

## HYPERTONIC THERMOSTABLE VACCINE COMPOSITIONS

### Technical Field

[0001] This invention relates to hypertonic thermostable vaccine compositions.

### Background

5 [0002] Vaccines generally come as liquids that require cold transport and storage. This is particularly true for vaccines that contain microbial and viral antigens adsorbed to particulate adjuvants such as aluminium hydroxide, aluminium phosphate and calcium phosphate. Furthermore, many of these vaccine preparations are destroyed not only by exposure to high temperatures but are also very sensitive to damage caused by freezing.

10 [0003] Dry pharmaceutical compositions are usually protected against damage caused by high temperatures and therefore many medicines are provided in a dry state for reconstitution prior to use. Effective drying generally requires the addition of pharmaceutical excipients that are recognised as not interfering with the mechanism of action of the medicine while providing a solid soluble matrix in which the vaccines and medicines are stabilised.

15 Examples of commonly used pharmaceutical excipients include sugars such as glucose, sucrose and lactose, and sugar alcohols such as mannitol.

[0004] Vaccines often incorporate adjuvants such as aluminium hydroxide, aluminium phosphate or calcium phosphate in the form of nanoparticles which also need to be effectively stabilised in order to retain vaccine efficacy.

20 [0005] Generally, dry compositions of medicines contain a combination of elements, including drug substance, stabilisers, buffers and salts that once rehydrated provide a solution that is isotonic with the intended site of injection. This is generally accepted to be between 300 and 312 mOsm/L. This avoids undesirable osmotic effects from hypoosmotic and hyperosmotic formulations that can cause pain or even tissue damage, particularly for larger injection  
25 volumes (Wang, *Int J Pharm.* 490(1-2): 308-315, 2015). However, hyperosmotic formulations of a combined diphtheria, tetanus, acellular pertussis and inactivated poliomyelitis vaccine with aluminium hydroxide adjuvant have been found to be tolerated (Nony *et al.*, *Vaccine* 19(27): 3645-3651, 2001).

30 [0006] In the case of particulate adjuvants, the state of aggregation of the adjuvant is crucial for the effective adsorption of antigen and effective adjuvant activity (Shardlow *et al.*, *Allergy Asthma Clin Immunol* 14: 80, 2018). This state of aggregation of the adjuvant has a great impact on its biological efficacy and is affected by the surface charge of the adjuvant nanoparticles, which is in turn dependent on the presence and concentration of inorganic ions in solution. Typically, vaccine compositions with aluminium hydroxide have a sodium

chloride concentration between 3 and 6 g/L. This concentration has been found to provide the greatest stability of the aluminium hydroxide gel structure while at the same time contributing to an osmolality that is close to isotonic, i.e. about 310 mOsm/Kg (Art *et al.*, *Int J Pharm.* 517(1-2): 226-233, 2017).

5 [0007] US 6890512 discloses the use of trehalose to prevent aggregation of aluminium phosphate and aluminium hydroxide adjuvants on freezing and on drying, to permit long-term preservation in the dry or frozen state.

[0008] De la Torre *et al.* (*Vaccine* 41(22): 3413-3421, 2023) describe a dry tetanus/diphtheria vaccine with aluminium hydroxide adjuvant made from a solution containing 0.243 M  
10 trehalose and the surfactant polysorbate 80, that is impregnated in a sponge and air-dried by forced evaporation. The dry vaccine was highly stable but has a long reconstitution time, and despite having the surfactant requires vigorous flicking to ensure dissolution and vaccine recovery. Moreover, the resulting product is severely limited as the user is not able to discern if complete dissolution has taken place because of interference by the sponge, and as a  
15 result of this cannot have confidence that a full dose is administered.

### **Summary of the Invention**

[0009] The invention relates to dry vaccine compositions that are thermostable and may be stored for long periods of time without refrigeration. Furthermore, the vaccine compositions of the invention are not damaged by freezing.

20 [0010] A first aspect of the invention provides a thermostable lyophilizate vaccine composition that contains an antigen, 80% to 97% w/w (weigh/weight) trehalose, 2% to 10% w/w of a soluble inorganic salt and 0.7% to 12.5% w/w of an insoluble particulate adjuvant. Dry vaccine compositions of this first aspect of the invention are safe and form an injectable product following reconstitution with water for injection.

25 [0011] The thermostable dry vaccine composition of the first aspect may, upon reconstitution with water, provide a liquid vaccine product containing a dose of vaccine in 0.4 to 0.6 mL and comprising:

- (a) between 90 and 130 g/L of trehalose;
- (b) between 3 and 10 g/L of a soluble inorganic salt; and
- 30 (c) between 1 and 13.5 g/L of an insoluble particulate adjuvant.

[0012] A second aspect of the invention provides a method of preparing the thermostable dry vaccine composition of the first aspect, the method comprising:

- (a) providing a liquid antigen formulation comprising an antigen and:
  - (i) a liquid solvent;
  - 35 (ii) an insoluble particulate adjuvant; and

- (iii) a soluble inorganic salt;
- (b) diluting the liquid antigen formulation by adding trehalose solution to the liquid antigen formulation to produce a compounded liquid antigen formulation; and
- (c) lyophilizing the compounded liquid antigen formulation to produce a thermostable dry vaccine composition containing between 80% and 97% in weight (i.e. w/w) trehalose, 2% to 10% in weight (i.e. w/w) of a soluble inorganic salt and 0.7% to 12.5% in weight (i.e. w/w) of an insoluble particulate adjuvant.

[0013] In some embodiments, the dry vaccine composition of the first aspect may be prepared using the method according to the second aspect.

- [0014] The thermostable dry vaccine composition of the first aspect, or produced according to the second aspect, is stable for prolonged periods of time without refrigeration, and remains stable and within specifications after storage at +40°C, for a period of at least 6 months, and is not damaged by freezing temperatures below 0°C.

- [0015] A third aspect of the invention provides a method to obtain a liquid vaccine product, the method comprising:

- (a) providing a liquid antigen formulation comprising an antigen and:
  - (i) a liquid solvent;
  - (ii) an insoluble particulate adjuvant; and
  - (iii) a soluble inorganic salt;
- (b) producing a compounded liquid antigen formulation by adding trehalose solution to the liquid antigen formulation;
- (c) dispensing a suitable volume of the compounded liquid antigen formulation into a vial;
- (d) lyophilising the compounded liquid antigen formulation to produce the thermostable lyophilizate vaccine composition of the first aspect; and
- (e) reconstituting the dry vaccine composition with water.

- [0016] The antigen of the vaccine compositions and antigen formulations of the first, second and third aspects is preferably from a pathogen. In particular, the antigen may be a toxoid, for instance tetanus toxoid or diphtheria toxoid. Other antigens which may be used include a *Bordetella pertussis* antigen, a poliovirus antigen, a *Haemophilus influenzae* antigen and/or a hepatitis B virus antigen.

[0017] Suitable particulate adjuvants for use in the first, second and third aspects include aluminium hydroxide, aluminium phosphate and calcium phosphate.

- [0018] Suitable soluble inorganic salts for use in the first, second and third aspects include sodium chloride.

[0019] The preferred liquid solvent for use in the second and third aspects is water.

[0020] In some preferred embodiments, the dry vaccine composition of the first aspect or produced by the second aspect, may be reconstituted, or is suitable for reconstitution, such that a single dose of injectable formulation has a volume of 0.5 ml and comprises 50 to 5 65 mg of trehalose and 1.5 to 5.0 mg of the soluble inorganic salt, preferably sodium chloride. In such embodiments, a single dose of injectable formulation may further comprise 0.5 to 6.75 mg of the insoluble particulate adjuvant, preferably aluminium hydroxide or aluminium phosphate.

[0021] Each dose of a tetanus and/or diphtheria vaccine composition of the invention may 10 comprise at least 20, 30 or 40 IU of tetanus toxoid and/or at least 2, 4, 8, 15, 20 or 30 IU of diphtheria toxoid.

[0022] A fourth aspect of the invention provides a liquid vaccine product produced by reconstituting in an aqueous solution a dry vaccine composition of the first aspect, or produced in the second aspect, that is suitable for injection for immunisation. The 15 reconstitution may be as described in the third aspect.

[0023] In particular, the liquid vaccine product of the fourth aspect is an aqueous composition comprising an antigen and:

- (a) between 90 and 130 g/L of trehalose;
- (b) between 3 and 10 g/L of a soluble inorganic salt; and
- 20 (c) between 1 and 13.5 g/L of an insoluble particulate adjuvant.

[0024] The liquid vaccine product of the fourth aspect, or produced by the third aspect, may provide a single dose for immunisation in 0.3-0.6 mL, e.g. 0.4-0.6 mL.

[0025] The liquid vaccine product of the fourth aspect, or produced by the third aspect may have an osmolality of between 550-750 mOsm/Kg. Trehalose may for example contribute 25 200-400 mOsm/Kg to the osmolality and the inorganic salt may contribute 200-400 mOsm/Kg to the osmolality. In some embodiments tonicity may be in the range of 650-750 mOsm/Kg.

[0026] A fifth aspect of the invention provides a liquid vaccine product, according to any of the previous aspects of the invention, for use in immunising a subject against a pathogen.

[0027] A sixth aspect of the invention provides the use of a liquid vaccine product, according to 30 any of the previous aspects of the invention, in the manufacture of a medicament for immunising a subject against a pathogen.

