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Vaccines

The present invention relates to novel vaccine formulations, methods for preparing them and to their use in therapy. In particular the present invention relates to novel combination vaccine formulations including a Hepatitis B vaccine component for treating Hepatitis B infections.

Infection with Hepatitis B (HB) virus is a widespread problem but vaccines which have been used for mass immunisation are now available, for example the product 'Engerix-B' (SmithKline Beecham plc). Engerix B has as the Hepatitis B antigenic component Hepatitis B surface antigen (HBsAg) which is obtained by genetic engineering techniques.

However it is often necessary or desirable to administer Hepatitis B vaccine at the same time as other vaccines and this can involve multiple injections. Problems associated with multiple injections include a more complicated administration procedure and a large total injection volume.

There is therefore a need for a combined vaccine comprising a Hepatitis B antigen in combination with other antigens. The other antigens are in particular those capable in a vaccine formulation of preventing Hepatitis A (HA), diphtheria (D), tetanus (T), whole cell pertussis (Pw), acellular pertussis (Pa), Haemophilus influenzae b (Hib) and polio (P).

Aluminium hydroxide (AH) is widely used as an adjuvant in the formulation of vaccines. For example, Engerix B uses Hepatitis B surface antigen (HBsAg) adsorbed to aluminium hydroxide. We have also used AH successfully in the formulation of Hepatitis A vaccine and in the combined vaccines DT, DTPw and DTPa. However, when AH -adsorbed HBsAg is used in combination with other vaccines in a combined formulation there is a substantial decrease of the immune response to HBsAg , resulting in lower or insufficient seroprotection after vaccination. In addition the stability of the HBsAg component of the combined vaccine is poor.

Aluminium phosphate (AP) adsorbed HBsAg has been used in a commercially available monovalent vaccine (HEPPACINE) made by Korean Cheil Sugar Co Ltd. We have found that there is no significant difference in immunogenic properties between an AH- adsorbed HBsAg monovalent vaccine (Engerix B) and an AP-adsorbed HBsAg monovalent vaccine.

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European patent application publication number 0 339 667 discloses a bivalent vaccine comprising HBsAg and a Hepatitis A antigen in which either aluminium hydroxide or aluminium phosphate is used as adjuvant. There appears, however, to be no appreciation of the need to avoid aluminium hydroxide as an adjuvant for a multivalent vaccine comprising HBsAg. Furthermore there appears to be no disclosure in this document or elsewhere of a bivalent or multivalent hepatitis B vaccine in which at least one antigen other than HBsAg is adsorbed on aluminium hydroxide and the HBsAg is adsorbed on aluminium phosphate.

Indeed there appears to be no prior enabling disclosure of a stable and effective multivalent vaccine comprising HBsAg at all.

In one aspect the present invention provides a combined vaccine composition comprising Hepatitis B surface antigen (HBsAg) and a number (n) of other antigens in combination with an adjuvant comprising one or more aluminium salts in which the value of n is 1 or greater and in which the adjuvant used to adsorb the HBsAg is not aluminium hydroxide, with the proviso that when n is 1 the other antigen is not an antigen against Hepatitis A.

Preferably n is 2, 3, 4, 5 or 6.

The advantage of the invention is that no substantial decrease in the immunogenicity of the HBsAg occurs in the combined vaccine formulation. Avoiding the use of AH to adsorb the HBsAg component in the vaccine formulation also gives rise to a product of markedly superior stability. A further advantage of the invention is that the aforesaid problems associated with multiple injections are overcome or at least mitigated and a stable, highly immunogenic combined formulation is provided. The compositions of the invention are particularly suitable for administration to children.

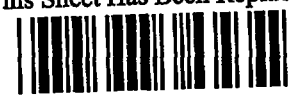
Preferably the HBsAg is adsorbed on AP. In particular we have found in human clinical studies that when AP-adsorbed HBsAg is combined with one or more AH-adsorbed or AP-adsorbed antigens in a combined vaccine no substantial decrease in immunogenicity occurs. The stability of the AP-adsorbed HBsAg in the formulation is also greater than AH-adsorbed HBsAg.

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Accordingly in a further aspect there is provided a vaccine composition according to the invention in which at least one of the antigens other than HBsAg is adsorbed to aluminium phosphate.

In a further preferred aspect at least one of the antigens other than HBsAg is adsorbed to AH.

