_4)$ or $\text{Al}(\text{OH})_3$.

- 5 When the aluminium containing adjuvant is aluminium phosphate or aluminium hydroxide, then it is preferred that the immune stimulating complex is a Posintro, such as any of the Posintros described herein above.

10 Further Adjuvant

In addition to the aluminium adjuvant described above and the immune stimulating complex described above, it is also comprised within the scope of the present invention, that the vaccine compositions may comprise further adjuvants.

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The term "adjuvant" as used herein is any substance whose admixture with an administered antigen modifies the immune response. Modification of the immune response means augmentation, intensification, or broadening the specificity of either or both of antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses such as the induction of tolerance.

20

The further adjuvant could for example be of mineral, bacterial, plant, synthetic or host origin or they could be oil in water emulsions.

25

Further adjuvants could be selected from the silica, $\text{Ca}_3(\text{PO}_4)_2$, kaolin, carbon, muramyl dipeptides, N-bacetyl-muramyl-L-threonyl-D-isoglutamine (thr-DMP), N-acetyl-nornuramyl-L-alanyl-D-isoglutamine (CGP 11687, also referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, also referred to as MTP-PE), RIBI (MPL+TDM+CWS) in a 2% squalene/Tween-80.RTM. emulsion, lipopolysaccharides, lipid A, Freund's Complete Adjuvant (FCA), Freund's Incomplete Adjuvants, Merck Adjuvant 65, polynucleotides, wax D from Mycobacterium tuberculosis, substances found in Corynebacterium parvum, Bordetella pertussis, and members of the genus Brucella, liposomes, Titermax, Lipid A derivatives, Heat Shock Proteins, HSP

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derivatives, LPS derivatives, synthetic peptide matrixes, GMDP, IL-1, IL-2, IL-5, IL-10, IL-12, IL-15 and lipopeptides.

5 **Saccharide antigen**

The saccharide antigen according to the present invention is in general oligosaccharides comprising or preferably consisting of associated saccharide monomers. Typically, oligosaccharides have a low number of repeat units (typically 5-
10 30 repeat units) and are typically hydrolysed polysaccharides, such as polysaccharides purified from for example a bacterial capsule, which have been hydrolysed.

Frequently, the saccharide antigen is a polysaccharide purified from a bacterial capsule of a fragment thereof. Polysaccharides from bacterial capsules may be purified
15 according to conventional methods, for example as described in Example I of US patent 4,496,538. Alternatively, many capsular bacterial polysaccharides may be commercially available.

After purification bacterial polysaccharide may be directly used as saccharide antigens,
20 however, preferably the bacterial polysaccharides are fragmented into shorter oligosaccharides, preferably by hydrolysis. Thus the saccharide antigen is preferably an oligosaccharide. Hydrolysis may be performed by a number of methods known to the skilled person, for example by treatment with hydrogen peroxide, for example as described in international patent application WO2005/105141, p. 5, l. 5-17. The skilled
25 person will be able to adapt that description of WO2005/105141 to other polysaccharides.

The vaccine compositions may comprise more than one different saccharide antigen, such as in the range of 2 to 200, for example in the range of 2 to 150, such as in the
30 range of 2 to 100, for example in the range of 2 to 75, such as in the range of 2 to 50, for example in the range of 2 to 30, such as in the range of 5 to 30 for example in the range of 10 to 30, such as in the range of 15 to 30, for example at least 7, such as at least 10, for example at least 15, such as at least 20, for example at least 23, such as at least 30, for example at least 40, such as at least 50, for example in the range of 20
35 to 90, such as in the range of 10 to 90 different saccharide antigens. The different

saccharide antigens may be derived from different microbial organisms, such as from different bacteria, for example from bacteria of different order, such as from bacteria of different family, such as from bacteria of different genus. It is however also possible that all or at least some of the different saccharide antigens are derived from bacteria of a specific species, preferably from different serotypes of bacteria of a specific species.

The saccharide antigen(s) to be comprised within the vaccines of the present invention are preferably derived from a microbial organism (such as a microbial organism, which is a pathogen), more preferably from a microbial organism (preferably microbial organisms, which are pathogens) selected from the group consisting of amoebae and bacteria, even more preferably from a bacteria, preferably a bacteria, which is a pathogen.

By the term "derived from" is meant in the context of this section that the saccharide is isolated from said microbial organism or that said saccharide is identical to saccharides isolated from said microbial organism. Thus, in this context, the term "derived from" is not intended to include saccharides isolated from microbial organisms, which have been subsequently been modified by for example substitution or otherwise. However, as described herein below the saccharide antigen may be covalently linked to carrier protein B. Furthermore, saccharides derived from a given microbial organism may also be fragments of saccharides obtainable by purification from bacteria, such as encapsulated bacteria, wherein said fragment preferably is an oligosaccharide comprising at least 5, such as at least 8 sugar residues. The fragments may be obtained by fragmentation, for example by hydrolysis.

Capsular polysaccharides of encapsulated bacteria, such as *Streptococcus pneumoniae* comprise repeating oligosaccharide units which may in general contain up to 8 sugar residues. In one embodiment, a saccharide antigen may be a full length polysaccharide, however in others it may be one oligosaccharide unit, or a shorter than native length saccharide chain of repeating oligosaccharide units. Preferably, the saccharide antigen is an oligosaccharide comprising at least one repeating unit, and thus for example if the saccharide antigen is derived from *Streptococcus pneumoniae*, the saccharide antigen is an oligosaccharide preferably comprising at least 8 sugar residues.

The size of the saccharide antigen may be any suitable size, such as an average size molecular weight; Mw of above 80kDa, for example above 100kDa, such as above 200kDa, for example above 300kDa, such as above 400kDa, for example above 500kDa, such as above 1000kDa, for example in the range of 200 and 2000 KDa.

The saccharide antigen may preferably be derived from a bacteria, which carries polysaccharides on the surface, more preferably, the saccharide antigen may be derived from an encapsulated bacteria. The term "encapsulated bacteria" as used herein covers any bacterium which have an outer covering (capsule) made of polysaccharide.

When the vaccine compositions according to the invention comprises more than one different saccharide antigen, such as in the range of 2 to 200, for example in the range of 2 to 150, such as in the range of 2 to 100, for example in the range of 2 to 75, such as in the range of 2 to 50, for example in the range of 2 to 30, such as in the range of 5 to 30 for example in the range of 10 to 30, such as in the range of 15 to 30, for example at least 7, such as at least 10, for example at least 13, such as at least 15, for example at least 20, such as at least 23, for example at least 30, such as at least 40, for example at least 50, such as in the range of 20 to 90, for example in the range of 10 to 90 different saccharide antigens, then it is preferred that at least 50%, preferably at least 60%, more preferably at least 70%, even more preferably at least 80%, yet more preferably at least 90%, even more preferably at least 95%, yet more preferably essentially all, even more preferably all saccharide antigens are derived from encapsulated bacteria.

The saccharide antigen(s) may preferably be derived from one or more selected from the group consisting of *haemophilus influenza type B*, *streptococcus pneumoniae*, *neisseria meningitides*, *group B streptococcus* and *Salmonella enterica*.

Thus, the saccharide antigen(s) may be derived from *Haemophilus influenzae*, which may also be referred to as *Pfeiffers bacillus* or *Bacillus influenzae*, preferably the saccharide antigen may be derived from *H. influenzae* selected from the group consisting of type a, b, c, d, e, and f, preferably from *H. influenzae* type b. One non-limiting example of a saccharide antigen from *H. influenzae* is the PRP polysaccharide,

which is a heteropolymer of ribitol, ribose and phosphate. A vaccine composition comprising one or more saccharide antigens derived from *H. influenzae* may in particular be useful for reducing the risk of infection by *H. influenzae*. Thus, a vaccine composition comprising one or more saccharide antigens derived from *H. influenzae* may in particular be useful for reducing the risk of an individual encountering one or more of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis or joint infections, preferably bacteremia or bacterial meningitis.

10 The saccharide antigen may also be derived from *Neisseria meningitides* also referred to as *meningococcus*. For example, the saccharide antigen may be derived from meningococcus of any of the serotypes A, B, C, H, I, K, L, 29E, W135, X, Y and/or Z, preferably from serotypes A, C, W135 and/or Y. A vaccine composition comprising one or more saccharide antigens derived from *Neisseria meningitides* may in particular be
15 useful for reducing the risk of infection by *Neisseria meningitides*. Thus, a vaccine composition comprising one or more saccharide antigens derived from *Neisseria meningitides* may in particular be useful for reducing the risk of an individual encountering one or more of bacterial meningitis and meningococcal septicaemia.

20 Thus, the saccharide antigen(s) may also be derived from *Group B Streptococcus*, which may also be referred to as GBS or *Streptococcus agalactiae*. A vaccine composition comprising one or more saccharide antigens derived from *Group B Streptococcus* may in particular be useful for reducing the risk of infection by *Group B Streptococcus*. Thus, a vaccine composition comprising one or more saccharide
25 antigens derived from *Group B Streptococcus* may in particular be useful for reducing the risk of an individual encountering one or more of mastitis, such as bovine mastitis (if the individual is bovine) and perinatal GBS disease.

Thus, the saccharide antigen(s) may also be derived from *Salmonella enterica*, for
30 example the saccharide antigen may be derived from *Salmonella enterica* of any serotype, for example selected from the saccharide antigen may be derived from the Serovar Typhi or serovar typhimurium, such as typhimurium LT2. One non-limiting example of a saccharide antigen from *Salmonella* Typhi is the Vi polysaccharide. A vaccine composition comprising one or more saccharide antigens derived from
35 *Salmonella enterica* may in particular be useful for reducing the risk of infection by

Salmonella enterica. Thus, a vaccine composition comprising one or more saccharide antigens derived from *Salmonella enterica* may in particular be useful for reducing the risk of an individual encountering one or more of typhoid fever (in particular if the saccharide antigen is derived from serovar Typhi) or gastroenteritis (in particular if the saccharide antigen is derived from serovar typhimurium).

In a very preferred embodiment of the invention the vaccine compositions comprises at least one antigen derived from *pneumococcus*, such as at least 2, for example at least 5, such as at least 7, for example at least 13, such as in the range of 5 to 100, for example in the range of 7 to 91, such as in the range of 10 to 91, for example in the range of 13 to 91, such as in the range of 20 to 91 different saccharide antigens derived from *pneumococcus*, preferably from different serotypes of *pneumococcus*.

The saccharide antigen may be derived from any serotype of *pneumococcus*, such as from one or more selected from the group consisting of serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F, for example one or more selected from the group consisting of serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F, such as one or more selected from the group consisting of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, for example one or more selected from the group consisting of serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. *Pneumococcus* is also referred to as *Streptococcus pneumoniae* and presently at least 91 different serotypes are known.

The serotypes differ in their polysaccharide capsule and thus they carry different saccharide antigens. Capsular polysaccharides of *Streptococcus pneumoniae* comprise repeating oligosaccharide units which may in general contain up to 8 sugar residues. For a review of the oligosaccharide units for the key *Streptococcus pneumoniae* serotypes see JONES, Christopher. Vaccines based on the cell surface carbohydrates of pathogenic bacteria. An. Acad. Bras. Cienc, June 2005, vol.77, no.2, p.293-324. ISSN 0001-3765. A vaccine composition comprising one or more saccharide antigens derived from *pneumococcus* may in particular be useful for reducing the risk of infection by *pneumococcus*. Thus, a vaccine composition comprising one or more saccharide antigens derived from *pneumococcus* may in particular be useful for reducing the risk of an individual encountering one or more of pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic

arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis and brain abscess, more preferably one or more of pneumonia, otitis media and meningitis.

In one embodiment of the invention the vaccine composition is a vaccine composition comprising

5

a. an immune stimulating complex comprising

i. at least one saponin, and

ii. at least one sterol, and

iii. at least one contacting group capable of binding a nucleic acid,

10

wherein said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, and

iv. optionally a lipophilic moiety

said complex being associated with at least one carrier protein designated

15

carrier protein A; and

b. at least one saccharide antigen attached to a carrier protein designated carrier protein B; and

c. at least one aluminium containing adjuvant

20

In another embodiment of the invention the vaccine composition is a vaccine composition comprising

a. an immune stimulating complex comprising

i. at least one saponin, and

ii. at least one sterol, and

25

iii. at least one contacting group capable of binding a nucleic acid,

wherein said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, and

said complex being associated with at least one carrier protein designated

30

carrier protein A; and

b. at least one saccharide antigen (which for example may be any of the saccharide antigens described herein above in this section) attached to a carrier protein designated carrier protein B; and

c. at least one aluminium containing adjuvant.

35

Optionally the immune stimulating complex further comprises a lipophilic moiety.

In a preferred embodiment of the invention the vaccine composition is a vaccine composition comprising

- 5 a. an immune stimulating complex comprising
 - i. at least one saponin, and
 - ii. at least one sterol, and
 - iii. at least one contacting group capable of binding a nucleic acid,
10 wherein said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, andsaid complex being associated with at least one carrier protein designated carrier protein A; and
 - 15 b. one or more different *Streptococcus pneumoniae* saccharide antigens attached to a carrier protein designated carrier protein B; and
 - c. at least one aluminium containing adjuvant,
- wherein each dosage unit of said vaccine composition comprises at the most 1 µg of each of said *Streptococcus pneumoniae* saccharide antigens.

20 Optionally the immune stimulating complex further comprises a lipophilic moiety.

In another preferred embodiment the vaccine composition is a vaccine composition comprising

- 25 a. an immune stimulating complex comprising
 - i. at least one saponin, and
 - ii. at least one sterol, and
 - iii. at least one contacting group capable of binding a nucleic acid,
30 wherein said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, andsaid complex being associated with at least one carrier protein designated carrier protein A; and
- b. one or more different *Neisseria meningitidis* saccharide antigens attached to a carrier protein designated carrier protein B; and
- 35 c. at least one aluminium containing adjuvant.

Optionally the immune stimulating complex further comprises a lipophilic moiety.

In a further embodiment the vaccine composition is a vaccine composition comprising

- 5 a. an immune stimulating complex comprising
 - i. at least one saponin, and
 - ii. at least one sterol, and
 - 10 iii. at least one contacting group capable of binding a nucleic acid, wherein said contacting group may be covalently attached to said at least one saponin or to said at least one sterol or to at least one lipophilic moiety, and
 - said complex being associated with at least one carrier protein designated carrier protein A;
- b. at least one saccharide antigen, and
- 15 c. at least one aluminium containing adjuvant.

Optionally the immune stimulating complex further comprises a lipophilic moiety.

20 The at least one saccharide antigen may for example be any of the saccharide antigen described herein above in this sections, preferably one or more different *Neisseria meningitidis* saccharide antigens and/or one or more different *Streptococcus pneumoniae* saccharide antigens.