[0028] A seventh aspect of the invention provides a method of immunising a subject against a pathogen using, e.g. by injection of, a liquid vaccine product according to any of the previous aspects of the invention.

[0029] Pathogens against which the subject may be vaccinated include one or more of:

*Clostridium tetani*, *Corynebacterium diphtheria*, *Bordetella pertussis*, poliovirus, *Haemophilus influenzae* and hepatitis B, depending on the antigens contained within the liquid vaccine product.

- 5 [0030] In an eighth aspect the invention provides a method of stimulating an immune response in a subject, comprising administering, e.g. by injection, to the subject the liquid vaccine product of according to any of the previous aspects.

[0031] The immune response may be against a pathogen, as described above, including against a toxin produced by a pathogen.

- 10 [0032] Immunisation of the subject with (e.g. by injection of) the liquid vaccine product according to any of the previous aspects of the invention may be pain-free, cause no significant pain, or be no more painful than immunisation with an equivalent, isotonic liquid reference vaccine. In some embodiments, immunising the subject with the liquid vaccine product may cause no local tissue damage or no greater tissue damage than immunisation  
15 with an equivalent, isotonic liquid reference vaccine.

[0033] Other aspects and embodiments of the invention are described in more detail below.

#### **Detailed Description of the Invention**

- [0034] Having vaccines that do not require temperature control on transport and storage is highly desirable. Current vaccines containing particulate adjuvants such as aluminium  
20 hydroxide, aluminium phosphate or calcium phosphate require refrigerated storage and are very sensitive to heat and freezing temperatures. Dry vaccine compositions of the present invention are porous, water-soluble compositions that contain 80% to 97% w/w trehalose, 2% to 10% w/w of a soluble inorganic salt and 0.7% to 12.5% w/w of an insoluble particulate adjuvant, along with one or more antigens.
- 25 [0035] Vaccines of the present invention may be produced from a “liquid antigen formulation” containing all the elements of a vaccine, including adjuvant and antigens, that are compounded to obtain a “compounded liquid antigen formulation” containing at least an antigen, a particulate adjuvant, an inorganic salt and trehalose (see **Fig. 1**). The “dry vaccine composition” (also referred to as the “lyophilised vaccine composition”) is produced by  
30 removing water from the compounded liquid antigen formulation by lyophilisation. The dry vaccine compositions of the present invention contain trehalose as a major component, a soluble inorganic salt, an insoluble particulate adjuvant and generally do not contain surfactants or insoluble supporting matrices. The dry vaccine compositions are highly porous and rapidly dissolve upon the addition of water. The “liquid vaccine product” is produced from

the process of resuspending the dry vaccine composition with an aqueous solution. In a preferred embodiment the aqueous solution is water for injection.

[0036] Particulate adjuvants are vaccine components that locally enhance the immune response in a non-specific manner. They are formed of loosely associated insoluble nanoparticles onto which vaccine antigens are adsorbed by surface charge. Particulate adjuvants which may be used herein include mineral adjuvants such as aluminium hydroxides [e.g.  $\text{AlO}(\text{OH})$  or  $\text{Al}(\text{OH})_3$ ], aluminium phosphates [e.g.  $\text{AlPO}_4$ , or  $\text{Al}(\text{OH})_x(\text{PO}_4)_y$ ], and calcium phosphates, commonly referred to as CAP, [e.g.  $\text{Ca}_3(\text{PO}_4)_2$  and  $(\text{CaHPO}_4)2\text{H}_2\text{O}$ ]. Integrity of the adjuvant structure and state of aggregation is essential for the efficacy of vaccines that contain them. The terms “insoluble particulate adjuvant”, “particulate adjuvant” and “mineral adjuvant” are used interchangeably herein.

[0037] As defined herein an aluminium hydroxide is a compound comprising aluminium and a hydroxide ion, an aluminium phosphate is a compound comprising aluminium and phosphorus, and a calcium phosphate is a compound comprising calcium and phosphorus.

[0038] In the dry vaccine composition, the proportion of particulate adjuvant is between 0.7% and 12.5% w/w, for example 0.7-4%, 0.7-7%, 0.7-10%, 4-7%, 4-10%, 4-12.5%, 7-10%, 7-12.5% and 10-12.5% w/w of the dry vaccine composition. Preferably between 3% and 9% w/w of the dry vaccine composition is the particulate adjuvant.

[0039] The amount and state of aggregation of particulate adjuvants may be determined by sedimentation columns. In some embodiments the volume occupied by the particulate adjuvant (particularly in the case of an aluminium hydroxide adjuvant) in the dry vaccine composition after reconstitution and being allowed to settle for 24 or 48 hrs is between 10% and 40% of the total volume (v). For example, 12-38% v, 15-35% v, 17-32% v, 20-30% v, 20-40% v, 15-30% v or 15-25% v. Preferably the volume is at least 15 % v or 20 % v, e.g. 15-25 % v or 15-30 % v. Sedimentation may be analysed at an ambient temperature, e.g. 20°C or 25°C.

[0040] In some embodiments the amount of insoluble particulate adjuvant in the dry vaccine composition (and so also in the liquid vaccine product) is between 0.5 and 7 mg/dose, e.g. 0.5-6.75 mg/dose, 0.5-6 mg/dose, 0.5-5 mg/dose, 0.5-4 mg/dose, 0.5-3 mg/dose, 0.5-2 mg/dose, 1.5-7 mg/dose, 1.5-6 mg/dose, 1.5-5 mg/dose, 1.5-4 mg/dose, 1.5-3 mg/dose, 2.5-7 mg/dose, 2.5-6 mg/dose, 2.5-5 mg/dose, 2.5-4 mg/dose, 3.5-7 mg/dose, 3.5-6 mg/dose, 3.5-5 mg/dose, 4.5-7 mg/dose, 4.5-6 mg/dose or 5.5-7 mg/dose, preferably 1.5-5 mg/dose.

[0041] The concentration of the insoluble particulate adjuvant in the reconstituted liquid vaccine product may be between 1 and 13.5 g/L, for example 1-4 g/L, 1-7 g/L, 1-10 g/L, 1-12 g/L,

3-5 g/L, 3-8 g/L, 3-11 g/L, 3-13.5 g/L, 5-9 g/L, 5-11 g/L, 5-13.5 g/L, 7-9 g/L, 7-11 g/L, 7-13.5 g/L, 9-12 g/L or 9-13.5 g/L. Preferably the concentration of the adjuvant in the liquid vaccine product is 3-11 g/L.

[0042] For aluminium containing adjuvants, the amount of particulate adjuvant in the dry vaccine composition may be measured indirectly by determining the concentration of elemental aluminium per dose. One mg of aluminium typically corresponds to 2.2-2.9 mg of aluminium hydroxide or 4.5 mg of aluminium phosphate. For aluminium containing adjuvants, the concentration of aluminium in the liquid vaccine product may be any value between 0.4 g/L and 3.0 g/L, for example 0.4-1.0 g/L, 0.4-2.0 g/L, 0.4-3.0 g/L, 0.6-1.0 g/L, 0.6-2.0 g/L, 0.6-3.0 g/L, 0.8-1.0 g/L, 0.8-2.0 g/L, 0.8-3.0 g/L, 1.0-2.0 g/L, 1.0-3.0 g/L, 2.0-3.0 g/L, 2.5-3.0 g/L of the vaccine. In some embodiments the concentration of Al in the liquid vaccine is between 1.5 and 2.5 g/L.

[0043] Soluble inorganic salts, excluding the adjuvant, provide the necessary ionic strength to preserve adjuvant structure and state of particle aggregation as well as adsorption of the antigens. That is to say, the soluble inorganic salt(s) used in the vaccine compositions herein is distinct from the insoluble particulate adjuvant. These soluble inorganic salts may be phosphate, calcium, sodium or chloride salts. Soluble inorganic salts include those that upon dissolution provide any of the following inorganic ions:  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $(\text{H}_2\text{PO}_4)^-$ ,  $\text{H}(\text{PO}_4)^{2-}$ ,  $(\text{PO}_4)^{3-}$ ,  $(\text{PO}_3)^-$ , that contribute to the stabilisation of the particulate adjuvant and adsorption of the antigen to the adjuvant. Preferred soluble inorganic salts include sodium chloride. In the dry vaccine composition, the proportion of soluble inorganic salts is between 2% and 10% in weight (w/w) of the dry vaccine composition, for example, 3.0-10.0%, 5.0-10.0%, 7.0-10.0%, 9.0-10.0%, 3.0-9.0%, 5.0-9.0%, 7.0-9.0%, 3.0-7.0%, 5.0-7.0% or 3.0-5.0% w/w of the dry vaccine composition. Preferably the soluble inorganic salt accounts for between 4.0% and 8.0% w/w of the dry vaccine composition.

[0044] In some embodiments the amount of soluble organic salt in the dry vaccine composition (and so also in the liquid vaccine product) is between 1.5 and 5 mg/dose, e.g. 1.5-3 mg/dose, 1.5-4 mg/dose, 2-3 mg/dose, 2-4 mg/dose, 2-5 mg/dose, 3-4 mg/dose or 3-5 mg/dose, preferably 2-4 mg/dose.