In a further aspect, the invention provides a combined vaccine comprising Hepatitis B surface antigen (HBsAg) adsorbed to AP and an antigen adsorbed to AP or to AH selected from an antigen providing immunity against one or more of the following viruses: diphtheria (D); tetanus (T); pertussis (P); Inactivated Polio (IPV); Haemophilus influenzae b (Hib); and Hepatitis A (HA).

In a paediatric vaccine other compatible antigens may also be included, eg antigens known to be effective against meningitis B, meningitis A and C, and otitis media.

As used herein the term 'bivalent' is used to refer to a vaccine comprising a combination of two antigens in total (including HBsAg). The term 'multivalent' is applied to a vaccine composition comprising more than two antigens, for example three, four or five or six antigens.

The meaning of the terms 'aluminium phosphate' and 'aluminium hydroxide' as used herein includes all forms of aluminium hydroxide or aluminium phosphate which are suitable for adjuvanting vaccines.

For example, aluminium phosphate can be a precipitate of insoluble aluminium phosphate (amorphous, semi-crystalline or crystalline), which can be optionally but not exclusively prepared by mixing soluble aluminium salts and phosphoric acid salts. "Aluminium hydroxide" can be a precipitate of insoluble (amorphous, semi-crystalline or crystalline) aluminium hydroxide, which can be optionally but not exclusively prepared by neutralizing a solution of aluminium salts. Particularly suitable are the various forms of aluminium hydroxide and aluminium phosphate gels available from commercial sources for example, Alhydrogel (aluminium hydroxide, 3% suspension in water) and Adju-fos (aluminium phosphate, 2% suspension in saline) supplied by Superfos (Vedbaek, 2950 Denmark).

It will be appreciated that for the first time we are able to provide a stable and effective multivalent vaccine composition comprising HBsAg.

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Accordingly, in a further aspect of the invention there is provided a stable and effective combined vaccine composition directed to the prevention of more than two diseases, comprising HBsAg and at least two other antigens.

As regards choice of adjuvant, excellent results are obtained when the HBsAg is adsorbed on AP and at least one of the antigens other than HBsAg is adsorbed to AH. Other suitable adjuvants may, however, be used. For example one or all of the antigens other than HBsAg may be adsorbed to AP.

Preferred stable combination vaccines according to the invention are

Diphtheria-Tetanus- Pertussis-Hepatitis B (DTP-HB)
Diphtheria-Tetanus-Hepatitis B (DT-HB)
DTP - IPV (inactivated polio vaccine) - Hepatitis B

It will be appreciated that for a vaccine containing a Hib component the Hib antigen may be used extemporaneously by formulating the vaccine just prior to administration. In this way the following combined vaccine compositions within the scope of the invention may, for example, be prepared:

Hib-Hepatitis B
DTP-Hib-Hepatitis B
IPV - DTP-Hib-Hepatitis B.

More specifically particular vaccines within the scope of the invention are:

Diphtheria - Tetanus - Pertussis (DTP adsorbed on AH or AP) - Hepatitis B (HBsAg adsorbed on AP)

Diphtheria - Tetanus (DT adsorbed on AP or AH) - Hepatitis B (HBsAg adsorbed on AP).

By 'stable' as used herein to describe a vaccine according to the invention is meant a vaccine which can be kept a 37°C for one week without any substantial loss of immunogenicity of the HBsAg component.

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By 'effective' as used herein is meant a vaccine composition, characterised in that the immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of at least 200 mIU/ml, preferably 300 mIU/ml or greater, is found in human infants one month after the third dose of the vaccine when the vaccine is administered at one month intervals in an appropriate vaccination schedule.

In a further aspect the invention provides a multivalent vaccine composition comprising HBsAg and a stabilising adjuvant selected such that the vaccine can be kept at 37° C for one week without any substantial loss in immunogenicity of the HBsAg component. Preferably the multivalent vaccine composition is further characterised by giving rise to a geometric mean titre of at least 200 mIU/ml (one month post third dose), preferably 300 mIU/ml or greater, in human infants when the vaccine is administered at one month intervals in an appropriate vaccination schedule.

As used herein the term 'appropriate vaccination schedule' means a schedule known to those of skill in the art for administering a course of doses of a vaccine, especially for paediatric doses. A schedule of 3, 4 and 5 months may for example be used. This is particularly appropriate for example for DTP - HBsAg containing vaccines according to the invention.