25 **Preparing saccharide antigen/carrier protein/adjuvant**

The vaccine compositions according to the invention in general comprises at least one saccharide antigen, which may be any of the saccharide antigens described in the section "Saccharide Antigen" herein above, attached to a carrier protein B, which may
 30 be any of the carrier proteins described herein above in the section "Carrier Protein". Preferably, said saccharide antigen is covalently bound to said carrier protein B, optionally through a linker. Hereinafter saccharide antigen covalently bound to carrier protein B is also referred to as "saccharide antigen/carrier protein conjugate". Preferably, the saccharide antigen/carrier protein conjugate is adhered to an aluminium
 35 containing adjuvant. Preferably, adherence of the saccharide antigen/carrier protein

conjugate to the aluminium containing adjuvant is by non-covalent interactions, such as electrostatic interactions.

5 The saccharide antigen/carrier protein conjugate may be prepared by any suitable method known to the skilled person. For example, some saccharide antigens comprise reactive groups, for example amine, carboxyl or aldehyde groups which may conveniently be reacted with a reactive group of the carrier protein B for form a covalent linkage. Thus, carrier protein B preferably comprises one or more reactive groups, preferably selected from the group consisting of primary amine groups (for
10 example at the N-terminus or in the side chains of the amino acids arginine or lysine), carboxyl groups (for example at the C-terminus or in the side chains of aspartate or glutamate) and amide groups with primary amines (for example in the side chains of asparagine or glutamine). It is also comprised within the present invention that the saccharide antigen may be coupled to the carrier protein B via a linker.

15 However, some saccharide antigens require activation before they can be conveniently coupled to carrier protein B. One suitable method for activating saccharide antigens for coupling to carrier protein B is cyanylation for example with CNBr or 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP).

20 Following activation of saccharide antigen, for example by cyanylation, the saccharide antigen may optionally be coupled to a linker.

Suitable linkers for linking a saccharide antigen to carrier protein B includes for
25 example ADH, B-propionamido, nitrophenyl-ethylamine, haloalkyl halides, glycidic linkages, 6-amino-caproic acid, hexanediamine or adipic dihydrazide, preferably the linker may be hexane diamine or adipic dihydrazide.

30 Examples of suitable methods for preparing the saccharide antigen/carrier protein B conjugate are for example described in Shafer et al., 2000, Vaccine 18:1273-1281 or Lees et al., 1996, Vaccine 14(3):190-198. Other suitable methods are described in WO2007 071711 and references therein, in particular the methods described on p. 14, l. 32 to p. 18, l. 35 may be used for preparing saccharide antigen/carrier protein B
35 conjugates according to the present invention. Yet other suitable methods are described in US patent 4,496,538, in particular in Examples II, III and IV. The skilled

person will be able to adapt these methods to a given saccharide antigen/carrier protein B. General methods for preparing saccharide conjugates are described in US patent 6,011,008. These methods are also suitable for preparing conjugates of saccharide antigens and carrier protein B, in particular the methods described in the
5 "Brief Summary of the invention" and in Example X with some adaptations straight forward to the skilled person may be useful.

Following conjugation, excess reactive groups may be reacted with for example glycine.
10

Carrier protein B may also be linked to the saccharide antigen by means of a labile linker. Suitable methods for preparing conjugates of saccharide antigen linked to carrier protein B via a labile linker are disclosed in WO 97/49425.

15 Preferably the ratio of carrier protein B to saccharide antigen is between 1 :5 and 5:1 ; e.g. between 1 :0.5-4:1 , 1 :1-3.5:1 , 1.2:1-3:1 , 1.5:1-2.5:1 ; e.g. between 1 :2 and 2.5:1 ; 1 :1 and 2:1 (w/w). In an embodiment, the majority of the conjugates, for example 6, 7, 8, 9 or more of the conjugates have a ratio of carrier protein B to saccharide antigen that is greater than 1 :1 , for example 1.1 :1 , 1.2:1 , 1.3:1 , 1.4:1 , 1.5:1 or 1.6:1.

20 The vaccine compositions according to the present invention may comprise more than one different kind of carrier protein B and more than one kind of saccharide antigen. If so, it is possible that different kinds of saccharide antigen are attached to different carrier protein B. Thus for example all saccharide antigens of a specific kind may be
25 attached to only one kind of carrier protein B and each kind of carrier protein B may be attached to only this kind of saccharide antigen or each kind of carrier protein B may be attached to different kinds of saccharide antigens. In another example each kind of carrier protein B is only attached to one kind of saccharide antigen, and each kind of saccharide antigen may be attached only to this kind of carrier protein B or each kind of
30 saccharide antigen may be attached to different kinds of carrier protein B.

It is also comprised within the present invention that the vaccine composition comprises only one kind of carrier protein B and more than one kind of saccharide antigen in which case all different kinds of saccharide antigen preferably are attached
35 to, preferably covalently bound to this particular kind of carrier protein B

Some useful saccharide antigen optionally linked to carrier protein and optionally adhered to adjuvant are also commercially available. Useful commercially available saccharide antigen compositions include for example MeningoVax A+C from Sanofi
5 Pasteur MSD, Prevenar from Wyeth and pneumovax from Sanofi Pasteur MSD.

Clinical conditions

10 The present invention relates to vaccine compositions comprising

- i) an immune stimulating complex comprising at least one saponin and at least one sterol associated with at least one carrier protein A and
- ii) at least one saccharide antigen attached to carrier protein B adhered to an aluminium containing adjuvant.

15 In general these vaccine compositions are employed for generation of a protective immune response recognising the saccharide antigen comprised within the vaccine composition. Thus, the vaccine compositions are in general used for prophylactic treatment of an individual before said individual actually encounters a clinical condition.

20 When the vaccine composition comprises more than one saccharide antigen it is contemplated that the vaccine compositions are employed for generation of a protective immune response recognising all of the saccharide antigens comprised within the vaccine composition.

25 In one preferred embodiment the vaccine compositions of the invention may be employed for generation of an immune response, preferably a protective immune response against carrier protein A or carrier protein B, preferably against carrier protein A.

30 The vaccine compositions can also be employed for generation of an immune response, preferably a protective immune response against both carrier protein A and the saccharide antigen.

In these embodiments it is preferred that protein A is derived from a pathogen against
35 which an immune response is desirable. Thus for example in these embodiments,

protein A is preferably a bacterially derived or a virally derived protein, such as any of the bacterially derived or virally derived proteins described herein above in the section "Carrier Protein".

5 As used herein the term "prophylactic treatment" means administration of the vaccine composition to an individual which does not yet suffer from the clinical condition against which treatment is directed and that said administration inhibits or at least reduces the risk of said individual encountering said clinical condition.

10 Thus, it is preferred that a vaccine composition comprising a given saccharide antigen according to the present invention when administered to an individual (preferably a human being) reduces the risk that said individual encounters a clinical condition caused by infection of a microorganism carrying said saccharide antigen on the
15 surfaced is reduced by at least 50%, preferably at least 60%, more preferably at least 70%, even more preferably at least 80%, yet more preferably at least 90%, such as at least 95%, for example at least 98%.

Although less preferred the vaccine compositions of the invention may in some instances also be used in an ameliorating or curative treatment of a clinical condition.

20 During an ameliorating or curative treatment of a clinical condition the vaccine composition is administered to an individual already suffering from said clinical condition.

The clinical condition to be treated, preferably prophylactically treated is preferably
25 infection by one or more microbial organism(s) (preferably encapsulated bacteria, in particular said microbial organism(s) from which the saccharide antigens comprised in the given vaccine composition are derived.

Thus by way of example if the vaccine composition comprises one or more saccharide
30 antigen(s) derived from *Streptococcus pneumonia*, then the clinical condition to be treated (preferably prophylactically) is infection by *Streptococcus pneumonia*. If the vaccine composition comprises one or more saccharide antigen(s) derived from *Streptococcus pneumonia* and one or more saccharide antigens derived from *Haemophilus influenzae*, then the clinical condition to be treated (preferably

prophylactically) is infection by *Streptococcus pneumonia* and/or *Haemophilus influenzae*.

5 Thus, depending on the specific saccharide antigen(s) of a given vaccine composition the clinical condition is preferably one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis or joint infections meningitis and meningococcal septicaemia mastitis, such as bovine mastitis (if the individual is bovine) and perinatal GBS disease, typhoid fever, gastroenteritis, pneumonia, invasive pneumococcal disease, bacteremia, chronic
10 obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis and brain abscess.

15 The vaccine composition can also be used to treat the combination of a viral and a bacterial infection. By way of example the vaccine composition may comprise one or more saccharide antigen(s) and at least one carrier protein A which is capable of inducing an immune response.

20 Thus, in embodiments of the invention wherein Carrier Protein A is a protein derived from Hepatitis type B, such as HBsAg, then the clinical condition is preferably hepatitis. If the composition in addition also comprises one or more saccharide antigens, then the vaccine composition is useful for treating (preferably prophylactically treating) hepatitis as well as one or more additional clinical conditions.

25 Depending on the specific saccharide antigens and the specific carrier protein A of a given vaccine composition the clinical conditions are preferably one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis or joint infections meningitis and meningococcal septicaemia mastitis, such as bovine mastitis (if the individual is bovine)
30 and perinatal GBS disease, typhoid fever, gastroenteritis, pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis, brain abscess, polio, tetanus, hepatitis B, and diphtheria.

35

Administration and formulation

5 The individual to receive the vaccine composition according to the present invention may be any individual in need thereof, preferably a mammal in need thereof, more preferably a human being in need thereof. In embodiments wherein the vaccine composition comprises one or more saccharide antigens derived from *Group B Streptococcus*, then the individual may preferably be a human being or the individual may be bovine. In embodiments wherein one or more saccharide antigens are derived
10 from one or more encapsulated bacteria selected from the group consisting of *Neisseria meningitides*, *H. influenzae*, *Salmonella enterica*, and *Streptococcus pneumoniae* then the individual is preferably a human being.

15 In one preferred embodiment the vaccine composition is administered prophylactically (see above) and in this embodiment the individual in need thereof, may be any individual at risk of encountering the given clinical condition against which the vaccine composition is directed. The individual may thus be any human being and in this embodiment the individual will frequently be a young human being, such as a child, for example a child below the age of 10, such as below the age of 5, for example below
20 the age of 2. One advantage of the vaccine compositions of the present invention is that they are capable of inducing a protective immune response even when administered to young children with an immature immune system, for example to children below the age of 2.

25 The vaccine compositions may be administered in any suitable manner. For example the vaccine compositions may be administered enterally, parenterally, transdermally, orally, by inhalation and/or over a mucosal membrane.

30 In a preferred embodiment, the vaccine compositions are administered parenterally. Examples of parenteral routes of administration include injections and infusions, e.g. intravenous, intraarterial, intramuscular, intracardial, subcutaneous, intraosseous, intradermal, intraperitoneal or intrathecal. A preferred route of parenteral administration of the vaccine composition is by subcutaneous injection. A suitable administration form for parenteral administration is a solution or dispersion.

35

In some embodiments of the present invention it is desirable that the pharmaceutical composition comprises an isotonic agent. In particular when the pharmaceutical composition is prepared for administration by injection or infusion it is often desirable that an isotonic agent is added.

5

Accordingly, the composition may comprise at least one pharmaceutically acceptable additive which is an isotonic agent.

10

The pharmaceutical composition may be isotonic, hypotonic or hypertonic. However it is often preferred that a pharmaceutical composition for infusion or injection is essentially isotonic, when it is administered. Hence, for storage the pharmaceutical composition may preferably be isotonic or hypertonic. If the pharmaceutical composition is hypertonic for storage, it may be diluted to become an isotonic solution prior to administration.

15

The isotonic agent may be an ionic isotonic agent such as a salt or a non-ionic isotonic agent such as a carbohydrate.

20

Examples of ionic isotonic agents include but are not limited to NaCl, CaCl₂, KCl and MgCl₂. Examples of non-ionic isotonic agents include but are not limited to mannitol, sorbitol and glycerol.

25

It is also contained within the present invention that at least one pharmaceutically acceptable additive is a buffer. For some purposes, for example, when the pharmaceutical composition is meant for infusion or injection, it is often desirable that the composition comprises a buffer, which is capable of buffering a solution to a pH in the range of 4 to 10, such as 5 to 9, for example 6 to 8.

30

However, in other embodiments of the invention the pharmaceutical composition may comprise no buffer at all or only micromolar amounts of buffer.

The buffer may for example be selected from the group consisting of TRIS, acetate, glutamate, lactate, maleate, tartrate, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate and triethanolamine buffer.

TRIS buffer is known under various other names for example tromethamine including tromethamine USP, THAM, Trizma, Trisamine, Tris amino and trometamol. The designation TRIS covers all the aforementioned designations.

- 5 The buffer may furthermore for example be selected from USP compatible buffers for parenteral use, in particular, when the pharmaceutical formulation is for parenteral use. For example the buffer may be selected from the group consisting of monobasic acids such as acetic, benzoic, gluconic, glyceric and lactic, dibasic acids such as aconitic, adipic, ascorbic, carbonic, glutamic, malic, succinic and tartaric, polybasic acids such
10 as citric and phosphoric and bases such as ammonia, diethanolamine, glycine, triethanolamine, and TRIS.

- The vaccine composition may also comprise antioxidants and/or reducing agents for
15 example acetone sodium bisulfite, ascorbate, bisulfite sodium, butylated hydroxy anisole, butylated hydroxy toluene, cystein/cysteinate HCL, dithionite sodium, gentisic acid, gentisic acid ethanolamine, glutamate monosodium, formaldehyde sulfoxylate sodium, metabisulfite potassium, metabisulfite sodium, monothioglycerol, propyl gallate, sulfite sodium and thioglycolate sodium.

- 20 In another embodiment the vaccine composition is administered transdermally. Examples of useful administration forms for transdermal administration include ointment, gel, cream, gel-like cream, paste, liquid, lotion, aerosol, spray, liniment, plaster, poultice, foam, bath admixture, a patch and a bandage. Preferably, if the
25 vaccine composition is to be administered transdermally, it is preferably in the form of a patch.

- Ointments, lotions, creams and the like may be prepared as described in Remington "The science and practice of pharmacy", chapter 44 pages 845-851, 20th edition.
30 Patches for transdermal vaccines may be prepared by any conventional methods, for example as described in EP1384403 or WO2004/030696. Preferably patches for transdermal vaccines are prepared essentially as described in Examples 4 to 13 of WO2004/03069 except that the transdermal patches should comprise the vaccine composition according to the present invention. The skilled person will readily be able
35 to make the required adaptations.

For inhalation the vaccine composition may be administered in the form of an aerosol and/or spray dosage form which can be prepared, for example, by filling an aerosol container with above-mentioned solution for example, together with an injection agent
5 such as liquefied petroleum gas. A poultice can be prepared by adding the above mentioned vaccine composition to an ointment base formed from a partially neutralized polyacrylic acid, sodium polyacrylate, and the like.

For oral administration, the vaccine composition may be administered in the form of a
10 tablet, capsule, drop, liquid mixture or powder. Tablet, capsules, and drops may be swallowed or chewed. Oral administration may result in uptake via the mucosa in the mouth, such as buccal or sublingual uptake, and/or in uptake via the gastro-intestinal route, such as uptake over the mucosa of the intestines.