[0045] The concentration of soluble inorganic salts in solution in the reconstituted liquid vaccine product may vary between 3 g/L and 10 g/L, for example 4-10 g/L, 5-10 g/L, 6-10 g/L, 7-10 g/L, 8-10 g/L, 9-10 g/L, 3-9 g/L, 4-9 g/L, 5-9 g/L, 6-9 g/L, 7-9 g/L, 8-9 g/L, 3-8 g/L, 4-8 g/L, 5-8 g/L, 6-8 g/L, 7-8 g/L, 3-7 g/L, 4-7 g/L, 5-7 g/L, 6-7 g/L, 3-6 g/L, 4-6 g/L, 5-6 g/L, 3-5 g/L, 4-5 g/L or 3-4 g/L. Preferably the concentration of soluble inorganic salts in the liquid vaccine produce is between 4 and 9 g/L.

- [0046] The ions of the soluble inorganic salts can be present in the liquid antigen formulation, added to the liquid antigen formulation, or generated, totally or partially, in the production of the adjuvant by precipitation reactions, such as by the reaction of aluminium chloride ( $\text{AlCl}_3$ ) with sodium hydroxide ( $\text{NaOH}$ ) to produce aluminium hydroxide adjuvant particles, with a positive surface charge at neutral pH, and  $\text{Cl}^-$  and  $\text{Na}^+$  ions in solution (in which case the solution can be considered to comprise the soluble inorganic salt  $\text{NaCl}$ ).
- [0047] Trehalose is a chemically inert, water-soluble, non-ionisable disaccharide that when dry forms an amorphous glass with a high glass transition temperature ( $T_g = 106^\circ\text{C}$ ) and excellent stabilising properties against damage by freezing, drying and storage. Incorporation of trehalose in the dry vaccine composition contributes to increased stability at high temperatures. In the dry vaccine composition, the proportion of trehalose (% weight/weight) is between 80% w/w and 97% w/w, for example it may be: 80-96% w/w, 80-94% w/w, 80-92% w/w, 80-90% w/w, 80-88% w/w, 80-86% w/w, 80-84% w/w, 80-82% w/w, 82-96% w/w, 82-94% w/w, 82-92% w/w, 82-90% w/w, 82-88% w/w, 82-86% w/w, 82-84% w/w, 84-96% w/w, 84-94% w/w, 84-92% w/w, 84-90% w/w, 84-88% w/w, 84-86% w/w, 86-96% w/w, 86-94% w/w, 86-92% w/w, 86-90% w/w, 86-88% w/w, 88-96% w/w, 88-94% w/w, 88-92% w/w, 88-90%, 90-96% w/w, 90-94% w/w or 90-92% w/w of the dry vaccine composition. Preferably the proportion of trehalose in the dry vaccine composition is 85-92% w/w.
- [0048] In some embodiments the amount of trehalose in the dry vaccine composition (and so also in the liquid vaccine product) is between 50 and 65 mg/dose, for example 50-62 mg/dose, 50-59 mg/dose, 50-56 mg/dose, 50-53 mg/dose, 53-65 mg/dose, 53-62 mg/dose, 53-59 mg/dose, 53-56 mg/dose, 55-65 mg/dose or 55-60 mg/dose, preferably 53-62 mg/dose or 52-63 mg/dose. Lyophilisation of the compounded liquid antigen formulation is preferably carried out with a primary drying step between  $-30^\circ\text{C}$  and  $-35^\circ\text{C}$  in which the majority of water is removed while the product is maintained frozen. A preferred lyophilisation program includes a secondary drying step between  $+35^\circ\text{C}$  and  $+45^\circ\text{C}$ . As a result of the lyophilisation, the dry vaccine composition contains less than 3% w/w residual water. Preferably the dry vaccine composition contains less than 2% w/w residual water. Surprisingly, freezing of the compounded liquid antigen formulation during the lyophilisation process does not destroy the vaccine, as would be expected for the liquid antigen formulation that does not contain trehalose, allowing for effective removal of water by sublimation.
- [0049] The dry vaccine composition of the present invention is highly porous as a result of the removal of frozen water by sublimation from the compounded liquid antigen formulation by the lyophilisation process. Porosity of the dry vaccine composition measured by the available

interconnected open space is above 60% in volume. Preferably open porosity within the dry vaccine composition is above 70% in volume. Furthermore, open porosity may be above 80% in volume. This open porosity greatly facilitates rapid dissolution of the dry vaccine composition upon reconstitution by addition of water to obtain the liquid vaccine product.

5 [0050] The dry vaccine composition does not comprise, and is not located or embedded in, a supporting matrix, such as a water-insoluble sponge. Commonly, the dry vaccine composition is comprised within a container, such as a vial. The absence of any interfering insoluble supporting matrices within the dry vaccine composition, such as the sponge described in J. de la Torre *et al.* (*supra*), greatly facilitates a rapid and complete dissolution upon reconstitution without the need for sustained flicking (or other technique for vigorous mixing to promote entry of water into the hydrophobic sponge and dissolution of the entrapped trehalose). Furthermore, the absence of a sponge facilitates visual verification of complete dissolution of the dry lyophilizate plug, ensuring a complete dose is delivered without flicking. An additional advantage of avoiding the aforementioned sponge is that no surfactants are required in the composition eliminating possible interference of binding of the antigens and the particulate adjuvants. Thus the vaccine compositions and products of the invention generally do not contain a surfactant, such as a polysorbate.

[0051] The liquid vaccine product, obtained after reconstitution of the dry vaccine composition, may comprise between 90 and 130 g/L of trehalose. For example, it may contain 90-100 g/L, 20 90-110 g/L, 90-120 g/L, 100-110 g/L, 100-120 g/L, 100-130 g/L, 110-120 g/L, 110-130 g/L or 120-130 g/L. Preferably the concentration of trehalose in the liquid vaccine product is 95-125 g/L.

[0052] The liquid vaccine product may thus comprise 90-130 g/L trehalose, 3-10 g/L soluble inorganic salt and 1-13.5 g/L insoluble particulate adjuvant. Preferably, the liquid vaccine product comprises 95-125 g/L trehalose, 4-9 g/L soluble inorganic salt and 3-11 g/L insoluble particulate adjuvant.

[0053] The dry vaccine composition (and so also the liquid vaccine product) may comprise 50-65 mg/dose trehalose, 1.5-5 mg/dose soluble inorganic salt and 0.5-6.75 mg/dose insoluble particulate adjuvant. Preferably the dry vaccine composition (and so also the liquid vaccine product) comprises 53-62 mg/dose trehalose, 2-4 mg/dose soluble inorganic salt and 1.5-5 mg/dose insoluble particulate adjuvant.

[0054] In some embodiments, the dry vaccine composition (and so also the liquid vaccine product) comprises less than 10 times as much trehalose as insoluble particulate adjuvant. For example, the dry vaccine composition may comprise at most 9.5, 9, 8.5, 8, 7.5, 7 or 6.5 times as much trehalose as insoluble particulate adjuvant on a w/w basis. For example, the

dry vaccine composition may comprise 8.5-12.5% w/w of an insoluble particulate adjuvant, 80-89.5 % w/w trehalose and 2-10% w/w of a soluble inorganic salt.

5 [0055] The dry vaccine composition has a high porosity and high solubility. As a result of this, upon addition of a suitable volume of water the dry vaccine composition reconstitutes in less than a minute. Typically, the dry vaccine composition reconstitutes in 5 to 20 seconds (without need for flicking) and complete reconstitution is clearly visible within the containing glass vial.

10 [0056] An “antigen” as defined herein is any substance which, upon administration to a subject (generally a human), stimulates an immune response. Such an immune response may be a humoral/antibody response or a cellular response. The antigen may be a protein or peptide, a nucleic acid molecule, an attenuated or inactivated pathogen (particularly virus), a polysaccharide or polysaccharide/protein conjugate, or any other suitable type of molecule.

15 [0057] The vaccine compositions and products of the invention may comprise one or more antigens. In such cases, the antigens may be from the same or different organisms and may be of the same or different types.

[0058] Preferably, the antigen is from a pathogen. Vaccines comprising antigens from pathogens may be used to vaccinate subjects against infectious diseases. The term “infectious disease” is used herein to mean any disease caused by a pathogen.

20 [0059] In preferred embodiments, the antigen is a toxoid. Most preferably, the vaccine compositions and products (and the antigen formulations used in their production) comprise a tetanus toxoid (from *Clostridium tetani*) and/or a diphtheria toxoid (from *Corynebacterium diphtheria*). Other antigens of interest include a *Bordetella pertussis* antigen (whooping cough), a poliovirus antigen, a *Haemophilus influenzae* antigen and a hepatitis B virus antigen. Any such antigen may be used in the vaccine compositions of the invention, or any combination thereof.

30 [0060] The liquid vaccine product may be a tetanus (T) vaccine, a tetanus – reduced diphtheria (Td) vaccine, a diphtheria-tetanus (DT) vaccine, a diphtheria – tetanus – acellular pertussis (DtaP) vaccine, a tetanus- reduced diphtheria – acellular pertussis (TdaP) vaccine, a diphtheria – tetanus – acellular pertussis – inactivated polio virus (DtaP-IPV) vaccine, a diphtheria – tetanus – acellular pertussis – hepatitis B – inactivated polio virus (DtaP-HepB-IPV) vaccine, a diphtheria – tetanus – acellular pertussis – haemophilus influenza B – inactivated polio virus (DtaP-HI-IPV) vaccine, or a diphtheria – tetanus – acellular pertussis – haemophilus influenza B – inactivated polio virus – hepatitis B (DtaP-HI-IPV-HepB) vaccine.