In one aspect the HBsAg can be adsorbed to an aluminium salt other than aluminium hydroxide. Preferably it is adsorbed to AP. The other antigens in the multivalent vaccine formulation may be adsorbed to AP or AH (or both) and are advantageously adsorbed to AH as shown in the examples hereinbelow.

Advantageously the vaccine formulation according to the invention comprises a pertussis vaccine.

The pertussis component is suitably a whole cell pertussis vaccine or an acellular pertussis vaccine containing partially or highly purified antigens.

The above combinations may optionally include a component which is protective against Hepatitis A, i.e. an HAV antigen.

Advantageously the Hepatitis B combination vaccine is a paediatric vaccine.

The preparation of the antigens and adsorption procedure with the adjuvants are well known in the art, see for example as given below.

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The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See for example, Harford *et al.(1983)* in Develop. Biol. Standard 54, page 125 , Gregg *et al.* (1987) in Biotechnology 5, page 479, EP A-0 226 846, EP A- 0 299 108 and references therein.

As used herein the expression 'Hepatitis B surface antigen' or 'HBsAg' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et al, Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. In particular the HBsAg may comprise a polypeptide comprising an amino acid sequence comprising residues 12-52 followed by residues 133-145 followed by residues 175-400 of the L-protein of HBsAg relative to the open reading frame on a Hepatitis B virus of ad serotype (this polypeptide is referred to as L*; see EP 0 414 374). HBsAg within the scope of the invention may also include the preS1-preS2 -S polypeptide described in EP 0 198 474 (Endotronics) or analogues thereof such as those described in EP 0 304 578 (Mc Cormick and Jones). HBsAg as herein described can also refer to mutants, for example the 'escape mutant' described in WO 91/14703 or European Patent Application Publication Number 0 511 855 A1, especially HBsAg wherein the amino acid substitution at position 145 is to arginine from glycine.

Normally the HBsAg will be in particle form. The particles may comprise for example S protein alone or may be composite particles, for example (L*,S) where L* is as defined above and S denotes the S-protein of HBsAg. The said particle is advantageously in the form in which it is expressed in yeast.

Suitable antigens for use in vaccines according to the invention are already commercially available and details may be obtained from the World Health Organisation. For example the IPV component may be the Salk inactivated polio vaccine. The pertussis vaccine may comprise a whole cell product , an acellular product or a recombinantly produced product. In particular the pertussis component can be PT (pertussis toxins) or subfractions thereof, FHA (filamentous haemagglutinin antigen), agglutinogens (fimbrial) and outer membrane proteins, including the 69kDa protein (pertactin, non fimbrial agglutinogen). *References*: Robinson, A., Irons, L. I. & Ashworth, A. E., Vaccines, 3, 1985, 11-22; and

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Brennan, H. J., Li, S. M., Cowell, J. L., Bishen, M. E., Steven, A. C. Novotny., P, Manclarck, C. R., Infection and Immunity, 56, 1988, 3189-3195.

The component affording protection against Hepatitis A is preferably the product known as 'Havrix' (SmithKline Beecham Biologicals) which is a killed attenuated vaccine derived from the HM-175 strain of HAV [see 'Inactivated Candidate Vaccines for Hepatitis A' by F.E. Andre, A. Hepburn and E.D'Hondt (1980), Prog. Med. Virol. Vol 37, pages 72-95 and the product monograph 'Havrix' published by SmithKline Beecham Biologicals (1991).

Flehmig et al (loc cit., pages 56-71) have reviewed the clinical aspects, virology, immunology and epidemiology of Hepatitis A and discussed approaches to the development of vaccines against this common viral infection.

As used herein the expression 'HAV antigen' refers to any antigen capable of stimulating neutralising antibody to HAV in humans. The HAV antigen preferably comprises inactivated attenuated virus particles or may be, for example an HAV capsid or HAV viral protein, which may conveniently be obtained by recombinant DNA technology.