As the pH adjuster, for example, lactic acid, citric acid, phosphoric acid and the like can
15 be mentioned for adjusting to a lower pH range, and sodium hydroxide, potassium hydroxide, sodium lactate, sodium citrate, monoethanolamine and diisopropanolamine and the like can be mentioned for adjusting to a higher pH range. Addition of carboxyvinyl polymer, which is a water-soluble polymer, can also achieve a lower pH.
20 Buffers such as acetate buffer, a phosphate buffer, a citrate buffer, a succinate buffer or TRIS may also be included for pH adjustment.

As the stabilizer, for example, ascorbic acid, dibutylhydroxytoluene, sodium thiosulfate, sodium thioglycolate, sodium thiomalate, erythorbic acid, sodium erythorbate, sodium
25 pyrosulfite, benzoic acid, sodium benzoate, sodium alginate, sodium caprylate, L-arginine, L-cysteine, dl-.alpha.-tocopherol, tocopherol acetate, propyl gallate, disodium edetate and the like can be mentioned.

As the preservative, for example, benzethonium chloride, benzalkonium chloride,
30 methylparaben, ethylparaben, propylparaben, chlorobutanol, benzyl alcohol, thimerosal and the like can be mentioned.

The vaccine compositions of the present invention may also be administered over a mucosal membrane. Thus, the vaccine composition may be applied to any mucous

membrane including the conjunctiva, nasopharynx, orthopharynx, vagina, colon, urethra, urinary bladder, lung, large (rectal) and small (enteral) intestine.

5 When administered ocularly or nasally, the compositions of the present invention can be formulated in an aqueous solution buffered to a pH of between 3.0 and 8.0, most preferably pH 5.0-5.4, by means of a pharmaceutically acceptable buffer system. Any pharmaceutically acceptable buffering system capable of maintaining the pH in the preferred ranges can be used in the practice of this invention. A typical buffer will be, for example, an acetate buffer, a phosphate buffer, a citrate buffer, a succinate buffer, 10 or the like. The concentration of buffer typically range from between 0.005 and 0.1 molar, most preferably about 0.02 molar.

The container, e.g. nasal applicator or eye drop bottle, may contain sufficient composition for a single nasal or ocular dosing or for several sequential dosages.

15 In one embodiment of the invention, the vaccine compositions of the present invention may be formulated for sustained release. For example, one or more of the immune stimulating complex, carrier protein, saccharide antigen and/or aluminium containing adjuvant may be combined with a silicone elastomer that releases the saccharide antigen over a long period of time. The silicone elastomer can also comprise albumin. 20 See U.S. Pat. No. 4,985,253, the contents of which are fully incorporated by reference herein. The release rate of the antigen from the silicone elastomer can be controlled by incorporation of a water soluble or fat soluble mixing agent or cosolvent (e.g., polyethylene glycol 400, polysorbate 80, sodium alginate, L-alanine, sodium chloride, 25 polydimethylsiloxane) into the silicone elastomer. Any other additive can also be incorporated into the silicone elastomer for the purpose of accelerating the release rate.

The vaccine composition according to the invention may be formulated into unit dosage 30 forms, wherein each unit, for example a sealed container, comprises one dosage of the vaccine composition.

The vaccine compositions may be administered once; however, more often the vaccine compositions are administered more than once, such as more than 2 times, for 35 example more than 5 times, such as more than 10, for example more than 15, such as

more than 20, for example more than 30 times, such as more than 50 times, for example more than 100 times. Preferably, the vaccine compositions are administered in the range of 2 to 10 times, more preferably in the range of 2 to 5 times, such as twice.

5

When the vaccine composition is administered more than once, the time period between two individual administrations is typically in the range of 1 week to 5 years, such as in the range of 1 month to 2 year, for example in the range of 1 month to 1 year, such as in the range of 2 months to 7 months. If the vaccine composition is administered more than twice, then the time period between the individual administrations may each individually be any of the aforementioned. Thus by way of example the interval between the first and second administration may be around 2 months and the interval between the second and the third administration may be around 7 months.

15

In a preferred embodiment of the present invention the immune stimulating complex is of the ISCOM type. In a more preferred embodiment the immune stimulating complex is an ISCOM. In a particularly preferred embodiment the immune stimulating complex is a Posintro. The immune stimulating complex is in general associated with carrier protein A. In said preferred embodiments, the immune stimulating complex associated with carrier protein A is administered in a vaccine composition that comprises a pharmaceutically effective concentration of said complex. Preferably, the vaccine compositions comprise a concentration from 0.01% to 10%, such as from 0.05% to 8%, for example from 0.1% to 7%, such as from 0.25% to 6%, for example from 0.5% to 5%, such as from 0.6% to 4%, for example from 0.7% to 2%, such as from 0.75% to 1.5%, for example from 0.8% to 1.2% w/w of immune stimulating complex. Furthermore in said preferred embodiments, the immune stimulating complex may be administered in a vaccine composition that comprises from 0.01% to 0.05%, such as from 0.05% to 0.1%, for example from 0.1% to 0.25%, such as from 0.25% to 0.5%, for example from 0.5% to 0.75%, such as from 0.75% to 1.0%, for example from 1.0% to 1.25%, such as from 1.25% to 1.5%, for example from 1.5% to 2.0% w/w of said complex.

The amount of saccharide antigen administered per dosage is dependent on the individual to whom the vaccine composition is administered. In general in the range of 0.01 to 100 µg, preferably in the range of 0.01 to 50 µg, more preferably in the range of

35

0.1 to 10 µg, for example in the range of 0.1 to 1 µg of each saccharide antigen comprised within a vaccine composition is administered per dosage. When the vaccine composition comprises more than one saccharide antigen, the composition may comprise the same or similar amount of each saccharide antigen preferably as
5 determined by weight, but it is also comprised within the invention that the vaccine composition comprises different amounts of the different saccharide antigens.

If the saccharide antigen is *Streptococcus pneumoniae* saccharide antigen, each dosage unit of the vaccine composition preferably comprises at the most 1 µg,
10 preferably at the most 0.5 µg, more preferably at the most 0.4 µg, such as at the most 0.3 µg, for example at the most 0.2 µg, such as at the most 0.1 µg of each of said *Streptococcus pneumoniae* saccharide antigens.

Accordingly, each dosage unit of said vaccine composition preferably comprises in
15 the range of 0.01 to 1 µg, such as in the range of 0.05 to 1 µg, for example in the range of 0.1 to 1 µg, such as in the range of 0.05 to 1 µg, for example in the range of 0.1 to 1 µg, such as in the range of 0.05 to 0.8 µg, for example in the range of 0.05 to 0.6 µg, such as in the range of 0.05 to 0.4 µg, for example in the range of 0.05 to 0.2 µg, such
20 as in the range of 0.1 to 0.8 µg, for example in the range of 0.1 to 0.6 µg, such as in the range of 0.1 to 0.5 µg, for example in the range of 0.1 to 0.4 µg, such as in the range of 0.1 to 0.3 µg, for example in the range of 0.1 to 0.2 µg of each of said *Streptococcus pneumoniae* saccharide antigens.

When the vaccine composition comprises more than one *Streptococcus pneumoniae*
25 saccharide antigen, the composition may comprise the same or similar amount of each saccharide antigen preferably as determined by weight, but it is also comprised within the invention that the vaccine composition comprises different amounts of the different saccharide antigens. Each dosage unit of the vaccine composition preferably comprises in the range of a total of 0.1 to 20 µg, such as in the range of 0.1 to 16 µg,
30 for example in the range of 1 to 10 µg, such as in the range of 1 to 16 µg *Streptococcus pneumoniae* saccharide antigens.

Examples

Preparation of Posintro-HBsAg

- 5 A stock solution consisting of 1,17 mg/ml Hepatitis B surface Antigen, 200 mg/ml
Mega-10, 200 mg/ml SDS, and 400 μ M DTT is mixed with 200 mg/ml Mega -10, 20
mg/ml DC-CH in 200 mg/ml Mega -10, 20 mg/ml CH in 200 mg/ml Mega -10, 20 mg/ml
POPC in 200 mg/ml Mega -10, 17,5 mg/ml Quil A, and PBS. Everything except Quil A
10 formulation is injected into a Slide-a-Lyzer and dialysed according to the following
scheme:

Time	Volume	Temperature
0	4 liter, 0.1x PBS	37° C
24	4 liter, 0.1x PBS	RT
48	4 liter, 0.1x PBS	4° C
72	4 liter, 0.1x PBS	4° C
96	4 liter, 0.1x PBS	end

- After 96 hours of dialysis, the formulation is withdrawn from the Slide-a-Lyzer, and the
15 volume is estimated by weight of the formulation. There is an expected loss of 25% of
both saponin and protein.

Immunisation with Posintro™ in the combination with Prevenar

20

Intro:

- In these studies a commercial pneumococcal vaccine Prevenar (purchased from
Wyeth) is combined, at different doses, with the experimental adjuvant system
Posintro™. The study demonstrates that addition of Posintro™-HBsAg to Prevenar
25 allows reduction of the dose of Prevenar and still achieve the same or even higher
immune response.

Summary

- 30 The Posintro™ is an ISCOM (immune stimulatory complex) with the unique ability to
incorporate the antigen of interest. In these studies the surface Hepatitis B antigen is
incorporated into the Posintro™, referred to as Posintro™-HBsAg here after. The

pneumococcal vaccine Prevenar (which includes 7 streptococcus pneumoniae types conjugated to a carrier, CRM197 carrier protein mutated diphtheria toxin and adsorbed to Alum hydroxide) is used in high and low dose and the low dose is combined with the Posintro™-HBsAg.

5

Material and methods

In these studies mice, NMRI strain, are used as animal model. The mice are immunized twice, with three weeks interval, parenteral (subcutaneous) in the neck with only Prevenar or a mixture of Prevenar and Posintro™-HBsAg according to below table.

10

	Prevenar per injection/mouse	Posintro-HBsAg per injection/mouse
High dose Prevenar	0.2 ml undiluted	
Low dose Prevenar	0.2 ml of 11% prevenar in PBS	
Low dose Prevenar and Posintro-hepatitis	0.2 ml of 11% prevenar in PBS	4 µg HBsAg,

Blood were taken before priming, just before boosting and 3 weeks after boosting.

15

Serum is extracted from the blood.

- Titer of antibodies binding two different polysaccharides, 19F and 23F, both included in the Prevenar vaccine, were analyzed. Presence of antibody was determined by ELISA. Briefly, antigen was immobilised in a microtiterplate and serum was added. After washing presence of specific antibodies was detected with anti mouse IgG antibodies coupled to phosphatase. Phosphatase converts substrate into a coloured substance which can be quantified by absorbance measurement. The antibody titer is calculated from interpolation of the linear part of the titration curves as the dilution of serum at 50% saturation at a log₁₀ scale for the dilution.
- The average of the highest absorbance (MAX-OD) from each serum sample is shown as results. OD was determined at 405 nm.

20

25

Results

Figure 1 shows that the anti-19F IgG titer is significantly higher in mice immunised with Prevenar+Posintro™-HBsAG, than in mice immunised with Prevenar only., even when a high dosis of Prevenar was administrered. The MAX-OD value of anti-19F is also significantly higher (see figure 3).

5

Similarly, figure 2 shows that the anti-23F IgG titer is significantly higher in mice immunised with Prevenar+Posintro™-HBsAG, than in mice immunised with prevanar only. In fact, anti-23F IgG was not detectable in mice immunised with Prevenar only. The MAX-OD value of anti-23F is also significantly higher (see figure 4).

10

Conclusions:

The Prevenar is a commercial pneumococcal vaccine including 7 streptococcus pneumoniae types, 19F and 23F being two of them. The addition of Posintro™-HBsAg to Prevenar increases both the anti-19F and anti-23F IgG titer and the Max-OD values to anti-19F and anti-23F.

15

Immunisation with Posintro™ in the combination with NeisVac-C®

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NeisVac-C® is a commercial meningococcal vaccine including serotype C. NeisVac-C® includes tetanus toxoid as carrierprotein, absorbed to alumininum hydroxide. In this study NeisVac-C® is combined, at different doses, with the experimental adjuvant system Posintro™-HBsAg.

25

Materials and methods

NMRI mice were immunized subcutaneously with NeisVac-C® together with HBsAg incorporated in Posintro™. The NeisVac-C® were administrated at two different dose levels; 40% and 4% NeisVac-C, corresponding to 40% and 4% respectively of normal human dose. Total IgG, IgG1, and IgG2 titers were measured after 2 and 3 immunizations.

30

Results

After 2 immunizations the total IgG titer for carbohydrate C was significantly increased (fig.19) and both the Th2 isotype IgG1 and Th1 isotype IgG2a were increased (figures 20 and 21, respectively).

35

Conclusions:

The combined vaccine Posintro-HBsAg and NeisVac-C® induced a strong enhanced effect of the immune response against polysaccharides serotype C in NeisVac-C® after 2 immunizations and preferably the isotype IgG2a. IgG2a is known as the primary isotype mediating optimal complement fixation and opsonisation leading to bacterial clearance.

10 **Induction of cellular responses by different adjuvant systems in combination with HBsAg**

Different adjuvant systems were tested in combination with HBsAg in their ability to induce an antigen specific cellular response. The different adjuvant systems tested were Twinrix®, a commercial Hepatitis B vaccine including Aluminium oxide hydrate and aluminium phosphate as adjuvant, Fendrix® with aluminium phosphate and 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) as adjuvant, Freund's incomplete adjuvant (FIA) and the Posintro™ a modified ISCOM.

The data demonstrate that Posintro™ is an equally strong inducer of hepatitis specific proliferation as the commercial vaccines Twinrix® and Fendrix®.

Two different proliferation analyses were performed, using either BrdU incorporation into proliferating cells from immunized mice or by flow cytometry analysis where proliferating cells were separated on cell size and granularization.

25 **Material and methods**

Mice were immunized with HBsAg combined with different adjuvant systems. Twelve days after the second immunization cells from the draining lymph nodes were cultured in vitro with HBsAg or medium only as control.

30 ***Measurement of HBsAg-specific cellular response***

Single cell suspensions of draining lymph nodes were plated in flat bottom 96-well plates and cultured for 5 days with 10 µg/ml HBsAg or only R5 medium. Five replicate cultures were performed for each assay. After the incubation time each well was pulsed with BrdU from a cell proliferation kit (Roche, Penzberg, Germany) and incubated for a further 6 hours. The cells were fixed and POD-labelled anti-BrdU was added to each

well and incubated for 90 minutes at RT. The wells were washed and substrate (from the kit) was added to each well and incubated for 30 minutes at RT. The colour development was stopped by the addition of 1 M H₂SO₄. The absorbance was read at 450 nm using a Vmax kinetic Microplate Reader (Molecular Devices Corp., CA).