35 [0061] In some preferred embodiments, the liquid vaccine product may be intended for immunization of a subject against bacterial and viral pathogens, particularly including tetanus

(T or t), diphtheria (D), whooping cough (P), polio (IPV), haemophilus influenza (Hib) or hepatitis B (HepB), or combinations thereof.

[0062] A liquid vaccine product of the present invention may be for animal or human use. The liquid vaccine product may be for paediatric or adult use.

5 [0063] Adjuvant structure and antigen adsorption is highly dependent on the surface charge of the adjuvant. Vaccine compositions of the present invention maintain the adjuvant gel structure and association of the adsorbed antigens by maintaining the ionic strength of the original vaccine and incorporating stabilising excipients that permit drying. Despite the contribution of the stabilising excipients to an increased osmolality upon reconstitution, the  
10 dry vaccine compositions of the present invention have been found to remain safe, stable, and effective after months of storage at +40°C and even as high as +45°C, and are not damaged by freezing temperatures below 0°C.

[0064] Following reconstitution of the dry vaccine composition with water for injection and dissolution of the stabilising excipients, the proportion of total antigen adsorbed to the  
15 adjuvant particles in the liquid vaccine product may be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%. Antigen concentration and degree of adsorption may be quantified as total protein (for example, European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.5.33. Total Protein), by standard immunochemical methods (for example, European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.1.  
20 Immunochemical Methods) including immunodiffusion, ELISA and flocculation tests, or by chromatographic methods such as HPLC.

[0065] Dry vaccine compositions of the present invention are provided as dry, mostly soluble compositions that after reconstitution with water for injection result in a liquid vaccine product with an osmolality between 550 and 750 mOsm/Kg, e.g. between 600 and 750 mOsm/Kg or  
25 650-750 mOsm/Kg. Trehalose may for example contribute 200-400 mOsm/Kg to the osmolality, the inorganic salt may contribute 200-400 mOsm/Kg to the vaccine osmolality. This hypertonicity is a result of the added contribution to osmolality of trehalose and inorganic ions from the soluble inorganic salt. Despite this high tonicity the liquid vaccine products of the present invention have not been found to result in increased pain or toxicity at  
30 the site of injection.

[0066] Following reconstitution of the dry vaccine, the pH of the resulting liquid vaccine product is generally between 6 and 7.

[0067] Dry vaccine compositions of the present invention may be provided in single or multiple dose containers. Typically, the reconstitution volume is such that one dose of liquid vaccine  
35 product for injection is finally contained in 0.1-1.5 mL of liquid, or more typically 0.4-0.6 mL of

liquid. In some embodiments the reconstitution volume of the dry composition is such that one vaccine dose for injection is contained in 0.5 ml of liquid vaccine product. Dry vaccine compositions of the present invention have been found to preserve the adjuvant structure, remain effective, and do not result in a significantly increased level of pain or local necrosis compared to an equivalent, isotonic liquid reference vaccine despite the increased osmolality. By an "equivalent, isotonic liquid reference vaccine" is meant a liquid vaccine containing the same antigen and adjuvant, but which is isotonic rather than hypertonic.

[0068] The thermostable dry vaccine composition of the invention may thus comprise amounts of trehalose, soluble inorganic salt and insoluble particulate adjuvant such that upon reconstitution in water, a dose of the liquid vaccine product has a volume of 0.4-0.6 ml and the concentrations and amounts of trehalose, soluble inorganic salt and insoluble particulate adjuvant are as set out above.

[0069] Incorporation of trehalose to the liquid antigen formulation, to obtain the compounded liquid antigen formulation, may be carried out after adsorption of the antigens to the adjuvant. Trehalose may be incorporated in the compounded liquid antigen formulation after it has previously been made to contain the inorganic ions in solution and antigens adsorbed to the adjuvant. Typically, as a first step the antigen is added to a suspension containing the adjuvant and the sodium chloride component, and gently mixed to facilitate adsorption. In a second step trehalose is added. Addition of the trehalose may be direct, or preferably, the trehalose may be dissolved in water to produce a concentrated solution that is filter sterilised before aseptic addition to the liquid antigen formulation to obtain the "compounded liquid antigen formulation". Trehalose may be added to the liquid antigen formulation as a 50-60% w/v sterile solution in water before drying. In preferred embodiments trehalose is added as one part of a 60% w/v trehalose solution in water to five parts of liquid antigen formulation. Addition of trehalose as a solution permits sterile filtration and provides a larger volume of compounded liquid antigen formulation containing the vaccine that results in greater porosity of the lyophilisation plug and a more rapid dissolution.

[0070] The liquid antigen formulation may comprise the soluble inorganic salt and the insoluble particulate adjuvant at the same concentrations as they are intended to be in the final liquid vaccine product. Thus, the liquid antigen formulation may comprise tetanus toxoid (and optionally one or more further antigens), 1-13.5 g/L insoluble particulate adjuvant and 3-10 g/L soluble inorganic salt. Preferably the liquid antigen formulation is aqueous. In a preferred embodiment of the invention, the compounded liquid antigen formulation is produced by adding 1 volume of a 60% w/v trehalose solution in water to five parts of a liquid antigen formulation comprising a tetanus toxoid, 1-13.5 g/L insoluble particulate adjuvant and 3-10 g/L soluble inorganic salt.

[0071] In some embodiments trehalose is added in sufficient amount to the liquid antigen formulation to provide a dry weight ratio of trehalose to the components of the liquid antigen formulation (soluble inorganic salt, adjuvant and antigen) of over 10, for example, a dry weight ratio of trehalose to the components of the liquid antigen formulation of 10-200, e.g. 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-100, 100-150 or 150-200.

[0072] The compounded liquid antigen formulation containing trehalose can then be dispensed into vials and lyophilised, as described above, thereby producing the lyophilizate vaccine composition of the invention. A "vial" as defined herein is a container suitable for lyophilisation, storage and reconstitution of the vaccine composition. A vial is commonly made of glass or plastic but may be made of any other stable, inert material. Commonly a vial is colourless and transparent to allow visualisation of reconstitution, although it may also be amber to prevent photodegradation. A vial may be of any suitable shape or size, though commonly is a small container holding sufficient vaccine for a single dose of vaccine or a small number of doses, e.g. up to 5, up to 10 or up to 50 doses, and allowing for the addition of the corresponding volume of water for reconstitution.

[0073] In embodiments the compounded liquid antigen formulation has a relative strength (i.e. concentration of the adjuvant and antigen) of 80-85% of the liquid antigen formulation. In embodiments, the dry vaccine composition is reconstituted by addition of a smaller volume of water for injection relative to the original fill volume with compounded liquid antigen formulation. For example, the dry vaccine composition may be reconstituted with 65-80% (e.g. 70-80% or 72-77%) of the volume of the compounded liquid antigen dispensed in the vial. That is to say, the dry vaccine composition may be reconstituted in a volume of water that is 20-35% (e.g. 20-30% or 23-28%) less than the volume of the compounded liquid antigen dispensed in the vial. In embodiments the final vaccine product after reconstitution (i.e. the liquid vaccine product) contains the same concentration of vaccine components as the liquid antigen formulation used in the manufacture.

[0074] In embodiments, during manufacture vials are filled with a volume of the compounded liquid antigen formulation that is greater than the final volume of the liquid vaccine product intended for administration after reconstitution. For example the volume of the compounded liquid antigen formulation may be between 15% and 25% greater than that of the final volume of the liquid vaccine product after reconstitution.

[0075] In a preferred embodiment vials are made to provide a single dose of vaccine. In embodiments, vials are filled with 0.75 to 0.85 ml of the compounded liquid antigen formulation and indicated to be reconstituted by addition of 0.6 ml of water for injection from which one dose of liquid vaccine product for administration, equivalent to the original liquid antigen formulation, is contained in 0.5 ml that can be easily extracted from the containing

glass vial. In other embodiments, vials are filled with 0.65 to 0.75 ml of the compounded liquid antigen formulation and indicated to be reconstituted with 0.5 ml of water for injection from which one dose of liquid vaccine product for administration, equivalent to the original liquid antigen formulation, is contained in 0.5 ml that can be easily extracted from the containing glass vial.

[0076] Aside from the antigens, which typically represent a negligible proportion of the bulk of the compositions, the dry vaccine composition of the invention comprises 80% to 97% w/w trehalose, 2% to 10% w/w of a soluble inorganic salt, and 0.7% to 12.5 % w/w of the mineral adjuvant.

[0077] One vial of dry vaccine composition may be made to contain one human dose, the dose containing between 0.5 to 6.75 mg of aluminium hydroxide, between 1.5 and 5 mg of NaCl and between 50 and 65 mg of trehalose. In embodiments the dry vaccine composition contains a proportional additional amount of all components, e.g. between 10% and 30%, to account for losses upon extraction from the vial. The additional amount can be achieved with a corresponding 10% to 30% excess fill volume per vial with the compounded liquid antigen formulation.