Vaccine preparation is generally described in New Trends and Developments in Vaccines (1978), edited by Voller *et al.*, University Park Press, Baltimore, Maryland U.S.A.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant , adverse side effects in typical vaccinees. Such amount will vary depending on which specific immunogens are employed. Generally it is expected that each dose will comprise 1-1000 µg of total immunogen, preferably 2-100 µg, more preferably 1 - 40 µg, most preferably 1 - 5 µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. A primary vaccination course may include 2 or 3 doses of vaccine, given one to two months apart, following the WHO recommendations for DTP immunization.

The invention thus provides a method of preventing hepatitis B and other infections in humans, especially infants, which method comprises treating a human subject in need thereof with an immunologically effective dose of a vaccine according to any aspect of the invention as hereinabove described.

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In a further aspect of the invention there is provided a vaccine composition according to the invention for use in medicine.

In a further aspect of the invention there is provided the use of HBsAg for the manufacture of a combination vaccine according to the invention for the prophylaxis of Hepatitis B viral infections.

In a further aspect the invention provides the use of AP for the purpose of acting as a stabiliser for, and/or to maintain the efficacy of, HBsAg in a multivalent vaccine according to the invention.

Specifically the invention provides the use of aluminium phosphate for the purpose of preparing a stable combined vaccine comprising HBsAg and at least one other antigen (preferably at least two other antigens) whereby the stability and/or immunogenicity of the HBsAg component is greater than in the corresponding combined vaccine in which the HBsAg component is adsorbed on AH.

More specifically the invention provides the use whereby the vaccine can be kept at 37° C for 1 week (i.e. 7 days) without substantial loss of immunogenicity of the HBsAg.

Also provided is the use whereby the geometric meant titre (GMT) found one month after the third dose of a course of vaccinations given at one month intervals in an appropriate vaccination schedule to human infants is greater than 200, preferably greater than 300, mIU/ml.

In a further aspect of the present invention there is provided a method of manufacture of a combined (i.e. bivalent or multivalent) vaccine effective in preventing hepatitis B infection as illustrated in the examples hereinbelow.

In one preferred aspect the antigens other than HBsAg are all adsorbed on AH. A very effective DTPa - Hepatitis B vaccine can, for example, be made in this way.

In general, the combined vaccine compositions according to any aspect of the invention can be prepared as follows. The required DT, DTPw, DTPa, HA or other components are adsorbed onto a suitable adjuvant, especially AH or AP; HBsAg is adsorbed onto a suitable stabilising adjuvant, selected as hereinabove described,

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especially an aluminium salt other than AH. Preferably it is adsorbed onto AP. After allowing time for complete and stable adsorption of the respective components, the different components are combined under appropriate conditions.

It will be appreciated that certain components, for example the DT, DTPw and DTPa components can be combined separately before adding the adsorbed HBsAg component. Multivalent vaccines comprising HBsAg and other or additional antigens to those mentioned hereinabove may be prepared in a similar manner.

In a preferred aspect there is provided a method of preparing a combined vaccine composition according to the invention wherein the method comprises mixing aluminium phosphate - adsorbed HBsAg with one or more aluminium hydroxide or aluminium phosphate adsorbed antigens.

The following examples illustrate the invention.

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EXAMPLES 1-5

Formulations

Particular formulations according to the present invention were prepared as described below.

Example 1 HBsAg adsorption on $AlPO_4$ as concentrate for formulation of combined vaccines.

A suspension of aluminium phosphate containing 0.03 to 0.3 g aluminium (as aluminium phosphate) in isotonic saline, is mixed with a HBsAg concentrate, containing 10 mg HBsAg protein, in a final volume of 10 to 100 ml. After adjusting the pH to 5 - 6.5 the mixture is left 10 - 24 hrs at room temperature with stirring. Antiseptic is then optionally added (i.e. merthiolate, 1 : 20,000 to 1 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) and the volume is brought to 50 ml with isotonic saline.

Example 2 Formulation of combined Diphtheria-Tetanus-Hepatitis B vaccine.