5

Flow cytometry analysis of HBsAg specific T-cells

Lymph node cells were washed twice in PBS and incubated together with 0.5µM CFSE (Invitrogen), diluted in PBS, for 5 minutes at RT. The reaction was stopped with 0.5 % BSA (Sigma-Aldrich) in PBS. The cells were diluted in R10 culture medium. Lymph node cells (10⁵ cells/well) were plated in flat bottom 96-well plates and cultured for 5 days with 10 µg/ml HBsAg or only R5 medium. Five replicate cultures were performed for each assay. After the incubation time, the five replicate wells were pooled and the cell number was counted using the Trucount tubes (Becton Dickinson). The rest of the cells were incubated with either phycoerythrin conjugated anti-CD4 or CD8 and 2 µg/ml 7AAD (Sigma-Aldrich) (detecting dead cells). PBS containing BSA (Sigma-Aldrich) was used in all cell labelling and washing steps. Cells were stained for 20 minutes at 4°C, washed and re-suspended in PBS/BSA. Flow cytometry analysis was performed on a FACScaliber (Becton Dickinson) using the FCS Express V3 software (De Novo Software, Ontario, Canada) for data analysis.

Proliferating cells were calculated by counting the blast cells detected by FCS and SSC analysis. The percentage blasted cells were multiplied with the total cell number both for cells incubated with HBsAg and cells incubated with medium only. The HBsAg induced number of proliferating cells was obtained by subtracting the number of positive cells incubated with medium only from the number of positive cells incubated with HBsAg.

HBsAg induced CD4+ and CD8+ T-cells

To measure the phenotype of the antigen specific T-cells, which were primed by the different vaccine formulations, a CFSE labelling assay was performed. Mice were immunized intradermally and cells from draining lymph nodes were stained with CFSE and incubated *in vitro* together with HBsAg to detect proliferating cells. After 5 days incubation the proliferating CD4⁺ and CD8⁺ T-cells were separated by surface antigen staining and analyzed on a flow cytometer (Fig. 8a and b).

Approximately 5.000 CD4⁺ or CD8⁺ cells were analyzed in each sample. Percentage CFSE^{low} CD4^{high} or CD8^{high} positive cells were multiplied with the total cell number, both for cells incubated with HBsAg and cells incubated with medium only. The HBsAg induced number of CD4⁺ and CD8⁺ cells was obtained by subtracting the number of positive cells incubated with medium only from the number of positive cells incubated with HBsAg.

Delayed type hypersensitivity test (DTH)

The well known delayed type hypersensitivity test was performed with HBsAg combined with different adjuvant systems. Mice were immunized intradermally in the base of the tail with 4 µg HBsAg (the commercial vaccines antigen dose was also set to 4 µg) in 100 µl saline (the same dilution was performed as for immunizations) followed by a boost dose with the same amount 3 weeks later. One week after the last immunization all mice were immunized s.c. in both hind footpads. Before injection the footpad thickness was measured. In the left footpad 50 µl PBS was injected and in the right footpad 50 µl Hepatitis B (5 µg in 50 µl PBS). 24 hours later the footpad thickness was measured. The average value in mm of the footpad thickness was recorded for each adjuvant.

Results

The results from the BrdU incorporation into proliferating cells from immunized mice are shown in figure 6. Results from the flow cytometry analysis where proliferating cells were separated on cell size and granularization are presented in figure 7.

The data demonstrate that Posintro[™] is an equally strong inducer of hepatitis specific proliferation as the commercial vaccines Twinrix[®] and Fendrix[®].

The Posintro[™] adjuvant system induced activated CD4⁺ antigen specific T-cells to a significantly higher degree than the commercial Aluminium oxide hydrate and aluminium phosphate adjuvant system (Twinrix[®]) (with a p-value of 0.0117) and higher, but not significant, induction of CD8⁺ antigen specific T-cells compare to Twinrix[®]. The other commercial vaccine Fendrix[®] induced almost the same level of both CD4 and CD8 positive T-cells as Posintro[™] and with significantly higher CD4⁺ antigen specific T-cells than the PBS control group.

Results from the delayed type hypersensitivity test are shown in figure 9. The thickness of the footpad was estimated for each adjuvant. The Posintro™ adjuvant system induced stronger DTH than the commercial vaccine Fendrix® and as strong DTH as the commercial vaccine Twinrix®.

5

Comparing different adjuvant systems in combination with HBsAg at inducing antigen specific B-cells.

10 The induction of antigen specific B-cells was detected using ELISPOT. Mice were immunized intradermally and the spleen cells were incubated on HBsAg coated membrane plate. The spots detected were antigen specific B-cells producing antibodies binding to the HBsAg coated membrane.

15 **Material ad methods**

The ELISPOT technique was used to measure the number of Hepatitis B specific antibody secreting B-cells. Briefly a PVDF membrane micro 96-well-plate (Pierce, Rockford, IL) was coated with 1 µg/well of HBsAg diluted in a carbonate coating buffer (0.1 M Na₂CO₃ and 0.1 M NaHCO₃, pH 9.6) over night at 4 °C. Tetanus Toxoid, 1 µg/well (SSI, Copenhagen, Denmark), was used as a negative control and medium only was used as background control. The wells were washed in washing buffer (Pierce) and a blocking buffer (Pierce) was added for 1 h at 37 °C. The blocking buffer was washed off and a single cell suspension from the spleen from each individual animal was added in an amount of 10⁶ cells/well diluted in R10 culture medium, with
 20 duplicates for each animal. The plate was wrapped in aluminium foil and incubated in an incubator at 37°C for 3 hours. The plate was washed 6 times in washing buffer and was shaken between each washing step. Biotinylated anti-mouse IgG (DakoCytomation, Glostrup, Denmark) was added and incubated for 1 hour at 37°C. The wells were washed and Streptavidine-alkaline phosphate (Pierce) was added for
 25 30 minutes at room temperature. The wells were washed and NBT/BCIP (nitroblue tetrazolium/bromochlorindophenol) substrate solution (Pierce) was added to each well for 15 minutes at room temperature. The colorimetric reaction was stopped by the addition of ultra pure water. The plates were dried over night and evaluated in high resolution (pixel size <5µm) using an automated ELISPOT reader system (Carl Zeiss)
 30 with KS Elispot Software 4.8. (Zellnet Consulting Inc., Fort Lee, NJ).

35

Anti-HBsAg specific B-cell spots were calculated by counting the spots in each well subtracting spots from wells coated with irrelevant antigen (Tetanus, ranging 0-3 spots in each well) and calculating an average from the duplicate.

5 **Results**

The numbers of positive spots were subtracted by the spots induced by an irrelevant antigen, tetanus toxoid (Fig. 4). The Posintro™ adjuvant system induced a higher number of positive cells with less variation between the animals, i.e. a more robust response of antigen specific B-cells, than both the commercial Hepatitis B vaccines
10 Twinrix® and Fendrix®.

Induction of systemic antibody response

15 Different adjuvant systems were tested in their ability to induce both antigen-specific IgG antibodies and the antigen-specific isotypes, IgG1 and IgG2a. The induction of total IgG antibody responses by different adjuvant systems were tested in two species, mice and guinea pigs. In both Guinea Pigs (Fig. 11) and mice (Fig. 12) a large panel of different adjuvant systems was compared to the Posintro™ adjuvant system. The
20 adjuvant system tested were FIA, Twinrix® (aluminium oxide hydrate and aluminium phosphate), Fendrix® (aluminium phosphate +MPL), QS21 (purified saponin fraction), the MF59 (oil-in-water emulsion) and Posintro™.

Material and methods

25 Antibody levels, both total IgG titer and the isotypes IgG1 and IgG2a antibody titer, were assessed in serum and measured by ELISA. Serum was obtained from the blood by centrifugation at 2160 g for 10 minutes. Total anti-HBsAg mouse IgG antibody levels and total anti-HBsAg Guinea Pig IgG antibody levels and mouse isotype IgG1 and IgG2a antibody level were measured by coating microtiter plates (96 well, MaxiSorb,
30 Nunc, Roskilde, DK) with 50µl of 0.4µg/ml HBsAg (serum Institute of India) in carbonate buffer (50mM, pH 9.6). Prior to use, the plates were washed three times with PBS containing 1% Triton X-100 and blocked with 1% bovine serum albumin (BSA) (Sigma-Aldrich) in PBS. Serum was added in a series of 5 fold dilutions ranging from 5 fold dilutions to 390625 fold dilutions and incubated for 1 hour at 37°C. The plate was
35 washed and alkaline phosphatase labelled Goat-anti-mouse IgG, IgG1 or IgG2a

(Southern biotech, Birmingham, AL) or alkaline phosphates labelled Goat-anti-Guinea Pig IgG (Southern biotech) diluted 1:4000 in PBS containing 1% (w/v) BSA was added and incubated for 1 hour at 37°C. The plate was washed and p-NPP (Kem-En-Tec Diagnostics, Copenhagen, Denmark) was added as substrate for 30 minutes at RT and the reaction was stopped by 1 M NaOH. The absorbance was read at 405 nm using a Vmax kinetic Microplate Reader (Molecular Devices Corporation, CA). Hepatitis B specific antibody titers were calculated from interpolation of the linear part of the titration curves as the dilution of serum at 50% saturation at a log₁₀ scale for the dilution. All values were correlated to an internal standard.

Results

In guinea pigs the Posintro™ adjuvant system induced the highest average IgG antibody titer compared to the other adjuvant systems, but only significantly ($p < 0.05$) higher than Twinrix®, Fendrix® and QS21 (Fig 11). In mice, the Posintro™ adjuvant system induced the highest total IgG antibody titer (Fig. 12). In mice two different IgG isotypes were analyzed, IgG1 (a Th2 induced isotype) and IgG2a (a Th1 induced isotype). The Posintro™ was the only adjuvant system that induced a strong Th1 antibody response with high IgG2a titers (Fig. 14). The other adjuvant systems only induced a Th2 antibody response with high IgG1 titer (Fig. 13). Posintro™ also induced a strong Th2 response.

Antigen specific cytokine production.

Draining lymph node cells from mice immunized intradermally with HBsAg in combination with FIA, Twinrix®, Fendrix®, Posintro™ or only PBS were cultured in vitro. The cytokine production was measured in the supernatant after 5 days of culture using the Luminex analysis system.

Results

The Posintro™ induces both IFN-gamma (a Th1 induced cytokine) (Fig. 15) and IL-5 and IL-13 (both Th2 induced cytokines) (Figs. 16 and 17, respectively). Posintro™ induces higher titer of IFN-gamma and significantly higher titers of IL-5 and IL-13 than the commercial vaccine Twinrix®. Posintro™ and Fendrix® induce the same amount of IFN-gamma but Posintro™ induced higher titers of both IL-5 and IL-13.

Potential combination vaccine, Posintro™-boost with the induction of a humoral immune response to Hepatitis B

- 5 NMRI mice were immunized parenteral with Prevenar® together with HBsAg combined with Posintro™ or the commercial Hepatitis vaccines Twinrix® (with AI as adjuvant) or Fendrix® (with AI and MPL as adjuvant). Anti-Hepatitis B IgG titer was measured three weeks after second immunization in serum (Fig 18).

Results

- 10 The combined vaccine Posintro™-HBsAg and Prevenar® induced a strong humoral response to Hepatitis B with a high titer of Hepatitis B IgG (Fig. 18).

Claims

- 5 1. A vaccine composition comprising
- a. an immune stimulating complex comprising
- i. at least one saponin, and
- ii. at least one sterol, and
- 10 iii. at least one contacting group capable of binding a nucleic acid,
 wherein said contacting group may be covalently attached to said at
 least one saponin or to said at least one sterol or to at least one
 lipophilic moiety,
 said complex being associated with at least one carrier protein designated
 carrier protein A; and
- 15 b. at least one saccharide antigen attached to a carrier protein designated
 carrier protein B; and
- c. at least one aluminium containing adjuvant.
2. A vaccine composition comprising
- 20 a. an immune stimulating complex comprising
- i. at least one saponin, and
- ii. at least one sterol, and
- iii. at least one contacting group capable of binding a nucleic acid,
 wherein said contacting group may be covalently attached to said at
- 25 least one saponin or to said at least one sterol or to at least one
 lipophilic moiety,
 said complex being associated with at least one carrier protein designated
 carrier protein A;
- b. at least one saccharide antigen, and
- 30 c. at least one aluminium containing adjuvant.
3. Vaccine composition according to claims 1 or 2, wherein said immune stimulating
 complex further comprises a lipophilic moiety.

4. Vaccine composition according to claims 1 to 3, capable of inducing an immune response to said saccharide antigen.
- 5 5. Vaccine composition according to claims 1 to 3, capable of inducing an immune response to said carrier protein A.
6. Vaccine composition according to claims 1 to 3, capable of inducing an immune response to said carrier protein A and said saccharide antigen.
- 10 7. Vaccine composition according to any one of the preceding claims, wherein said contacting group is capable of binding a nucleic acid via electrostatic or hydrophobic interactions.
- 15 8. Vaccine composition according to any one of the preceding claims, wherein said contacting group is a cationic moiety
9. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin is selected from the group consisting of synthetic saponins and naturally occurring saponins.
- 20 10. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin is selected from the group consisting of saponins comprising a triterpene glycoside, saponins comprising a steroid glycoside, and saponins comprising a steroid alkaloid glycoside.
- 25 11. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin comprises a triterpene glycoside comprising an aglycone compound and a saccharide compound.
- 30 12. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin comprises a triterpene glycosid isolated from a Quillaja plant species.
- 35 13. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin is isolated from a Quillaja saponaria species.

14. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin is isolated from *Quillaja saponaria* Molina or *Quillaja saponaria* *Officinalis*.
- 5
15. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin is Quillajabark Araloside A (Quil A).
16. Vaccine composition according to any one of the preceding claims, wherein said at least one saponin comprises at least one saponin selected from the group consisting of QA-7 (QS-7), QA-17 (QS-17), QA-18 (QS-18) and QA-21 (QS-21).
- 10
17. Vaccine composition according to any one of the preceding claims, wherein said at least one sterol is selected from the group consisting of synthetic sterols and naturally occurring sterols.
- 15
18. The vaccine composition according to any one of the preceding claims, wherein said immune stimulating complex comprises at least two different sterols.
19. Vaccine composition according to any one of the preceding claims, wherein at least one sterol is selected from the group of sterols consisting of cholesterol, lanosterols, lumisterols, stigmasterols, sitosterols, mycosterols, ergosterols, and thiocholesterols.
- 20
20. The vaccine composition according to any one of the preceding claims, wherein at least one sterol is covalently linked to the at least one contacting group.
- 25
21. The vaccine composition according to any one of the preceding claims, wherein at least one sterol is covalently linked to the at least one contacting group, wherein the contacting group is a cationic moiety.
- 30
22. The vaccine composition according to any one of the preceding claims, wherein at least one sterol is covalently linked to at least one contacting group, wherein the contacting group is a quaternary ammonium group.
- 35

23. The vaccine composition according to any one of the preceding claims, wherein at least one sterol is selected from the group consisting of DC-cholesterol and TC-cholesterol.
- 5 24. The vaccine composition according to any one of the preceding claims, wherein at least one sterol is DC-cholesterol.
- 10 25. The vaccine composition according to any one of the preceding claims, wherein said immune stimulating complex comprises at least two different sterols, wherein the first sterol is a sterol as defined in claim 17 and the second sterol is a sterol as defined in any of claims 18 to 22.
- 15 26. Vaccine composition according to any one of the preceding claims, wherein carrier protein A is selected from the group consisting of bacterially derived proteins, virally derived proteins, lipopeptides, members of the polyhistidine triad family of proteins and fusions thereof, keyhole limpet hemocyanin, PPD (purified protein derivative of Tuberculin), pneumococcal pneumolysin (PLY), OMPC (meningococcal outer membrane protein), PorB (from *N. meningitidis*), PD (*Haemophilus influenzae* protein D), heat shock protein, plasmodium flaciparum pfg27, lactate dehydrogenase peptide, gp120 of HIV, pertussis proteins, cytokines, lymphokines, artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens, pneumococcal surface protein, iron uptake proteins, serum proteins, immunoglobulins and hormones.
- 20 27. The vaccine composition according to any one of the preceding claims, wherein carrier protein A is a bacterially derived protein and variants thereof selected from the group consisting of bacterial toxins and toxoids.
- 25 28. The vaccine composition according to any one of the preceding claims, wherein the carrier protein A is a bacterial toxin or variant thereof selected from the group consisting of tetanus toxin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, toxin A and toxin B of *C. difficile*, tetanus toxoid, fragment C of tetanus toxoid, diphtheria toxoid, pertussis toxoid, pneumolysin s Mutations of pneumolysin (Ply) and mutants of pneumolysin with lower toxicity.
- 30 35

29. The vaccine composition according to any one of the preceding claims, wherein carrier protein A is selected from the group consisting of lipopeptides, HBsAg and variants of HBsAg sharing at least 70% sequence identity with HBsAg of SEQ ID NO: 1.
- 5
30. The vaccine composition according to claim 29, wherein the lipopeptide is a synthetic lipopeptide comprising a peptide comprising a lysine moiety and a lipid moiety linked to an amino group of said peptide.
- 10
31. Vaccine composition according to any one of the preceding claims, wherein carrier protein A is plasma-derived or genetically manufactured Hepatitis type B surface antigen (HBsAg).
- 15
32. Vaccine composition according to any one of claims 1, and 3-31, wherein carrier protein B is selected from the group consisting of bacterially derived proteins, virally derived proteins, lipopeptides, members of the polyhistidine triad family of proteins and fusions thereof, keyhole limpet hemocyanin, PPD (purified protein derivative of Tuberculin), pneumococcal pneumolysin (PLY), OMPC (meningococcal outer membrane protein), PorB (from *N. meningitidis*), PD (*Haemophilus influenzae* protein D), heat shock protein, plasmodium flaciparum pfg27, lactate dehydrogenase peptide, gp120 of HIV, pertussis proteins, cytokines, lymphokines, artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens, pneumococcal surface protein, iron uptake proteins, serum proteins, immunoglobulins and hormones.
- 20
- 25
33. The vaccine composition according to any one of claims 1, and 3-32, wherein carrier protein B is a bacterially derived protein and variants thereof selected from the group consisting of bacterial toxins and toxoids.
- 30
34. The vaccine composition according to any one of claims 1, and 3-33, wherein the carrier protein B is a bacterial toxin or variant thereof selected from the group consisting of tetanus toxin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, toxin A and toxin B of *C. difficile*, tetanus toxoid, fragment C of tetanus toxoid, diphtheria toxoid, pertussis toxoid, pneumolysin s Mutations of pneumolysin (Ply) and mutants of pneumolysin with lower toxicity.
- 35

35. The vaccine composition according to any one of claims 1, and 3-34 wherein carrier protein B is a diphtheria toxoid.
- 5 36. The vaccine composition according to any one of claims 1, and 3-35 wherein carrier protein B is diphtheria toxoid CRM197.
37. The vaccine composition according to any one of claims 1, and 3-34 wherein carrier protein B is a tetanus toxoid.
- 10 38. The vaccine composition according to any one of claims 1, and 3-37, wherein carrier protein A is different to carrier protein B.
39. The vaccine compositions according to any one of claims 1, and 3-39, wherein carrier protein A is as defined in any of claims 26 to 31 and carrier protein B is as defined in any of claims 32 to 37 and wherein carrier protein A is different to carrier protein B.
- 15 40. The vaccine composition according to any one of the preceding claims, wherein carrier protein A is incorporated into the immune stimulating complex.
- 20 41. Vaccine composition according to any one of the preceding claims, wherein said at least one saccharide antigen is derived from a microbial organism.
42. The vaccine composition according to any of the preceding claims, wherein at least one saccharide antigen is derived from encapsulated bacteria.
- 25 43. The vaccine composition according to any of the preceding claims, wherein at least one saccharide antigen is derived from *haemophilus influenza type B*.
- 30 44. The vaccine composition according to any of the preceding claims, wherein at least one saccharide antigen is derived from *streptococcus pneumoniae*.
45. A vaccine composition according to any of the preceding claims wherein one or more different *Streptococcus pneumonia* saccharide antigens is attached to carrier
- 35

protein B, and wherein each dosage unit of said vaccine composition comprises at the most 1 µg of each of said *Streptococcus pneumonia* saccharide antigens

- 5 46. The vaccine composition according to any of the preceding claims, wherein at least one saccharide antigen is derived from *Neisseria meningitides*.
47. The vaccine composition according to any of the preceding claims, wherein at least one saccharide antigen is derived from *group B streptococcus*.
- 10 48. The vaccine composition according to any of the preceding claims, wherein at least one saccharide antigen is derived from *Salmonella enterica*.
49. The vaccine composition according to any of the preceding claims comprising more than one different saccharide antigen, wherein at least 50%, such as at least 60%,
15 for example at least 70%, such as at least 80%, for example at least 90% of the saccharide antigens are derived from encapsulated bacteria.
50. The vaccine composition according to any of the preceding claims, wherein all saccharide antigens are derived from encapsulated bacteria.
- 20 51. The vaccine composition according to any one of the preceding claims, wherein the composition comprises in the range of 2 to 200, for example in the range of 2 to 150, such as in the range of 2 to 100, for example in the range of 2 to 75, such as in the range of 2 to 50, for example in the range of 2 to 30, such as in the range of
25 5 to 30 for example in the range of 10 to 30, such as in the range of 15 to 30, for example at least 7, such as at least 10, for example at least 13, such as at least 15, for example at least 20, such as at least 23, for example at least 30, such as at least 40, for example at least 50, such as in the range of 20 to 90, for example 10 to 90 different saccharide antigens.
- 30 52. Vaccine composition according to any one of the preceding claims, wherein said the aluminium containing adjuvant is selected from the group consisting of $\text{AlK}(\text{SO}_4)_2$, $\text{AlNa}(\text{SO}_4)_2$, $\text{AlNH}_4(\text{SO}_4)$, $\text{Al}(\text{PO}_4)$ and $\text{Al}(\text{OH})_3$.

53. The vaccine composition according to any one of the preceding claims, wherein said aluminium containing adjuvant is selected from the group consisting of aluminium phosphate and aluminium hydroxide.
- 5 54. The vaccine composition according to any one of the preceding claims, wherein said vaccine composition comprises an aluminium containing adjuvant to which said immune stimulating complex associated with carrier protein A and said saccharide antigen attached to said carrier protein B is adsorbed.
- 10 55. A vaccine composition according to any of claims 1 to 54 for use in treatment, preferably prophylactic treatment of a clinical condition in an individual in need thereof, wherein the clinical condition is infection by a microbial organism.
- 15 56. The vaccine composition according to claim 55, wherein the clinical condition is infection by encapsulated bacteria.
- 20 57. The vaccine composition according to any one of claims 55 to 56, wherein the clinical condition is one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis or joint infections meningitis and meningococcal septicaemia mastitis, such as bovine mastitis (if the individual is bovine) and perinatal GBS disease, typhoid fever, gastroenteritis, pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis and brain abscess,.
- 25 58. The vaccine composition according to any one of claims 55 to 57, wherein the vaccine composition comprises at least one saccharide antigen derived from, *Haemophilus influenzae*, and wherein the clinical condition is one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis and joint infections.
- 30 59. The vaccine composition according to claim 58, wherein the clinical condition is selected from the group consisting of bacteremia and bacterial meningitis.
- 35

60. The vaccine composition according to any one of claims 55 to 59, wherein the vaccine composition comprises at least one saccharide antigen derived from *Neisseria meningitides* and wherein the clinical condition is one or more selected from the group consisting of bacterial meningitis and meningococcal septicaemia.
- 5
61. The vaccine composition according to any one of claims 55 to 60, wherein the vaccine composition comprises at least one saccharide antigen derived from *Group B Streptococcus* and wherein the clinical condition is one or more selected from the group consisting of mastitis and perinatal GBS disease.
- 10
62. The vaccine composition according to any one of claims 55 to 61, wherein the vaccine composition comprises at least one saccharide antigen derived from *Salmonella enterica* Sero var Typhi and wherein the clinical condition is typhoid fever.
- 15
63. The vaccine composition according to any one of claims 55 to 62, wherein the vaccine composition comprises at least one saccharide antigen derived from *Salmonella enterica* Sero var typhimurium and wherein the clinical condition is gastroenteritis.
- 20
64. The vaccine composition according to any one of claims 55 to 63, wherein the vaccine composition comprises at least one saccharide antigen derived from *Streptococcus pneumoniae* and wherein the clinical condition is one or more selected from the group consisting of pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis and brain abscess.
- 25
65. The vaccine composition according to claim 64, wherein the clinical condition is one or more selected from the group consisting of pneumonia, otitis media and meningitis.
- 30
66. The vaccine composition according to claim 55, wherein the clinical condition is infection by virus and/or encapsulated bacteria.
- 35

67. The vaccine composition according to claim 64, wherein the clinical condition is one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis or joint infections meningitis and meningococcal septicaemia mastitis, such as bovine mastitis (if the individual is bovine) and perinatal GBS disease, typhoid fever, gastroenteritis, pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis, brain abscess, polio, tetanus, hepatitis B, and diphtheria.
68. The vaccine composition according to any one of claims 55 to 67, wherein the vaccine composition is for parenteral administration.
69. The vaccine composition according to any one of claims 55 to 67, wherein the vaccine composition is for administration by subcutaneous injection or by intramuscular injection.
70. The vaccine composition according to any one of claims 55 to 67, wherein the vaccine composition is for transdermal administration.
71. The vaccine composition according to claim 70, wherein the vaccine composition is comprised within an occlusive patch.
72. A method of treating a clinical condition in an individual in need thereof, wherein the method comprises administration of the vaccine composition according to any of claims 1 to 54 to said individual, wherein the clinical condition is infection by a microbial organism.
73. A method of reducing the risk of an individual encountering a clinical condition, wherein the method comprises administration of the vaccine composition according to any of claims 1 to 54 to said individual in an amount sufficient to generate a protective immune response, wherein the clinical condition is infection by microbial organisms.

74. The method according to any one of claims 72 and 73, wherein the clinical condition is infection by encapsulated bacteria.
75. The method according to any one of claims 72 and 73, wherein the clinical condition is infection by encapsulated bacteria and/or virus.
76. The method according to any one of claims 72 to 75, wherein the clinical condition is one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis or joint infections meningitis and meningococcal septicaemia mastitis, such as bovine mastitis (if the individual is bovine) and perinatal GBS disease, typhoid fever, gastroenteritis, pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis, brain abscess, polio, tetanus, hepatitis B, and diphtheria.
77. The method according to any one of claims 72 to 76, wherein the vaccine composition comprises at least one saccharide antigen derived from, *Haemophilus influenzae*, and wherein the clinical condition is one or more selected from the group consisting of bacteremia, acute bacterial meningitis, bacterial meningitis, cellulitis, osteomyelitis, epiglottitis and joint infections.
78. The method according to claim 77, wherein the clinical condition is selected from the group consisting of bacteremia and bacterial meningitis.
79. The method according to any one of claims 72 to 76, wherein the vaccine composition comprises at least one saccharide antigen derived from *Neisseria meningitides* and wherein the clinical condition is one or more selected from the group consisting of bacterial meningitis and meningococcal septicaemia.
80. The method according to any one of claims 72 to 76, wherein the vaccine composition comprises at least one saccharide antigen derived from *Group B Streptococcus* and wherein the clinical condition is one or more selected from the group consisting of mastitis and perinatal GBS disease.

81. The method according to any one of claims 72 to 76, wherein the vaccine composition comprises at least one saccharide antigen derived from *Salmonella enterica* Seroovar Typhi and wherein the clinical condition is typhoid fever.
- 5 82. The method according to any one of claims 72 to 81, wherein the vaccine composition comprises at least one saccharide antigen derived from *Salmonella enterica* Seroovar typhimurium and wherein the clinical condition is gastroenteritis.
- 10 83. The method according to any one of claims 72 to 82, wherein the vaccine composition comprises at least one saccharide antigen derived from *Streptococcus pneumoniae* and wherein the clinical condition is one or more selected from the group consisting of pneumonia, invasive pneumococcal disease, bacteremia, chronic obstructive pulmonary disease, acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, pharyngitis sepsis and brain abscess.
- 15 84. The method according to claim 83, wherein the clinical condition is one or more selected from the group consisting of pneumonia, otitis media and meningitis.
- 20 85. The method according to any one of claims 72 to 84, wherein the vaccine composition is administered parenterally.
- 25 86. The method according to any one of claims 72 to 84, wherein the vaccine composition is administered by subcutaneous injection or by intramuscular injection.
87. The method according to any one of claims 72 to 84, wherein the vaccine composition is administered transdermally.
- 30 88. The method according to claim 87, wherein the vaccine composition is comprised within an occlusive patch.
89. Use of the vaccine composition according to any of claims 1 to 54 for the preparation of a medicament for treatment, preferably prophylactic treatment of a

clinical condition in an individual in need thereof, wherein the clinical condition is infection by a microbial organism.

5 90. Use according to claims 79, wherein the vaccine composition and the clinical condition is as specified in any one of claims 56 to 71.

10 91. A vaccine composition for treatment, preferably prophylactic treatment of a clinical condition in an individual in need thereof comprising the composition according to any of claims 1 to 54 as an active ingredient, wherein the clinical condition is infection by a microbial organism.

15 92. The composition according to claim 91, wherein the vaccine composition and the clinical condition is as specified in any one of claims 55 to 71.