[0078] The dry vaccine composition preferably contains a tetanus toxoid. Reconstitution is typically carried out by addition of water for injection or any other suitable liquid. Upon rehydration, one dose of liquid vaccine product may be contained in 0.5 mL with an approximate mean potency for tetanus of 40 IU/0.5 mL (minimum 20 IU/0.5 mL with 95% confidence) (Ph.Eur.2.7.8), or  $\geq 4$  Lf/mL (Ph.Eur.2.7.27). In embodiments, upon rehydration the aluminium content per dose is between 0.2 and 1.5 mg / 0.5 mL (Ph.Eur.2.5.13); the sodium chloride content is between 1.5 and 5.0 mg / 0.5 mL dose (Ph.Eur.2.2.20); the trehalose content is between 50 and 65 mg / 0.5 mL dose; the osmolality is between 550 and 750 mOsm/Kg (Ph.Eur.2.2.35); the degree of adsorption for tetanus toxoid is  $\geq 80\%$  (Ph.Eur.2.7.27); and the pH is between 6.0 and 7.0. In particular embodiments, each vaccine dose contains a minimum of 20, 30 or 40 IU of tetanus toxoid.

[0079] The dry vaccine composition preferably contain a diphtheria toxoid. Upon rehydration, one dose of liquid vaccine product may be contained in 0.5 mL with an approximate mean potency for diphtheria of 4 IU/0.5 mL (Ph.Eur.2.7.6) (minimum 2 IU/0.5 mL with 95% confidence), or  $\geq 2$  Lf/mL (Ph.Eur.2.7.27). Alternatively, the liquid vaccine product may have a mean potency for diphtheria of 30 IU/0.5 mL (Ph.Eur.2.7.6) (minimum 15 IU/0.5 mL with 95% confidence). In embodiments, upon rehydration the aluminium content per dose is between 0.2 and 1.5 mg / 0.5 mL (Ph.Eur.2.5.13); the sodium chloride content is between 1.5 and 5.0 mg / 0.5 mL dose (Ph.Eur.2.2.20); the trehalose content is between 50 and 65 mg / 0.5 mL dose; the osmolality is between 550 and 750 mOsm/Kg (Ph.Eur.2.2.35); the degree

of adsorption for diphtheria toxoid is  $\geq 80\%$  (Ph.Eur.2.7.27); and the pH is between 6.0 and 7.0. In particular embodiments, each vaccine dose contains a minimum of 2, 4, 8, 15, 20 or 30 IU of diphtheria toxoid.

[0080] Most preferably, the dry vaccine composition contains a tetanus toxoid and a diphtheria  
5       toxoid. Upon rehydration, one dose of liquid vaccine product may be contained in 0.5 mL with  
an approximate mean potency for tetanus of 40 IU/0.5 mL (minimum 20 IU/0.5 mL with 95%  
confidence) (Ph.Eur.2.7.8), or  $\geq 4$  Lf/mL (Ph.Eur.2.7.27) and an approximate mean potency  
for diphtheria of 4 IU/0.5 mL (Ph.Eur.2.7.6) (minimum 2 IU/0.5 mL with 95% confidence), or  
10        $\geq 2$  Lf/mL (Ph.Eur.2.7.27). Alternatively, the liquid vaccine product may have a mean potency  
for diphtheria of 30 IU/0.5 mL (Ph.Eur.2.7.6) (minimum 15 IU/0.5 mL with 95% confidence).  
In embodiments, upon rehydration the aluminium content per dose is between 0.2 and  
1.5 mg / 0.5 mL (Ph.Eur.2.5.13 ); the sodium chloride content is between 1.5 and 5.0 mg /  
0.5 mL dose (Ph.Eur.2.2.20); the trehalose content is between 50 and 65 mg / 0.5 mL dose;  
the osmolality is between 550 and 750 mOsm/Kg (Ph.Eur.2.2.35); the degree of adsorption  
15       for tetanus toxoid is  $\geq 80\%$  (Ph.Eur.2.7.27); the degree of adsorption for diphtheria toxoid is  
 $\geq 80\%$  (Ph.Eur.2.7.27); and the pH is between 6.0 and 7.0. In particular embodiments, each  
vaccine dose contains a minimum of 20, 30 or 40 IU of tetanus toxoid, and a minimum of 2,  
4, 8, 15, 20 or 30 IU of diphtheria toxoid.

[0081] The dry vaccine composition may be for vaccination against tetanus, diphtheria,  
20       whooping cough, Haemophilus influenzae, hepatitis B, poliomyelitis or combinations thereof.  
Upon rehydration, one dose of liquid vaccine product may be contained in 0.5 mL with an  
approximate mean potency for tetanus of 40 IU/0.5 mL (minimum 20 IU/0.5 mL with 95%  
confidence) (Ph.Eur.2.7.8), or  $\geq 4$  Lf/mL (Ph.Eur.2.7.27); and approximate mean potency for  
diphtheria of 4 IU/0.5 mL (Ph.Eur.2.7.6) (minimum 2 IU/0.5 mL with 95% confidence), or  
25        $\geq 2$  Lf/mL (Ph.Eur.2.7.27), and  $\geq 5$   $\mu$ g/0.5ml of pertussis toxin (PT),  $\geq 3$   $\mu$ g/0.5ml filamentous  
haemagglutinin (FHA),  $\geq 1$   $\mu$ g/0.5ml peractin (PC) and  $\geq 3$   $\mu$ g/0.5ml fimbriae (FIM) pertussis  
antigens; an aluminium content between 0.2 and 1.5 mg / 0.5 mL (Ph.Eur.2.5.13 ); a sodium  
chloride content between 1.5 and 5.0 mg / 0.5 mL (Ph.Eur.2.2.20); a trehalose content  
between 50 and 65 mg / 0.5mL dose; an osmolality between 550 and 750 mOsm/Kg  
30       (Ph.Eur.2.2.35); a degree of adsorption for tetanus  $\geq 80\%$  (Ph.Eur.2.7.27), a degree of  
adsorption for diphtheria  $\geq 80\%$  (Ph.Eur.2.7.27); and a pH between 6.0 and 7.0.

[0082] Contrary to prior art liquid vaccines containing particulate adjuvants, the dry vaccine  
composition of the present invention has been found not to be affected by exposure to  
freezing temperatures such as  $-10^{\circ}\text{C}$  or  $-20^{\circ}\text{C}$ , or high temperatures such as  $+40^{\circ}\text{C}$ .  
35       Furthermore, dry vaccine compositions of the present invention have been demonstrated to  
maintain their potency unaffected following exposure at  $+45^{\circ}\text{C}$  for over 6 months. Potency is

typically determined by *in vivo* challenge tests by exposing animals to a standardised lethal dose of the relevant toxin. Maintenance of potency may be interpreted as a loss of less than 10% from the reference liquid antigen formulation. In a more stringent setting maintenance of potency may be interpreted as a loss of less than 5% from the reference liquid antigen formulation.

5 [0083] By “thermostable” is thus meant that the dry vaccine composition of the invention may be stable at 40°C or 45°C for a period of 3, 6, 9, 12 or 24 months or more. The dry vaccine compositions may also or alternatively be stable at 0°C, -10°C or -20°C for a period of 1, 2, 3 or 4 weeks, or 1, 2, 3, 4, 5 or 6 months or more. By “stable” is meant herein that during  
10 storage at that temperature for the specified period less than 10 % of the potency of the tetanus toxoid is lost, preferably less than 5 % of the potency. Where the composition also comprises a diphtheria toxoid, less than 10 % of the potency of either the tetanus or the diphtheria toxoid may be lost (i.e. more than 90 % of the potency of both toxoids is retained), preferably less than 5 % of either toxoid. As set out above, the retention/loss of potency can  
15 be calculated upon reconstitution of the dry vaccine in water, by comparing the potency of the reconstituted liquid vaccine product with the potency of the liquid antigen formulation used to produce the dry vaccine composition.

[0084] Thermostability after storage at high temperatures or upon freezing may also be assessed by adjuvant column sedimentation. The examples below show that generally,  
20 lyophilisation and reconstitution of a vaccine containing aluminium hydroxide adjuvant results in loss of column sedimentation, but that sedimentation is retained in the vaccine products of the invention. Thus “stable” may additionally or alternatively mean that following reconstitution of the dry vaccine composition, the adjuvant occupies at least 15 % v of a graduated settling column after 24 hrs incubation at 20°C, preferably at least 20 % v.

25 [0085] Stability of dry vaccine compositions within such broad temperature range, from  $\leq -20^{\circ}\text{C}$  to  $\geq +45^{\circ}\text{C}$ , opens the possibility for vaccine product specifications that altogether avoid temperature control on shipment and storage. This represents a significant contribution for the development of vaccines for the developing world and greatly reduces the cost and impact of maintaining the cold chain.

30 [0086] Addition of water results in rapid dissolution of the lyophilizate plug without the need to contain a surfactant in the formulation and without the requirement for significant flicking or agitation. Following reconstitution of the dry vaccine compositions in a vial, e.g. a glass vial, the reconstituted liquid vaccine product is clearly visible within seconds and may be administered via intramuscular injection, subcutaneous injection, intradermal injection, oral  
35 administration, nasal spray, or intravenous injection. Maintenance of the required concentration of inorganic ions to maintain adjuvant integrity in addition to the required

amount of stabilising excipient required to maintain particulate mineral adjuvant and antigen integrity in the dry state results in compositions with high osmolality. However, despite their high osmolality upon reconstitution and dissolution of the stabilising excipients, liquid vaccine product of the present invention have been found to be safe for administration.

- 5 [0087] Comparative studies against prior art liquid vaccine products containing particle-based adjuvants have unexpectedly shown that the liquid vaccine products of the present invention, despite their hypertonicity, do not induce any greater discomfort than the prior art liquid vaccines (see Examples).