A concentrate containing 25,000 Lf of diphtheria toxoid and 10,000 Lf of tetanus toxoid adsorbed to 0.35 g Al (as aluminium hydroxide or aluminium phosphate) is prepared in a final volume of 0.15 l of isotonic saline and adjusted to between pH 6 and 7, as specified by WHO for DT and DTP vaccines. This concentrate is combined with 0.05 l of the Hepatitis B concentrate of example 1.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

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D toxoid:	25Lf,
T toxoid:	10 Lf,
HBsAg:	10 µg protein

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 3 Formulation of combined Diphtheria - Tetanus - pertussis (whole cell vaccine) - Hepatitis B vaccine

A concentrate ex Behringwerke containing 7,500 Lf of diphtheria toxoid, 3,250 Lf of Tetanus toxoid and 15,000 capacity units of *B. pertussis* antigen adsorbed to 0.45 mg Al (as aluminium hydroxide and aluminium phosphate) is prepared in a final volume of 0.4 l of isotonic saline and adjusted to pH 6 - 7, as specified by WHO for DTP vaccines. This concentrate is combined with 0.05 l of Hepatitis B concentrate of example 1.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

D toxoid:	7.5Lf,
T toxoid:	3.25 Lf
Pw antigen:	15OU
HBsAg:	10 µg protein.

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 4 Formulation of Diphtheria-Tetanus-Pertussis (acellular component) vaccine.

A concentrate containing 25,000 Lf of diphtheria toxoid and 10,000 Lf of tetanus toxoid adsorbed to 0.35 g Al (as aluminium hydroxide or phosphate gel) is prepared

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in a final volume of 0.15 l of isotonic saline and adjusted to between pH 6 and 7, as specified by WHO for DTP vaccines. 25 mg of inactivated pertussis toxin (DTPa), 25 mg of filamentous hemagglutinin (FHA) and optionally 8 mg of 69kDa outer membrane protein (pertactin), each combined with 0.05 g Al (as aluminium hydroxide or aluminium phosphate) are added. The *B. pertussis* antigens PT, FHA and pertactin can be prepared as described by methods known in the art, for example European patent application 427 462, *PCT application WO 91/12020* or by other procedures giving physiologically acceptable and potent *B. pertussis* antigens.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

D toxoid:	25 Lf,
T toxoid:	10 Lf,
DTd toxoid:	25 µg,
FHA toxoid:	25 µg,
69kDa OMP:	8 µg (optional)

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 5 Formulation of combined Diphtheria - Tetanus - Pertussis (acellular component) - Hepatitis B vaccine

The procedure of example 4 is applied, with the exception that an additional 50 ml of HBsAg adsorbed concentrate as prepared in example. 1 is added to the final mixture.

The resulting mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

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D toxoid:	25 Lf,
T toxoid:	10 Lf
PTd toxoid:	25 µg,
FHA toxoid:	25 µg,
69kDaOMP:	8 µg (optional).

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

EXAMPLES 6-10

Animal and Human Studies

Example 6 Formulation of combined Hepatitis A - Hepatitis B vaccines

An inactivated Hepatitis A virus concentrate (460,000 Elisa units), adsorbed to 0.02 to 0.2 g, preferably 0.04 - 0.1 g aluminium (as aluminium hydroxide) in a final volume of about 125ml was combined to 50 ml of concentrate containing 10 mg HBsAg adsorbed to aluminium phosphate as described in example 1.

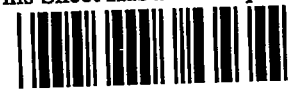
The resulting mixture was supplemented with isotonic saline and an amino acid concentrate (Travasol, Baxter-Travenol Inc) to obtain a final volume of 0.5 l containing 1.5 g amino acids. The resulting pH was between 6 and 7.

Our 1 ml dose of this bulk vaccine contains, as active ingredients:

Hepatitis A virus antigen:	800 Elisa units
HBsAg:	20 µg protein

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

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Results:

Clinical studies comparing aluminium hydroxide (AH) and aluminium phosphate (AP) adsorbed HBsAg (Monovalent vaccine)

Initially seronegative healthy adult volunteers were immunised with 3 doses of 20 µg HBsAg protein given at one month interval. Antibody levels were determined in sera obtained one month post 2 and 3 doses using the Ausab (Abbott) test. Responses were defined as subjects with titres significantly above background. Titres were expressed in mIU/ml.

Results are expressed as Geometric Mean Titres (GMT) in mIU/ml.