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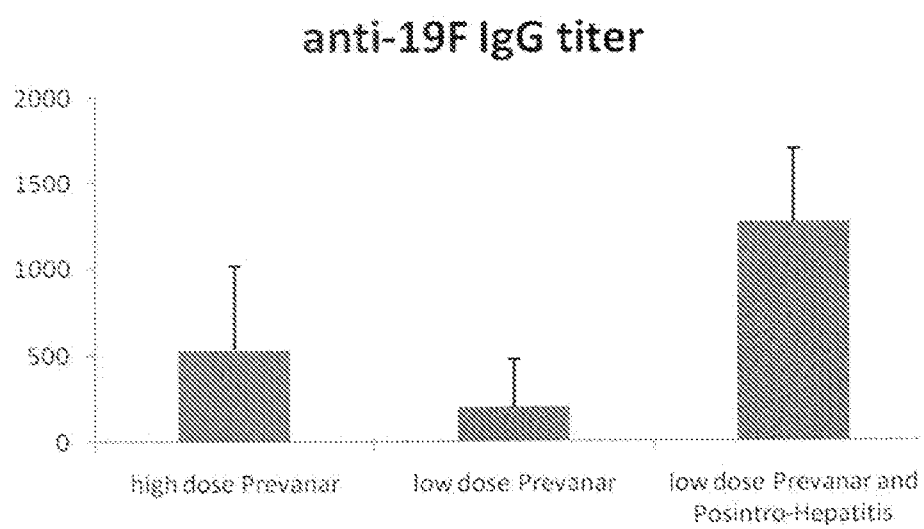


Fig. 1

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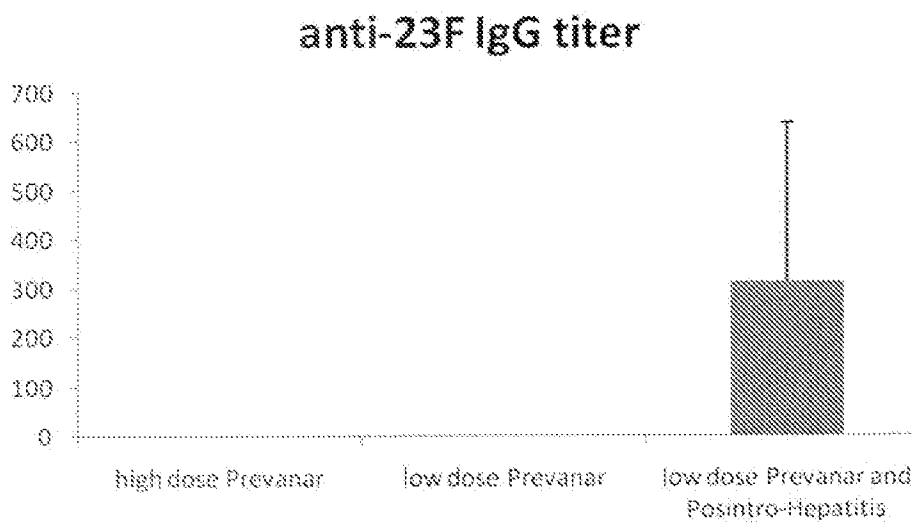


Fig. 2

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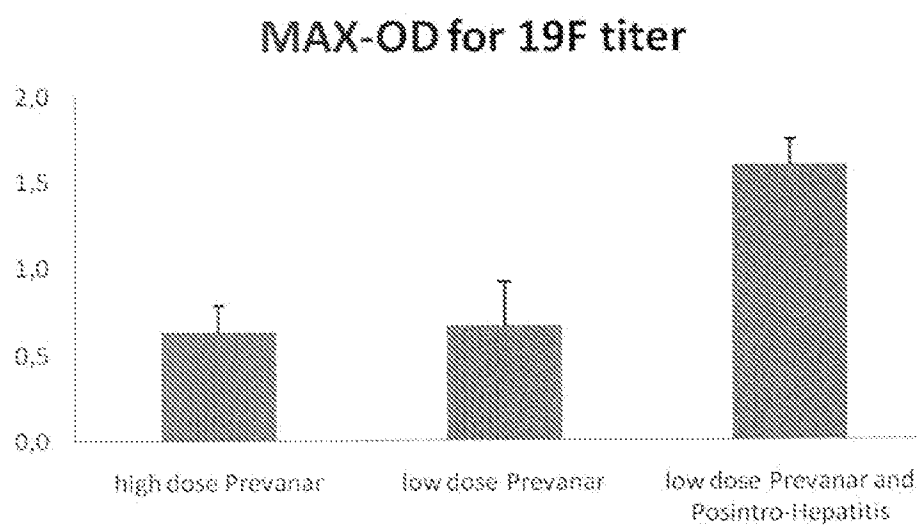


Fig. 3

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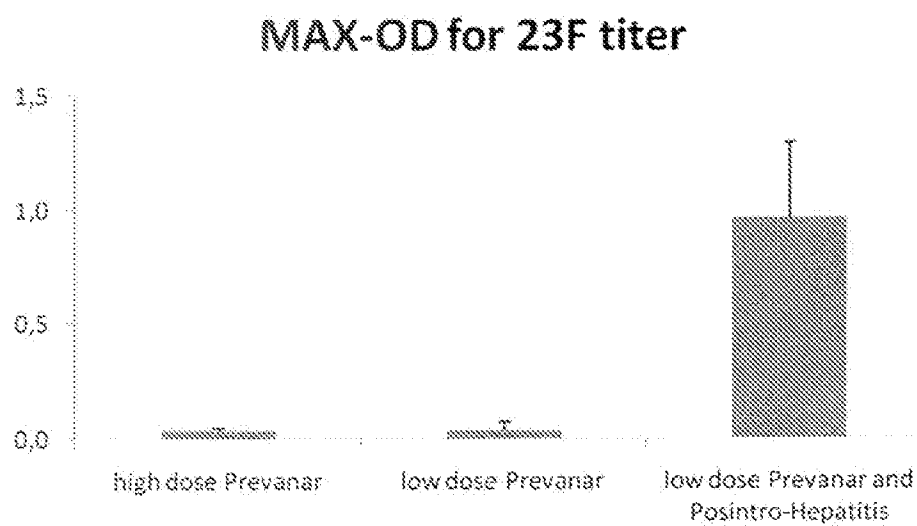
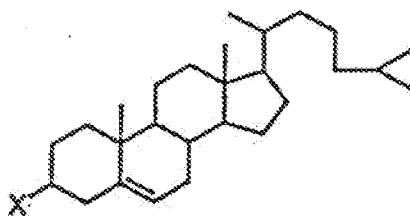
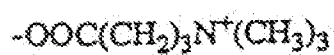


Fig. 4

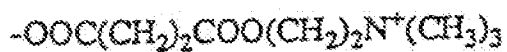
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X



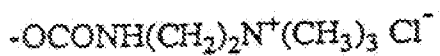
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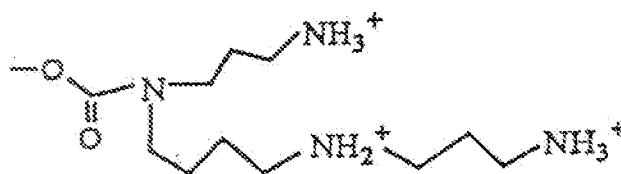
ChoSC



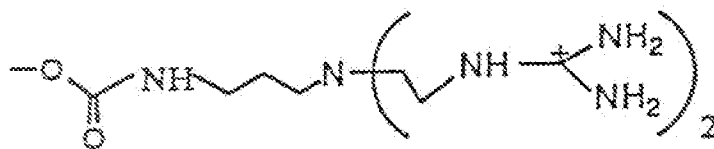
DC-Chol



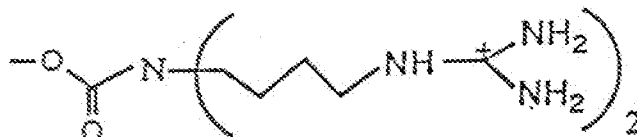
TC-Chol



Lipid 67



BGTC



BGSC

Fig. 5

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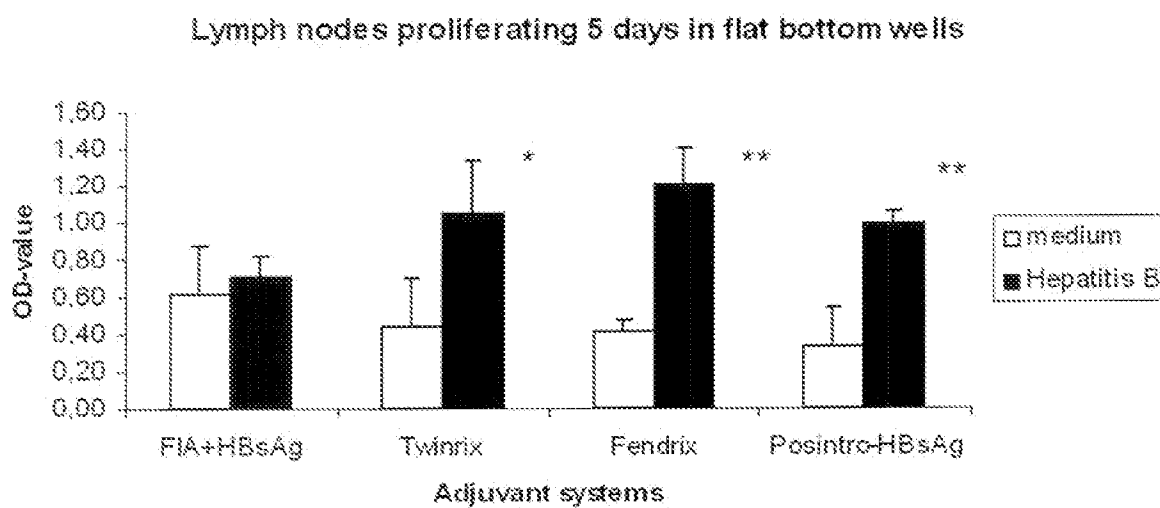


Fig. 6

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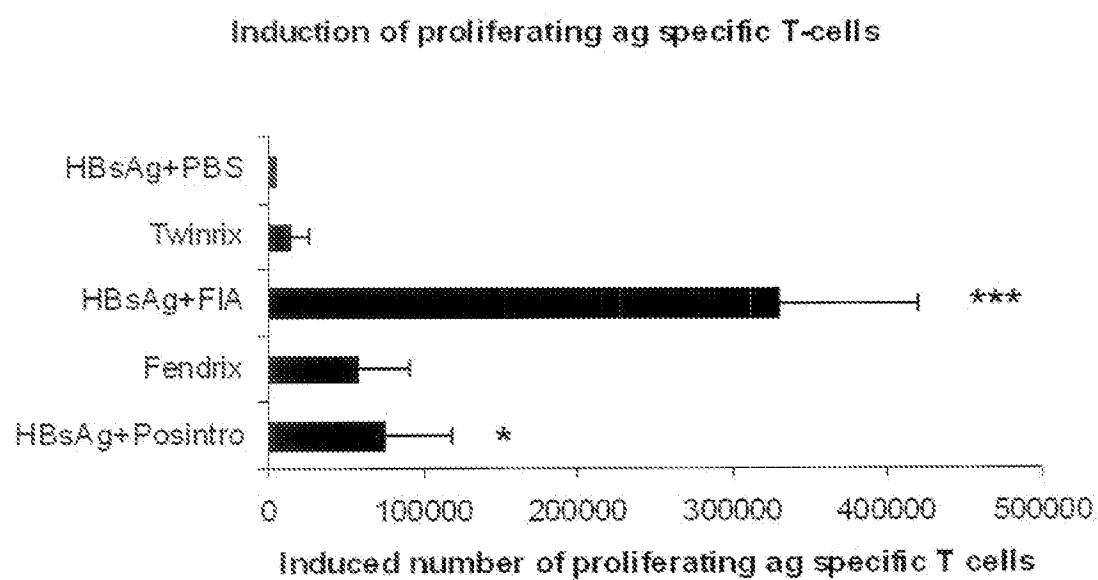


Fig. 7

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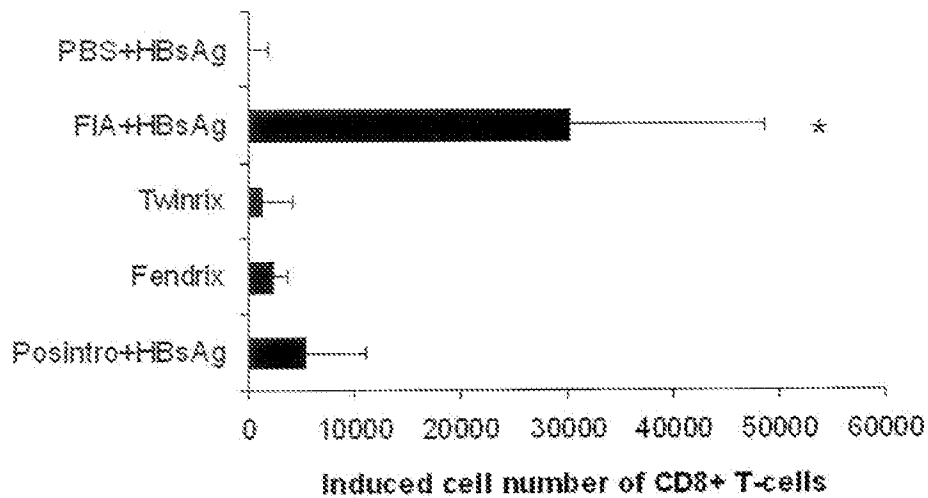
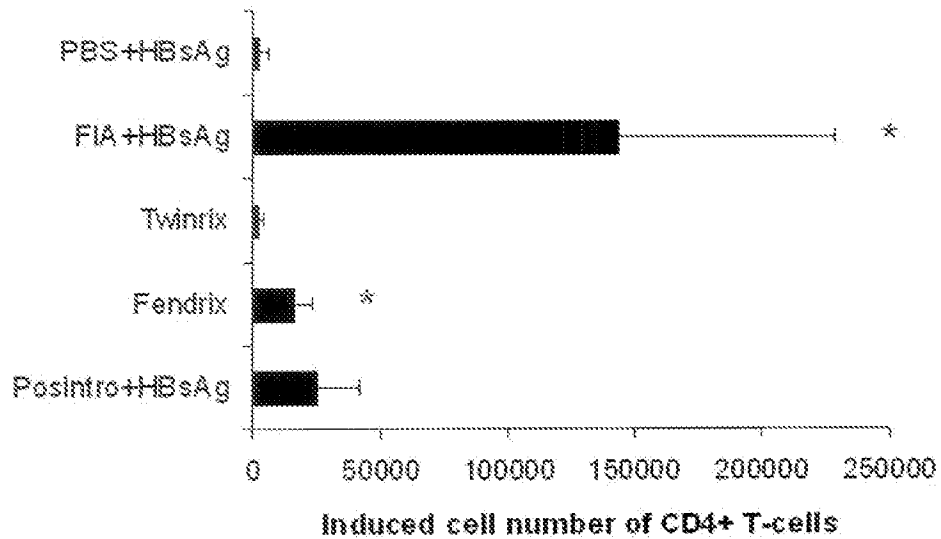