[0088] The liquid vaccine product of the invention is thus provided for use in therapy. For  
10 example, the liquid vaccine product comprising an antigen from a pathogen is provided for use in immunising a subject against an infectious disease. Similarly, a method of immunising a subject against an infectious disease is provided, the method comprising administering to the subject the liquid vaccine product of the invention. Similarly, also provided is use of the liquid vaccine product of the invention in the manufacture of a medicament for immunising a  
15 subject against an infectious disease.

[0089] The infectious disease(s) against which the vaccine can be used to immunise a subject depends on the antigen(s) it contains. For instance it may be used to immunise a subject against tetanus (if the vaccine comprises a tetanus toxoid), diphtheria (if the vaccine comprises a diphtheria toxoid), whooping cough (if it contains a *B. pertussis* antigen), polio (if  
20 it comprises a poliovirus antigen, *H. influenzae* infection (if it contains a *H. influenzae* antigen) and/or hepatitis B infection (if it contains a hepatitis B virus antigen).

[0090] By “immunising” (or “vaccinating”) as used herein is meant that the liquid vaccine product is administered to a subject and induces a protective immune response. Such an immune response may be an antibody and/or a T cell response. The immune response may  
25 prevent the subject from infection with a pathogen (depending on the nature of the antigen). In other cases the immune response prevents the subject from developing symptomatic disease caused by the relevant pathogen in the event of infection. Alternatively, a protective immune response may result in the subject developing milder disease in the event of infection by the relevant pathogen than they would have without the vaccination, e.g. the  
30 immune response may protect the subject from death, hospitalisation or other serious effects of a disease such as organ damage or suchlike.

[0091] Other aspects and embodiments of the invention provide the aspects and embodiments described above with the term “comprising” replaced by the term “consisting of” and the aspects and embodiments described above with the term “comprising” replaced by the term  
35 “consisting essentially of”.

[0092] It is to be understood that the application discloses all combinations of any of the above aspects and embodiments described above with each other, unless the context demands otherwise. Similarly, the application discloses all combinations of the preferred and/or optional features either singly or together with any of the other aspects, unless the context demands otherwise.

[0093] Modifications of the above embodiments, further embodiments and modifications thereof will be apparent to the skilled person on reading this disclosure, and as such, these are within the scope of the present invention.

[0094] All documents and sequence database entries mentioned in this specification are incorporated herein by reference in their entirety for all purposes.

[0095] “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

[0096] The term “between”, in the context of a range, is to be understood as an inclusive term encompassing both end points, e.g. the range “between 1 and 10” is the same as the range “1 to 10”.

### **Figure Legend**

[0097] Figure 1 shows the workflow for production of the liquid vaccine product from a liquid antigen formulation and a trehalose solution.

### **Examples**

#### **Example 1 – Preservation of Adjuvant Structure**

[0098] A liquid antigen formulation containing tetanus-diphtheria (Td) adsorbed to aluminium hydroxide as adjuvant was used as control “Reference” and compared to different test items. Physicochemical characteristics (conductivity, osmolality, aluminium content, sedimentation rate, particle size, aggregate size, adsorbed tetanus antigen, adsorbed diphtheria antigen) were analysed for different test items and compared to the Reference.

[0099] As a measure of adjuvant structure the aluminium hydroxide sedimentation rate was determined with a graduated settling column containing 5 ml of the test item following 24 hr incubation at room temperature. The adjuvant of the Reference liquid antigen formulation typically occupies 25% to 30% of the column. Column height values below 20% were regarded as unacceptable.

- [0100] The liquid antigen formulation (A) used as a comparator "Reference", and from which the rest of the test items (B to D) were derived, had a mean potency of 80 IU/ mL of tetanus toxoid, lower confidence limit  $p=0.95$  of 40 IU/ mL (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.8 Assay of tetanus vaccine – adsorbed), and a mean potency of 8 IU/ mL of diphtheria toxoid, lower confidence limit  $p=0.95$  of 4 IU/ mL (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.6 Assay of diphtheria vaccine – adsorbed), measured by lethal challenge test; 10 Lf/0.5 ml dose for tetanus, and 3 Lf/0.5 ml dose for diphtheria, measured by flocculation (Ramon) assay European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.2.27); an aluminium content 1.0 g/L (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.5.13 ); a sodium chloride content of 8.5 g/L (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.2.20); a theoretical osmolality of 255 mOsm/Kg and a measured osmolality of 272 mOsm/Kg (Ph.Eur.2.2.35), a degree of adsorption for tetanus  $\geq 80\%$  (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.27), and degree of adsorption for diphtheria  $\geq 80\%$  (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.27); and a pH between 6.0 and 7.0.
- 15 [0101] For test item (B); the Reference liquid antigen formulation (A) was dialysed with a 10,000 Nominal Molecular Weight Cutoff (NMWCO) membrane against a 10x volume of distilled water to reduce the concentration of sodium chloride. Reduction in the content of sodium chloride resulted in a drop in conductivity and osmolality, and resulted in the disappearance of the sedimentation column and adjuvant structure, and a reduced antigen adsorption.
- 20 [0102] For test item (C); the Reference liquid antigen formulation (A) was lyophilised and rehydrated to the same volume to obtain very similar osmolality, 275 mOsm/Kg, as the start material. Drying without a preservative resulted in the collapse of the sedimentation column to 10% of the Reference liquid antigen formulation and a decline in antigen adsorption.
- 25 [0103] For test item (D) "*dry vaccine composition*"; a volume of 0.10 ml of a 60% trehalose solution is added per 0.50 ml of the Reference Vaccine (A) to obtain a "*compounded liquid antigen formulation*", with a theoretical osmolality of 560 mOsm/Kg. The composition of the compounded liquid antigen formulation was 100 g/L of trehalose (10% w/v); 7 g/L sodium chloride; and 0.8 mg/ml of aluminium; containing a mean potency of 66.7 IU/ mL of tetanus toxoid, lower confidence limit  $p=0.95$  of 33.3 IU/ mL, and a mean potency of 6.7 IU/ mL of diphtheria toxoid, lower confidence limit  $p=0.95$  of 3.3 IU/ mL. A volume of 0.80 ml (8.3 g) of the compounded liquid antigen formulation was then dispensed into 2R glass vials and lyophilised to obtain a "*dry vaccine composition*" (D) with less than 3% residual moisture. The composition of the dry vaccine contained in the vial was 80 mg/vial of trehalose, 5.7 mg/vial sodium chloride; 0.7 mg/vial aluminium; and a mean potency of 53.3 IU/vial of tetanus toxoid, lower confidence limit  $p=0.95$  of 26.7 IU/ vial, and a mean potency of 5.3 IU/ vial of diphtheria toxoid, lower confidence limit  $p=0.95$  of 2.7 IU/ vial.
- 35

[0104] For testing, each vial of the test item (D) dry vaccine composition was reconstituted by addition of 0.60 ml water for injection (wfi), that is 70-75% of the volume of compounded liquid antigen formulation added to the vial in manufacture, to obtain a final approximate volume of 0.66 ml of "liquid vaccine product" with a theoretical potency that was within  $\pm 2\%$  IU/ml of the Reference Vaccine (A) and a full vaccine dose contained in a 0.50 ml volume. Reconstitution resulted in the complete dissolution of the trehalose lyophilizate in less than one minute, and a liquid vaccine product with a final osmolality of 656 mOsm/Kg. A vaccine dose of 0.5 ml of the liquid vaccine product having a final measured composition of 60.0 mg of trehalose; 4.3 mg of sodium chloride; 0.5 mg of aluminium, and a mean potency, measured by *in vivo* lethal challenge test, of 40 IU/ 0.5 mL dose of tetanus toxoid, and a mean potency of 4 IU/ 0.5 mL dose of diphtheria toxoid. Unexpectedly, drying in the presence of 10% trehalose while maintaining the salt content resulted in a hypertonic composition of 656 mOsm/Kg, which preserved adjuvant structure and a high degree of antigen adsorption necessary for vaccine efficacy.

|                                                            | (A) Liquid Td reference vaccine | (B) Liquid Td vaccine dialysed against water | (C) Dry and resuspended reference vaccine | (D) Dry and resuspended Td vaccine containing 10% trehalose |
|------------------------------------------------------------|---------------------------------|----------------------------------------------|-------------------------------------------|-------------------------------------------------------------|
| Conductivity $\mu\text{S}/\text{cm}$                       | 14,500                          | 223                                          | 14,600                                    | 14,600                                                      |
| Osmolality (mOsm/Kg)                                       | 272                             | 38                                           | 275                                       | 656                                                         |
| Aluminium content (mg/ml)                                  | 1.17                            | 1.13                                         | 1.18                                      | 1.18                                                        |
| Adjuvant structure (Sedimentation column)                  | 30%                             | No sedimentation and diffuse appearance      | <3%                                       | 28%                                                         |
| Particle size                                              | $\approx 10$ nm                 | $\approx 10$ nm                              | $\approx 10$ nm                           | $\approx 10$ nm                                             |
| Aggregate size                                             | $\approx 10$ $\mu\text{m}$      | > 50 $\mu\text{m}$                           | > 500 $\mu\text{m}$                       | $\approx 10$ $\mu\text{m}$                                  |
| Tetanus antigen (flocculation)                             | 20 Lf/ml                        | < 2.0 Lf/ml                                  | < 0.2 Lf/ml                               | 20 Lf/ml                                                    |
| Diphtheria antigen (flocculation)                          | 6 Lf/ml                         | < 0.6 Lf/ml                                  | < 0.6 Lf/ml                               | 6 Lf/ml                                                     |
| Tetanus adsorption                                         | $\approx 90\%$                  | < 60%                                        | < 60%                                     | $\approx 90\%$                                              |
| Diphtheria adsorption                                      | $\approx 90\%$                  | < 60%                                        | < 60%                                     | $\approx 90\%$                                              |
| Tetanus potency ( <i>in vivo</i> lethal challenge test)    | Mean 42 IU/ 0.5ml               | Mean < 20.0 IU/ 0.5ml                        | Mean < 20.0 IU/ 0.5ml                     | Mean 46 IU/ 0.5ml                                           |
| Diphtheria potency ( <i>in vivo</i> lethal challenge test) | Mean 5.5 IU/ 0.5ml              | Mean < 2.0 IU/ 0.5ml                         | Mean < 2.0 IU/ 0.5ml                      | Mean 5.6 IU/ 0.5ml                                          |