HBsAg Lot	Adjuvant	N.Subj.	Post 2, month 2		Post 3, month 3	
			GMT	% responders	GMT	% responders
100	AH	43	32	86	141	100
101	AH	45	26	93	198	98
102	AH	46	30	84	147	93
105/P	AP	7	43	83	380	100

HBsAg Lot	Adjuvant	N.Subj.	Post 2, month 2		Post 3, month 3	
			GMT	% responders	GMT	% responders
102	AH	51	14	82	126	98
103	AH	50	15	83	110	98
102	AH	54	17	83	133	96
104/P	AP	54	18	96	270	98
105/P	AP	51	14	90	156	96

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Example 7

Mouse immunogenicity tests and results of accelerated stability tests for combination vaccines comprising HBsAg with aluminium hydroxide (AH) or aluminium phosphate (AP) as adjuvant

Groups of 10 OF1 mice were immunised subcutaneously with 2 doses of 2.5 µg HBsAg (single component or combined) at days 0 and 14. Blood was drawn off at day 21 and titrated for anti-HBsAg using the Ausab (Abbott) test. Antibody titres were calculated in mIU/ml. The number of responding animals was defined as the number of those with antibody levels significantly above background values. The geometric mean titres was also calculated (GMT).

The results of DT-HB, DTPw-HB, DTPa-HB show that AP adsorbed HBsAg performed better than AH adsorbed HBsAg both in terms of number of responding animals and GMTs. The response to AP adsorbed HBsAg in the combination was comparable to that obtained by monovalent HBsAg administration.

Vaccine	4°C		7 days, 37°C		7 days, 45°C	
	N.resp.	GMT	N.resp.	GMT	N.resp	GMT
Engerix B(HB+AH)	7/10	30	9/10	17	6/10	2.7
Engerix B(HB+AH)	9/10	54	8/10	13	5/10	6
HB (AH)	9/10	45	10/10	55	9/10	32
HB (AP)	9/10	54	10/10	50	7/10	6.9
DTPw(AH)HB(AH)	4/10	1.4	nd	nd	nd	nd
DTPw(AH)HB(AP)	9/10	52	8/10	16	8/10	26
DT(AH)HB(AH)	6/10	1.7	nd	nd	nd	nd
DT(AH)HB(AP)	8/10	44	9/10	21	10/10	36
DTPa(AH)HB(AH)	5/10	1.7	nd	nd	nd	nd
DTPa(AH)HB(AP)	10/10	18	8/10	8	9/10	24

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Example 8

Immunogenicity of HBsAg combined to DTPw in monkeys

Results of aluminium hydroxide (AH) and aluminium phosphate (AP) adsorbed antigen

Cercopithenus aethiops monkeys received two injections of 10 µg HBsAg (alone or combined) at days 0 and 30. Sera were withdrawn at days 30 and 57 and titrated (Ausab, Abbott) for anti-HbsAg. Animals with antibody levels significantly above background (pre-vaccination sera) were considered responders. GMT were calculated in mIU/ml.

Results show AP adsorbed HBsAg performed better than AH adsorbed HBsAg.The response was comparable to that obtained by monovalent HBsAg administration.

	Post 1, day 30		Post 2, day 57	
Vaccine	N. resp.	GMT	N. resp.	GMT
Engerix B (HB)(AH)	4/5	10	5/5	666
DTPw(AH)HB(AH)	4/5	20	5/5	31
DTPw(AH)HB(AP)	5/5	12	5/5	414

Example 9

Clinical studies with combined DTPw vaccines using HBsAg adsorbed to aluminium hydroxide (AH) or aluminium phosphate (AP)

Subjects were immunised with 3 doses of 0.5 ml containing DTPw and 10µg HBsAg protein given at the age of 3, 4 and 5 months. Bleeding was at 6 months and sera were titrated with the Ausab test. Percentage responders (seroconversion) relates to subjects with antibody levels significantly above background. Percentage protection relates to subjects with titres equal to or greater than 10 mIU/ml. GMT in mIU/ml.

Results for DTPw-HB show AP adsorbed HBsAg produced a satisfactory response as opposed to AH adsorbed HBsAg. Seroconversion rates and GMT were comparable to data typically seen with monovalent HBsAg vaccine (Engerix B).

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Vaccine	N. subj.	Bleeding Time	% resp.	% prot.	GMT
DTPw(AH).HB(AH)	32	post 2	nd	nd	nd
		post 3	94	84	38.5
DTPw(AH).HB(AP)	29	post 2	97	97	63
	17	post 3	100	100	469

Example 10

Immunogenicity and stability of HBsAg adsorbed to aluminium hydroxide (AH) or aluminium phosphate (AP) in a hepatitis A-Hepatitis B combined vaccine

Groups of 10 OF1 mice were immunised subcutaneously with 2 doses of 2.5 µg HBsAg (single component or combined) at days 0 and 14. Blood was drawn at day 21 and titrated for anti-HBsAg as in Example 7.