Fig. 8a and b

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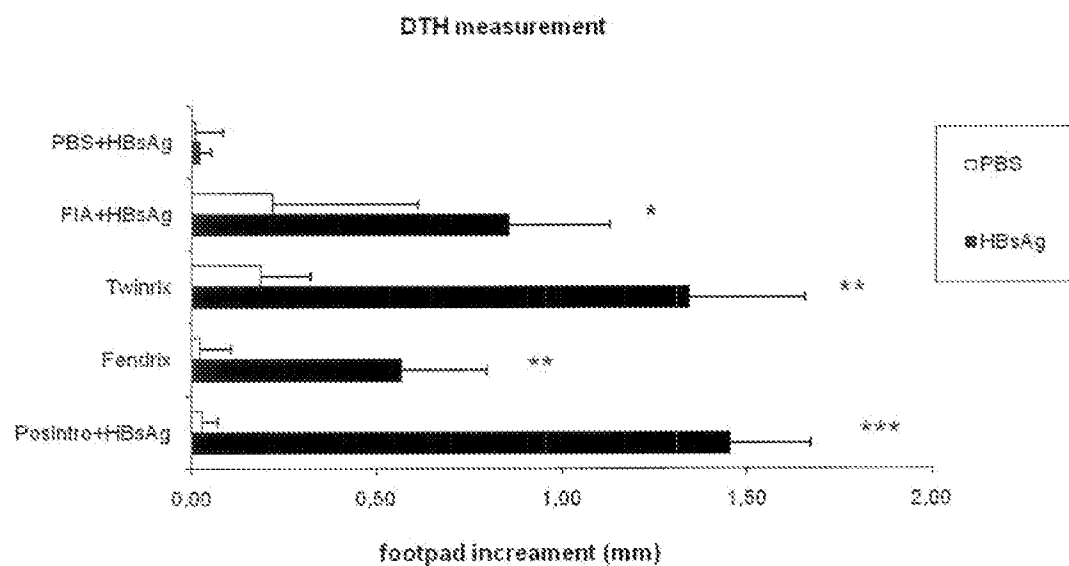
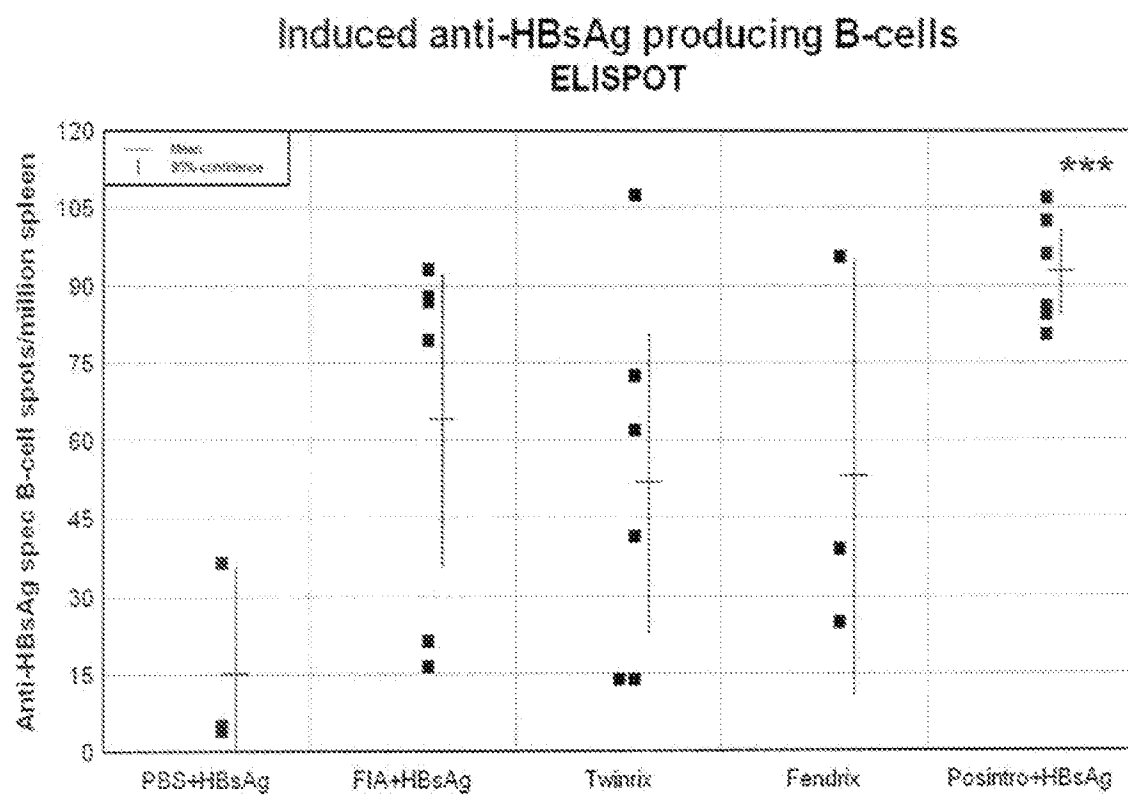


Fig. 9

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**Fig. 10**

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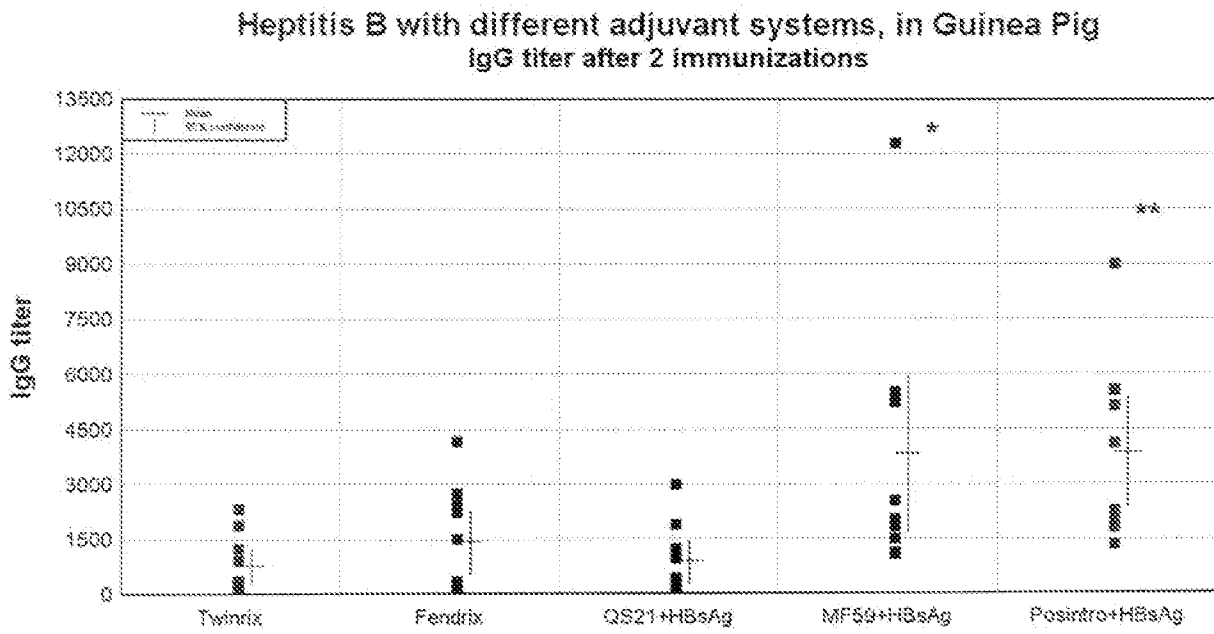


Fig. 11

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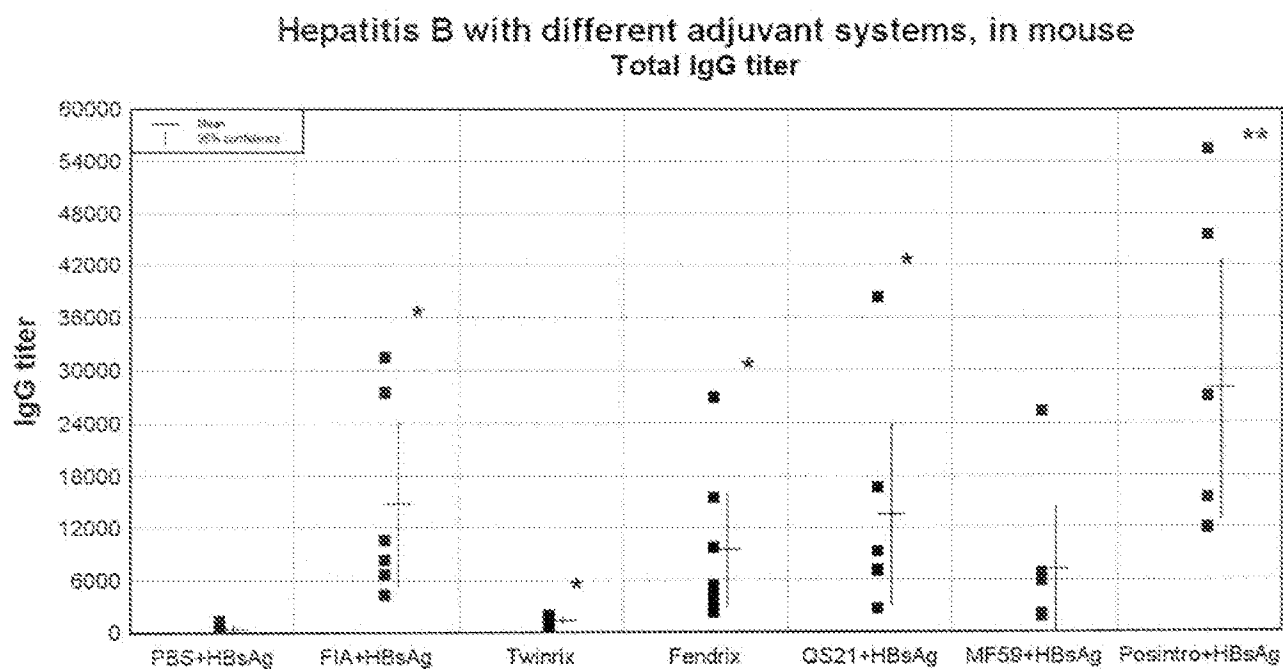


Fig. 12

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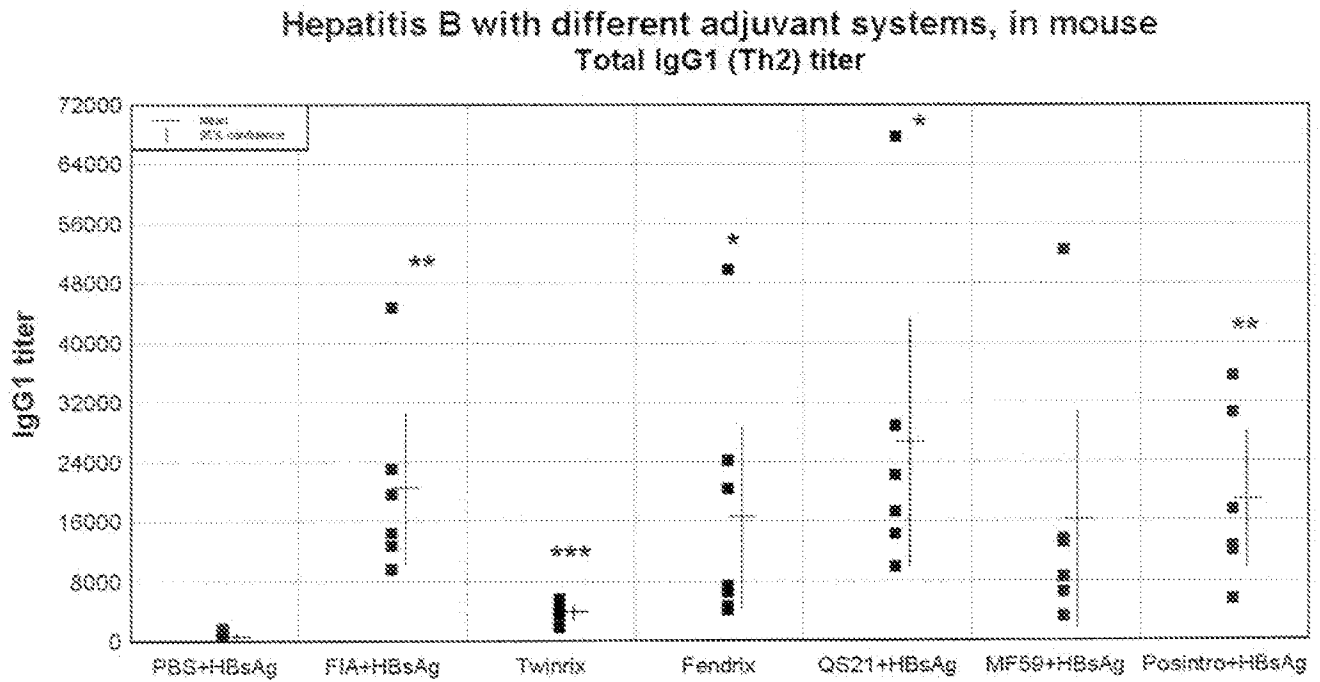


Fig. 13

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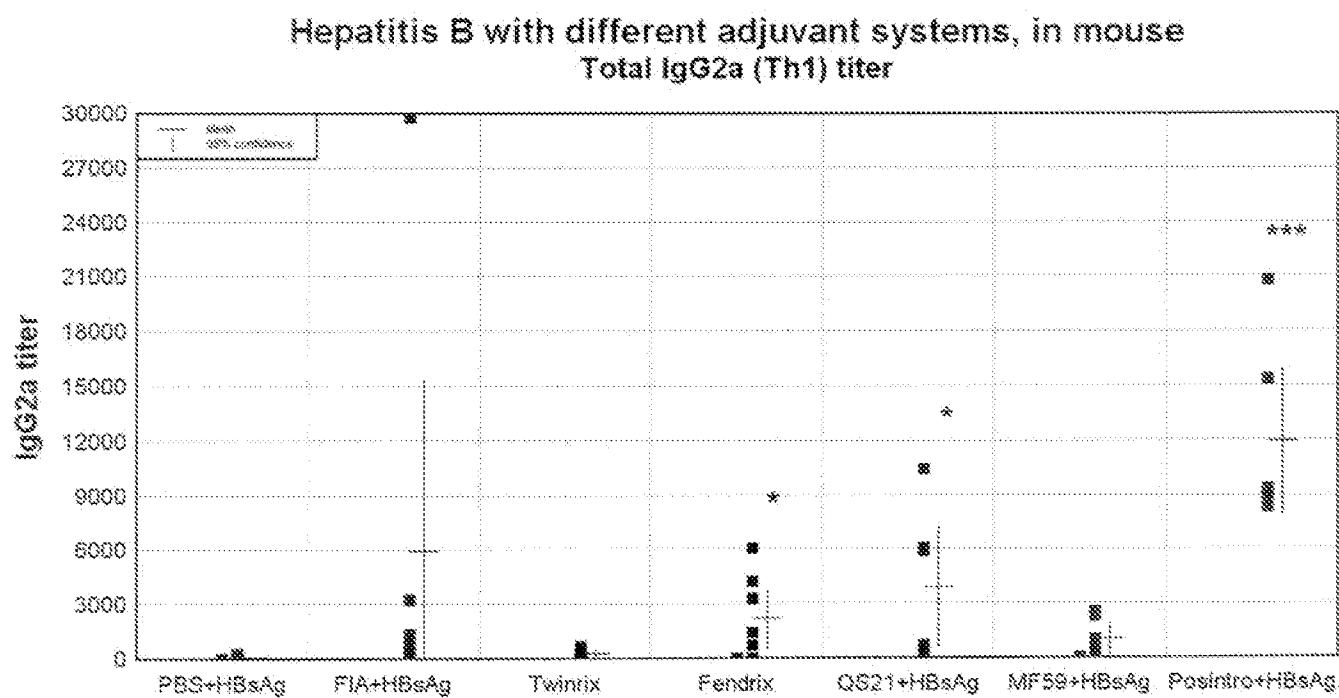