**Example 2 – Thermostability of tetanus-diphtheria vaccine**

[0105] Comparative evaluation of the thermostability of a tetanus-diphtheria (Td) “Reference” liquid antigen formulation (A), and a dry vaccine composition derived from it, “Test Item” (D) of the present invention was performed. Td Reference liquid antigen formulation (A) had a mean potency of 80 IU/ mL of tetanus toxoid, lower confidence limit  $p=0.95$  of 40 IU/ mL (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.8 Assay of tetanus vaccine – adsorbed), and a mean potency of 8 IU/ mL of diphtheria toxoid, lower confidence limit  $p=0.95$  of 4 IU/ mL (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.6 Assay of diphtheria vaccine – adsorbed), measured by lethal challenge test; 10 Lf/0.5 ml dose for tetanus, and 3 Lf/0.5 ml dose for diphtheria, measured by flocculation (Ramon) assay European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.2.27); an aluminium content 0.7 g/L (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.5.13 ); a sodium chloride content of 7.5 g/L (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.2.20); a theoretical osmolality of 240 mOsm/Kg and measured osmolality of 272 mOsm/Kg (Ph.Eur.2.2.35), a degree of adsorption for tetanus  $\geq 80\%$  (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.27), and degree of adsorption for diphtheria  $\geq 80\%$  (European Pharmacopoeia 8<sup>th</sup> Ed, Chapter 2.7.27); and a pH between 6.0 and 7.0. Aluminium hydroxide adjuvant column height was 28%.

[0106] For dry test item (D) “*dry vaccine composition*”; a volume of 0.10 ml of a 60% trehalose solution is added per 0.50 ml of the Td Reference Vaccine (A) to obtain a “*compounded liquid antigen formulation*” with a theoretical osmolality of 520 mOsm/Kg. The composition of the compounded liquid antigen formulation was 100 g/L of trehalose (10% w/v); 5.0 g/L sodium chloride; and 0.5 g/L of aluminium, containing a mean potency of 66.7 IU/ mL of tetanus toxoid, lower confidence limit  $p=0.95$  of 33.3 IU/ mL, and a mean potency of 6.7 IU/mL of diphtheria toxoid, lower confidence limit  $p=0.95$  of 3.3 IU/ mL. A volume of 0.69 ml of the compounded liquid antigen formulation was then dispensed into 2R glass vials and lyophilised to obtain a dry cake with less than 3% residual moisture. The composition of the dry vaccine composition was 69 mg/vial of trehalose, 4.3 mg/vial of sodium chloride; 0.4 mg/vial of aluminium; and a mean potency of 46.0 IU / vial of tetanus toxoid, lower confidence limit  $p=0.95$  of 23.0 IU/ vial, and a mean potency of 4.6 IU/ vial of diphtheria toxoid, lower confidence limit  $p=0.95$  of 2.3 IU/ vial.

[0107] For testing, each vial of the dry test item (D) dry vaccine composition was reconstituted by adding 0.50 ml water for injection (wfi) to obtain a final volume of 0.57 ml of “*liquid vaccine product*” with the same theoretical potency as the reference liquid antigen formulation (A) and a full vaccine dose within a 0.50 ml volume. Reconstitution resulted in the complete dissolution of the trehalose lyophilizate in less than one minute, and a liquid vaccine product with a final osmolality of 660 mOsm/Kg and an average adjuvant column height of 26%. A

vaccine dose of 0.5 ml of the liquid vaccine product had a final measured composition of 60.0 mg of trehalose; 3.8 mg of sodium chloride; 0.35 mg of aluminium, and a mean potency of 40 IU/ 0.5 mL dose of tetanus toxoid, and a mean potency of 4 IU/ 0.5 mL dose of diphtheria toxoid.

- 5 [0108] For the evaluation of stability, Reference Vaccine (A), and dry vaccine composition (D) were stored at +45°C for 1 and 9 months and the integrity of the adjuvant, antigens and potency evaluated. Liquid Td Reference Vaccine" (A) showed a clear degradation of adjuvant structure and loss of integrity for tetanus and diphtheria antigens established by *in vitro* ELISA and loss of potency of tetanus and diphtheria antigens determined by *in vivo* lethal
- 10 potency challenge tests. In contrast, unexpectedly, the liquid vaccine product resulting from the reconstitution of the dry vaccine composition (D) appeared to maintain all the essential specifications for an effective vaccine, including adjuvant structure, integrity of the antigens and *in vivo* potency.

| Time                     | Features                                                   | (A) Liquid Td Reference Vaccine | (D) Dry vaccine composition (reconstituted) |
|--------------------------|------------------------------------------------------------|---------------------------------|---------------------------------------------|
|                          | Osmolality (mOsm/Kg)                                       | 272                             | 660                                         |
| Day 0                    | Adjuvant structure (Sedimentation column)                  | 28%                             | 26%                                         |
|                          | Tetanus antigen ( <i>in vitro</i> ELISA)                   | 10 Lf/ 0.5 ml                   | 10 Lf/ 0.5 ml                               |
|                          | Diphtheria antigen ( <i>in vitro</i> ELISA)                | 3 Lf/ 0.5 ml                    | 3 Lf/ 0.5 ml                                |
|                          | Tetanus potency ( <i>in vivo</i> lethal challenge test)    | Mean 44 IU/ 0.5ml               | Mean 46 IU/ 0.5ml                           |
|                          | Diphtheria potency ( <i>in vivo</i> lethal challenge test) | Mean 6.0 IU/ 0.5ml              | Mean 6.5 IU/ 0.5ml                          |
| After 1 months at +45°C. | Adjuvant structure (Sedimentation column)                  | 24%                             | 27%                                         |
|                          | Tetanus antigen ( <i>in vitro</i> ELISA)                   | 7 Lf/ 0.5 ml                    | 9.8 Lf/ 0.5 ml                              |
|                          | Diphtheria antigen ( <i>in vitro</i> ELISA)                | 1.2 Lf/ 0.5 ml                  | 2.8 Lf/ 0.5 ml                              |
|                          | Tetanus potency ( <i>in vivo</i> lethal challenge test)    | Mean 40 IU/ 0.5ml               | Mean 44 IU/ 0.5ml                           |
|                          | Diphtheria potency ( <i>in vivo</i> lethal challenge test) | Mean 5 IU/ 0.5ml                | Mean 6.4 IU/ 0.5ml                          |
| After 9 months at +45°C. | Adjuvant structure (Sedimentation column)                  | < 3%                            | 28%                                         |
|                          | Tetanus antigen ( <i>in vitro</i> ELISA)                   | 0.2 Lf/ml                       | 6.7 Lf/ml                                   |
|                          | Diphtheria antigen ( <i>in vitro</i> ELISA)                | 0.3 Lf/ml                       | 2.7 Lf/ml                                   |
|                          | Tetanus potency ( <i>in vivo</i> lethal challenge test)    | Mean < 20 IU/ 0.5ml             | Mean 45 IU/ 0.5ml                           |
|                          | Diphtheria potency ( <i>in vivo</i> lethal challenge test) | Mean < 2 IU/ 0.5ml              | Mean 6.6 IU/ 0.5ml                          |
| After 1 week at -20°C.   | Adjuvant structure (Sedimentation column)                  | < 3%                            | 26%                                         |
|                          | Tetanus antigen ( <i>in vitro</i> ELISA)                   | 4 Lf/ml                         | 6.5 Lf/ml                                   |
|                          | Diphtheria antigen ( <i>in vitro</i> ELISA)                | 0.6 Lf/ml                       | 2.8 Lf/ml                                   |
|                          | Tetanus potency ( <i>in vivo</i> lethal challenge test)    | Mean < 2.0 IU/ 0.5ml            | Mean 44 IU/ 0.5ml                           |
|                          | Diphtheria potency ( <i>in vivo</i> lethal challenge test) | Mean < 2.0 IU/ 0.5ml            | Mean 6.4 IU/ 0.5ml                          |

**Example 3 – Td evaluation of pain and site of injection**

[0109] *In vivo* comparative evaluation of a tetanus-diphtheria (Td) “Reference” liquid antigen formulation (A), and a test Item (D) dry vaccine composition of the present invention was performed. “Reference” liquid antigen formulation (A), and from which the dry “Test Item” (D) dry vaccine composition was derived contained  $\geq 40$  IU/0.5 mL mean value of tetanus toxoid and  $\geq 4$  IU/0.5 mL mean value of diphtheria toxoid, and aluminium content of 7.0 g/L (Ph.Eur.2.5.13); a sodium chloride content of 3.7 g/L (Ph.Eur.2.2.20); a theoretical osmolality of 255 mOsm/Kg and a measured osmolality of 272 mOsm/Kg (Ph.Eur.2.2.35), a degree of adsorption for tetanus  $\geq 80\%$  (Ph.Eur.2.7.27), and degree of adsorption for diphtheria  $\geq 80\%$  (Ph.Eur.2.7.27); and a pH between 6.0 and 7.0.