Results for immunogenicity and stability of HA-HB combined product showed AP adsorbed HBsAg produced higher antibody levels and a more stable form.

Vaccine	Exposure	N. resp.	GMT
HA(AH).HB(AH)	4°C	9/10	41
	1 month, 37°C	6/10	5.6
	1 month, 45°C	5/10	6.4
HA(AH).HB(AP)	4°C	10/10	80
	1 month, 37°C	9/10	45
	1 month, 45°C	8/10	18
Engerix B HB(AH)	4°C	8/10	58

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Example 11: Further Clinical Results in humans

1. Immunogenicity of DTPw - Hepatitis B vaccines in infants

Experiment A

Studies in Slovakia: Schedule: 3-4-5 months. 10µg HBsAg; DTPw ex Behringwerke (DT on AH; Pw on a mixture of AH and AP)

Anti-Hbs titres

HBsAg adjuvant	Time	N	GMT	SP(%)
Hydroxide	Post II (5 months)	44	45	79.5
Hydroxide	Post III (6 months)	13	34	69.2
Phosphate	Post II (5 months)	32	80	97.0
Phosphate	Post III (6 months)	32	396	100

In this and other examples Post II means after the second dose, post III after the third dose. GMT is always measured one month after the injection time shown in the schedule. SP is the seroprotection rate.

Anti-Diphtheria, Tetanus, B pertussis titres

Post III results	N	GMT	%>0.1 IU/ml	GMT Post/Pre
Anti-Diphtheria	38	2.302	100	37.4
Anti-Tetanus	38	3.281	100	38.4
Anti-B pertussis	38	61	-	7.7

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Experiment B

Study in Greece: Schedule 2-4-6 months (same vaccine as for Experiment A)

Anti-HBs titres (interim results)

HBsAg adjuvant	Time	N	GMT	SP(%)
Hydroxide	Month 7	22	284	90.5
Hydroxide	Month 7	17	193	94.4
Phosphate	Month 7	23	1794	92.0

Experiment C

Study in Slovakia: Schedule 3-4-5 (HBsAg =5μg on aluminium phosphate; DTP ex Behringwerke as for Experiment A)

Anti-HBs titres

Time	N	GMT	SP(%)
Post II	21	94	90.5
Post III	18	311	100

Experiment D

Study in Slovakia: Schedule 3-4-5 months of age (HBsAg =10μg on aluminium phosphate; DTPw ex Behringwerke as for Experiment A)

Anti-HBs titres

Time	N	GMT	SP(%)
Pre	24	0	0
Post II (month 5)	13	259	92.3
Post III (month 6)	10	592	100.0

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Anti-diphtheria antibodies

Timing	N	GMT	SP (%)	GMT Post/Pre
Pre	32	0.054	6.3	1.0
Post II	16	1.094	93.8	20.4
Post III	11	2.314	100.0	43.1

Anti-tetanus antibodies

Timing	N	GMT	SP (%)	GMT Post/Pre
Pre	32	0.083	34.4	1.0
Post II	16	3.146	100.0	37.9
Post III	11	7.989	100.0	96.4

Anti-B pertussis antibodies

Timing	N	GMT	GMT Post/Pre
Pre	32	8	1.0
Post II	16	20	2.7
Post III	11	50	6.6

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2. Immunogenicity of DTPa - Hepatitis B vaccines in infants

Experiment A

Study in Turkey. HBsAg 10μg on AP; DTP (acellular) on AH. Preliminary results

Group 1 (DTPa - Engerix B combination)

Timing	N	S+	%	GMT
Pre	19	0	0	0
Post I	19	4	21.1	24
Post II	19	18	94.7	146
Post III	19	19	100.0	345

Group 2 (DTPa plus Engerix B; separate simultaneous injections)

Timing	N	S+	%	GMT
Pre	8	0	0	0
Post I	8	2	25.0	37
Post II	8	5	62.5	33
Post III	7	6	83.7	385

Key: N = number of subjects tested; S+ = number of subjects seropositive at a given blood sampling time; % = seroconversion rate and GMT = geometric mean antibody titre of seroconverters

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CLAIMS

1. A combined vaccine composition comprising Hepatitis B surface antigen (HBsAg) and a number (n) of other antigens in combination with an adjuvant comprising one or more aluminium salts in which the value of n is 1 or greater and in which the adjuvant used to adsorb the HBsAg is not aluminium hydroxide, with the proviso that when n is 1 the other antigen is not an antigen against Hepatitis A.