Fig. 14

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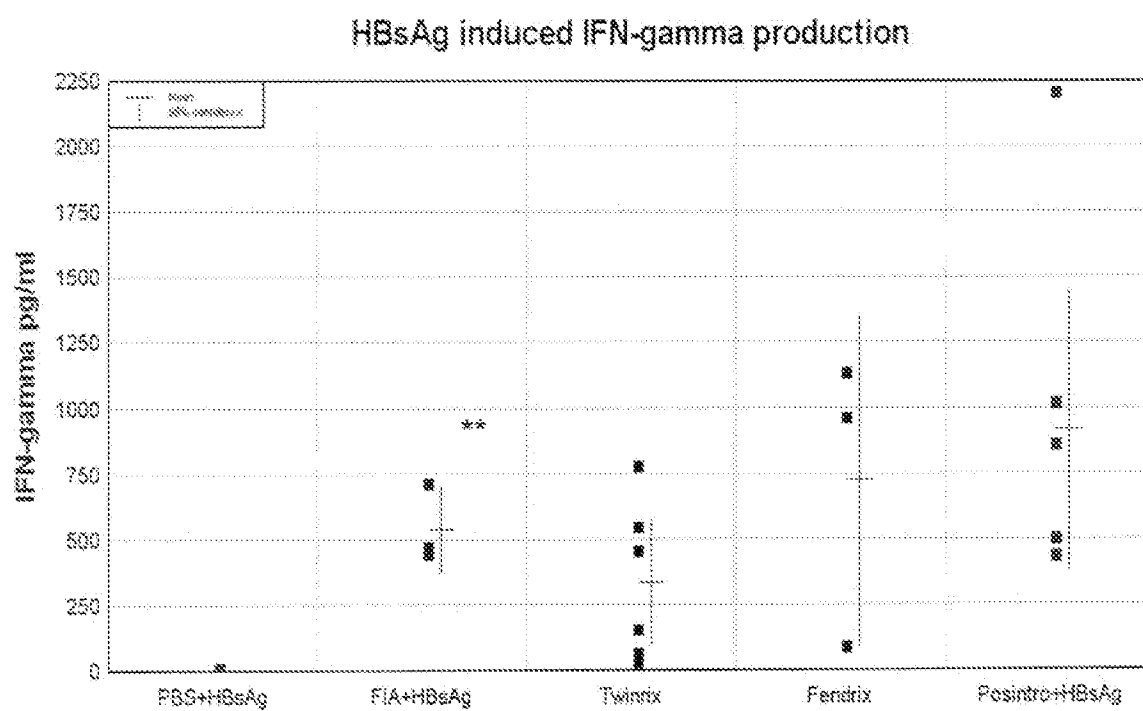


Fig. 15

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IL-5 production
HBsAg induced IL-5 production

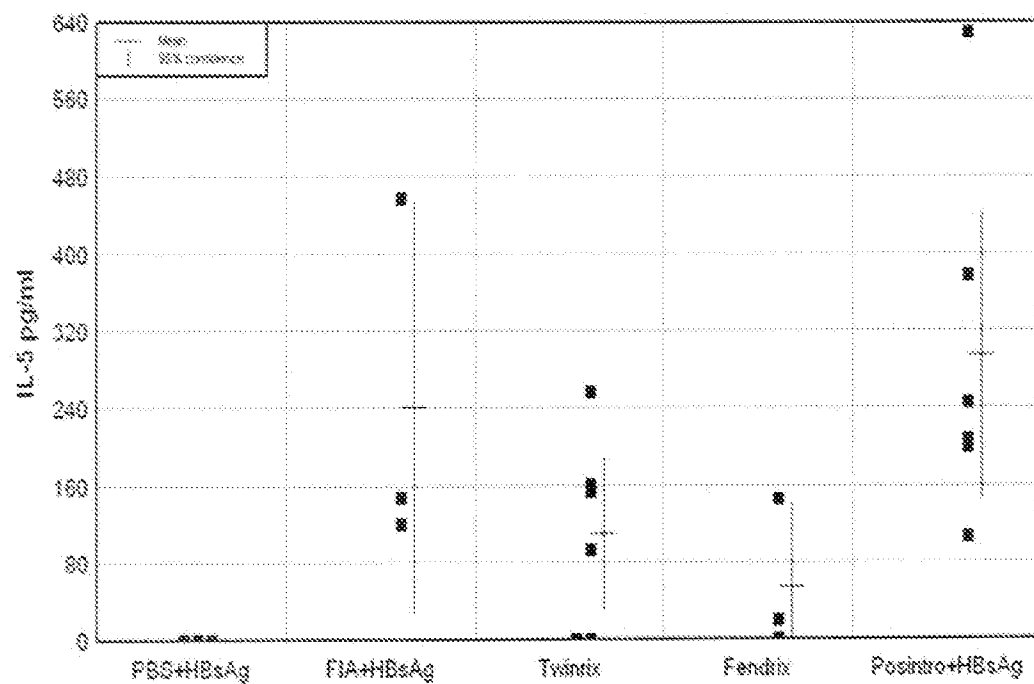


Fig. 16

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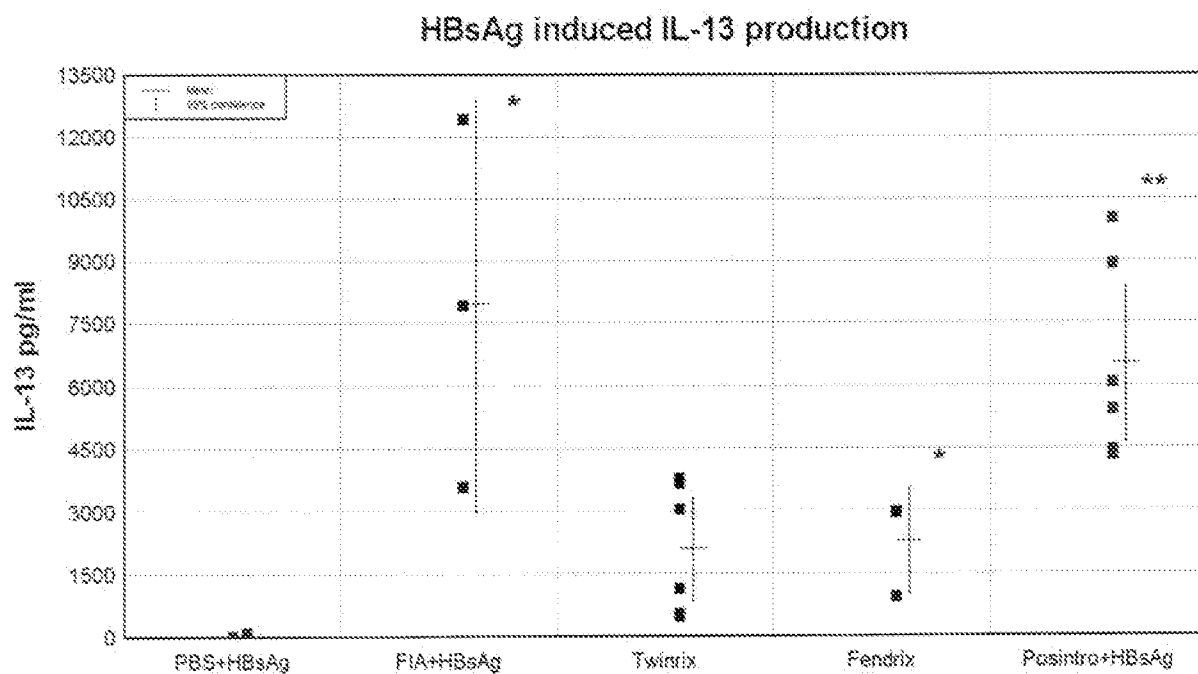


Fig. 17

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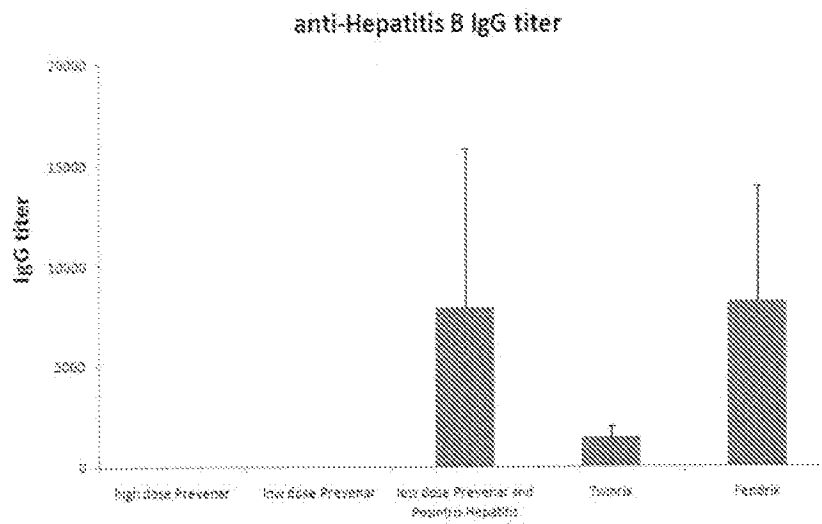


Fig. 18

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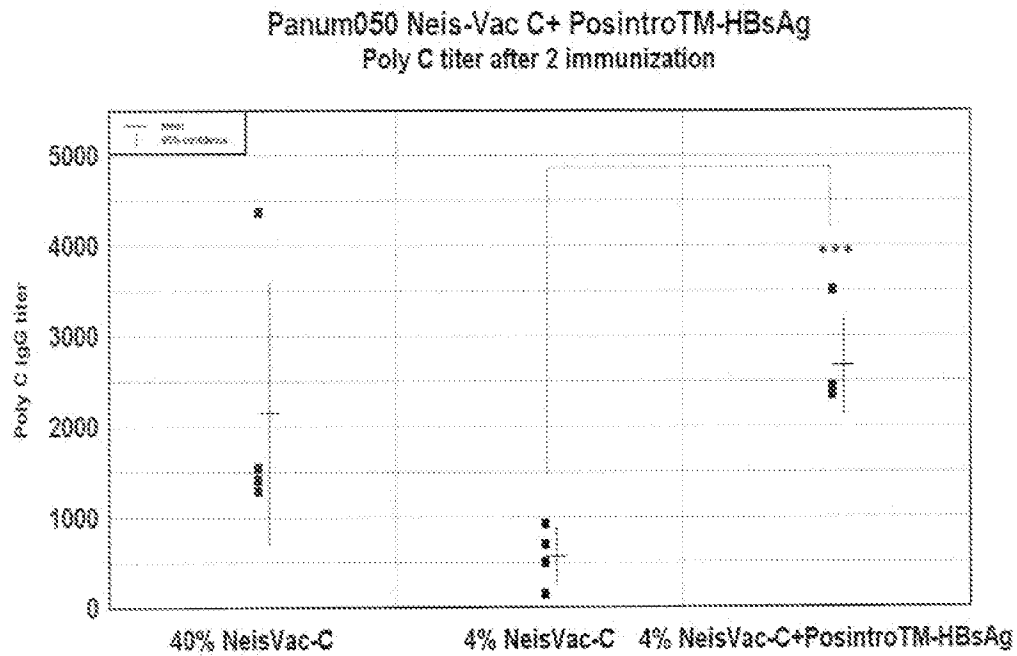


Fig. 19

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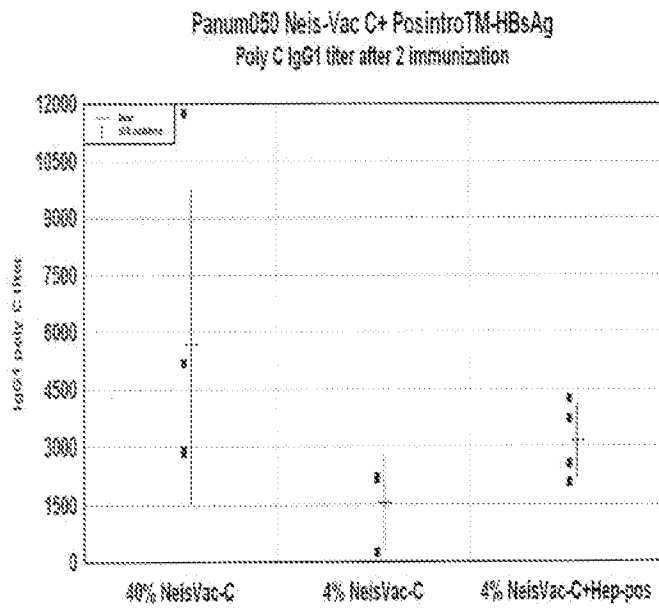


Fig. 20

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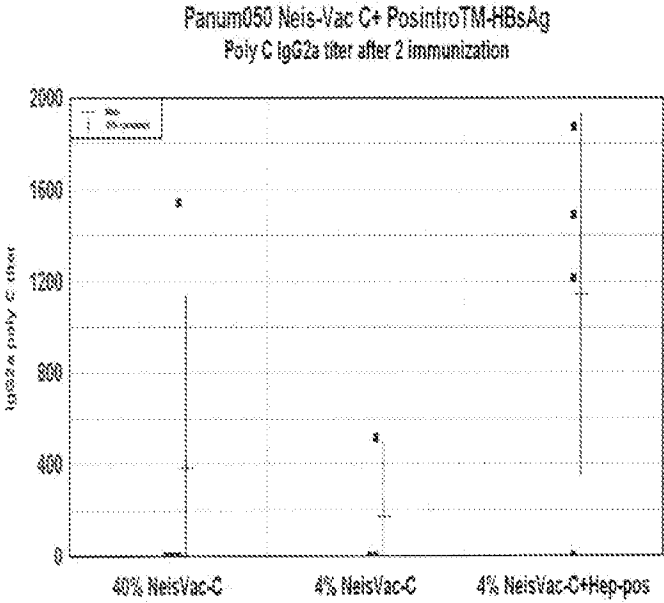


Fig. 21

INTERNATIONAL SEARCH REPORT

International application No

PCT/DK2009/050047

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/39 A61K39/09 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/030696 A (PHAROMED AS [DK]; COLOPLAST AS [DK]; KIRKBY NIKOLAI SOEREN [DK]; SAMUE) 15 April 2004 (2004-04-15) cited in the application page 5, lines 5-11 page 8, lines 20-24 page 13, lines 10-26 page 17, lines 21-27 page 21, line 16 page 22, lines 10-19 page 24, lines 24-27 page 28, line 21 - page 29, line 26 page 33, lines 1-27 page 35, line 20 - page 36, line 5 page 39, lines 19-26 example 18 claims 1,26,28,2937,38-40 ----- --/--	1-92

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

3 July 2009

Date of mailing of the international search report

14/07/2009

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Authorized officer

Noë, Veerle

INTERNATIONAL SEARCH REPORT

International application No

PCT/DK2009/050047

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	----- US 2006/228380 A1 (HAUSDORFF WILLIAM P [BE] ET AL) 12 October 2006 (2006-10-12) the whole document -----	1-92

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