[0110] For the manufacture of Test Item (D) dry vaccine composition, a volume of 1L of a 60% trehalose solution was added per 5L of the “Reference” liquid antigen formulation (A). A volume of 0.72 ml of the compounded mix was then dispensed into 2R glass vials and lyophilised to obtain a dry cake with less than 3% residual moisture. For testing, each vial of the Test Item (D) was reconstituted with 0.60 ml water for injection (wfi) to obtain a final volume of 0.625ml with the same theoretical potency as the Reference Vaccine (A) and a full vaccine dose within a 0.50 ml volume. Reconstitution resulted in rapidly visible complete dissolution and a liquid composition with the same potency values as the Reference (A), that is,  $\geq 40$  IU/0.5 mL mean value of tetanus toxoid and  $\geq 4$  IU/0.5 mL mean value of diphtheria toxoid, and a final osmolality of 630 mOsm/Kg and measured osmolality of 660 mOsm/Kg.

[0111] For the evaluation of potential adverse effects including pain, six Sprague Dawley rats per group were administered intramuscularly by injection with 0.1 ml of either Reference liquid antigen formulation (A) or reconstituted dry vaccine composition, Test Item (D). Animals were observed uninterruptedly for 6 hours and followed for 7 days and then sacrificed. Study conclusions were: a) that the administration of Test Item (D) or Reference (A) were well tolerated as there was no vaccine-related mortality recorded; b) no local discomfort, pain or other clinical signs related to the treatment were observed either in test animals receiving the reconstituted dry vaccine composition, Test Item (D), or control animals receiving the Reference (A); c) no differences in body weight were recorded between animals receiving Test Item (D) or controls receiving the Reference liquid antigen formulation (A). Animals gained weight throughout the study; d) at terminal sacrifice, neither gross lesions nor any relevant effects of the exposure on organ weights were observed in animals receiving either Test Item (D) or the Reference (A); e) no relevant differences in the microscopic findings between animals receiving Test Item (D) or the Td Reference Vaccine (A) were observed, and all other findings recorded were within the range of normal

background lesions. Altogether, the study demonstrated the absence of pain from the reconstituted dry vaccine composition of the present invention, Test Item (D), despite its composition and high osmolality.

**Claims**

1. A thermostable lyophilizate vaccine composition containing an antigen, the composition comprising:
  - 5 a) between 80% and 97% w/w of trehalose;
  - b) between 2% and 10% w/w of a soluble inorganic salt; and
  - c) between 0.7% and 12.5% w/w of an insoluble particulate adjuvant.
2. The thermostable vaccine composition of claim 1, wherein the soluble inorganic salt is  
10 sodium chloride.
3. The thermostable vaccine composition of claim 1 or 2, wherein the adjuvant is an aluminium hydroxide, an aluminium phosphate or a calcium phosphate.
- 15 4. The thermostable vaccine composition of any one of claims 1 to 3, wherein the composition comprises at most 9 times as much trehalose as adjuvant on a w/w basis.
5. The thermostable vaccine composition of any one of claims 1 to 4, wherein the antigen  
is from a pathogen.  
20
6. The thermostable vaccine composition of claim 5, wherein the antigen is a toxoid.
7. The thermostable vaccine composition of claim 5 or 6, wherein the antigen is selected from a tetanus toxoid, a diphtheria toxoid, a *Bordetella pertussis* antigen, a poliovirus antigen, a  
25 *Haemophilus influenzae* antigen and/or a Hepatitis B virus antigen.
8. The thermostable vaccine composition of claim 7, wherein the composition comprises a tetanus toxoid and/or a diphtheria toxoid.
- 30 9. The thermostable vaccine composition of claim 8, wherein following storage at 45°C for 6 months:
  - (a) less than 10 % of the potency of the tetanus toxoid is lost, preferably less than 5 %; and/or
  - (b) following reconstitution the adjuvant occupies at least 15% of a graduated settling  
35 column after 24 hr incubation at 20°C, preferably at least 20 %.

10. The thermostable vaccine composition of claim 8 or 9, wherein following storage at -20°C for 1 week:
- (a) less than 10 % of the potency of the tetanus toxoid is lost, preferably less than 5 %; and/or
- 5 (b) following reconstitution the adjuvant occupies at least 20 % of a graduated settling column after 24 hr incubation at 20°C, preferably at least 25 %.
11. The thermostable vaccine composition of any one of claims 1 to 10, comprising less than 3 % w/w water.
- 10 12. The thermostable vaccine composition of any one of claims 1 to 11, wherein the composition does not comprise a surfactant.
13. The thermostable vaccine composition of any one of claims 1 to 12, comprising:
- 15 a) between 50 and 65 mg/dose of trehalose;
- b) between 1.5 and 5 mg/dose of a soluble inorganic salt; and
- c) between 0.5 and 6.75 mg/dose of an insoluble particulate adjuvant.
14. The thermostable vaccine composition of any one of claims 1 to 13, which upon
- 20 reconstitution with water for injection provides a liquid vaccine product containing a dose of vaccine in 0.4 to 0.6 mL and comprising:
- a) between 90 and 130 g/L of trehalose;
  - b) between 3 and 10 g/L of a soluble inorganic salt; and
  - c) between 1 and 13.5 g/L of an insoluble particulate adjuvant.
- 25 15. The thermostable vaccine composition of claim 14, wherein the adjuvant is aluminium hydroxide or aluminium phosphate, and the concentration of aluminium in the liquid vaccine product is between 0.4 g/L and 3.0 g/L.
- 30 16. The thermostable vaccine composition of claim 14 or 15, wherein the liquid vaccine product has an osmolality between 550 and 750 mOsm/Kg.
17. The thermostable vaccine composition of any one of claims 14 to 16, wherein the pH of the liquid vaccine product is between 6 and 7.
- 35 18. A method of preparing the thermostable lyophilizate vaccine composition of any one of claims 1 to 17, the method comprising:

- (a) providing a liquid antigen formulation comprising:
- (i) an antigen;
  - (ii) between 1 and 13.5 g/L of an insoluble particulate adjuvant; and
  - (iii) 3 to 10 g/L of a soluble inorganic salt;
- 5 (b) producing a compounded liquid antigen formulation by adding 1 volume of a 50-60 % w/v trehalose solution to 5 volumes of the liquid antigen formulation; and
- (c) lyophilising the compounded liquid antigen formulation to produce the thermostable lyophilizate vaccine composition.
- 10 19. A method to obtain a liquid vaccine product, the method comprising:
- a) providing a liquid antigen formulation comprising:
- (i) an antigen;
  - (ii) between 1 and 13.5 g/L of an insoluble particulate adjuvant; and
  - (iii) 3 to 10 g/L of a soluble inorganic salt;
- 15 b) producing a compounded liquid antigen formulation by adding 1 volume of a 50-60 % w/v trehalose solution to 5 volumes of the liquid antigen formulation;
- c) dispensing a suitable volume of the compounded liquid antigen formulation into a vial;
- d) lyophilising the compounded liquid antigen formulation to produce the thermostable lyophilizate vaccine composition of any one of claims 1 to 17; and
- 20 e) reconstituting the dry vaccine composition with a volume of water 20% to 35% less than the volume of the compounded liquid antigen dispensed in the vial.
20. A liquid vaccine product as defined in any one of claims 14 to 17.
- 25 21. The liquid vaccine product of claim 20, wherein the antigen is from a pathogen.
22. The liquid vaccine product of claim 20 or 21, for use in therapy.
- 30 23. The liquid vaccine product of claim 21, for use in immunising a subject against an infectious disease.
24. The liquid vaccine product for use of claim 23, wherein the liquid vaccine product comprises a tetanus toxoid, a diphtheria toxoid, a *Bordetella pertussis* antigen, a poliovirus antigen, a *Haemophilus influenzae* antigen and/or a Hepatitis B virus antigen, and the immunisation is against tetanus, diphtheria, whooping cough, polio, *H. influenzae* infection and/or hepatitis B infection.
- 35

25. The liquid vaccine product for use of claim 24, wherein the liquid vaccine product comprises tetanus toxoid and/or diphtheria toxoid, and the immunisation is against tetanus and/or diphtheria.
- 5
26. Use of the liquid vaccine product of claim 21 in the manufacture of a medicament for immunising a subject against an infectious disease, optionally wherein the liquid vaccine product and immunisation are as defined in claim 24 or 25.
- 10
27. A method of immunising a subject against an infectious disease, comprising administering to the subject the liquid vaccine product of claim 21,  
optionally wherein the liquid vaccine product and immunisation are as defined in claim 24 or 25.
- 15
28. A method of stimulating an immune response in a subject, comprising administering to the subject the liquid vaccine product of claim 20 or 21.