2. A vaccine composition as claimed in claim 1 in which the HBsAg is adsorbed to aluminium phosphate.

3. A vaccine composition as claimed in claim 2 in which at least one of the other antigens is adsorbed to aluminium phosphate.

4. A vaccine composition as claimed in claim 2 in which at least one of the other antigens is adsorbed to aluminium hydroxide.

5. A vaccine composition as claimed in any preceding claim in which n is 2, 3, 4, 5 or 6.

6. A combined vaccine composition according to claim 5 wherein the antigen adsorbed to aluminium hydroxide or aluminium phosphate is selected from an antigen providing immunity against diphtheria (D); tetanus (T); pertussis (P); Inactivated Polio (IPV); Haemophilus influenzae b (Hib) and Hepatitis A (HA).

7. A stable and effective combined vaccine composition directed to the prevention of more than two diseases comprising HBsAg and at least two other antigens.

8. A vaccine composition according to claim 7 in which the HBsAg is adsorbed to aluminium phosphate.

9. A vaccine composition according to claim 8 in which at least one of the antigens other than HBsAg is adsorbed to aluminium hydroxide.

10. A combined vaccine composition as claimed in any one of claims 7 to 9 which is :

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Diphtheria-Tetanus-Pertussis (DTP) - Hepatitis B; or

Diphtheria - Tetanus (DT) - Hepatitis B ; or

DTP - IPV (inactivated polio vaccine) - Hepatitis B.

11. A combined vaccine composition according to any previous claim in which the stability of the vaccine is such that the vaccine can be kept at 37° C for 1 week without substantial loss of immunogenicity of the HBsAg component.

12. A combined vaccine composition according to any previous claim, characterised in that the immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of 200 mIU/ml (one month post third dose) or greater is found in human infants when a course of the vaccine is given at one month intervals in an appropriate vaccination schedule.

13. A combined vaccine composition as claimed in any preceding claim comprising an antigen component which is protective against Hepatitis A.

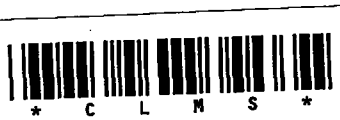
14. A combined vaccine composition according to any preceding claim which comprises a pertussis component.

15. A combined vaccine according to claim 14 in which the pertussis component is the whole cell pertussis vaccine or the acellular pertussis vaccine containing partially or highly purified antigens.

16. A multivalent vaccine composition comprising HBsAg and a stabilising adjuvant, the adjuvant being selected such that the vaccine can be kept at 37° C for one week without any substantial loss in immunogenicity of the HBsAg component.

17. A multivalent vaccine composition according to Claim 16 further characterised in that it gives rise to a geometric mean titre of at least 200 mIU/ml (1 month post third dose) when a course of the vaccine is given to human infants at one month intervals in an appropriate vaccination schedule.

18. A multivalent vaccine according to Claim 16 or 17 in which the adjuvant is selected from one or more aluminium salts with the proviso that the HBsAg component is not adsorbed on aluminium hydroxide.



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19. A multivalent vaccine according to Claim 18 in which the HBsAg is adsorbed to aluminium phosphate.

20. A multivalent vaccine according to Claim 18 or 19 in which the antigens present in the vaccine formulation other than HBsAg are adsorbed on aluminium hydroxide.

21. A vaccine formulation according to Claim 20 which is Diphtheria - Tetanus - Perrtussis (acellular) - HBsAg.

22. A multivalent vaccine formulation acording to Claim 18 or 19 in which the antigens present in the vaccine formulation other than HBsAg are adsorbed on aluminium phosphate.

23. A vaccine composition according to Claim 22 which is Diphtheria - Tetanus - Pertussis (whole cell) - HBsAg.

24. A composition as claimed in any one of claims 1 to 23 for use in medicine.

25. Use of HBsAg in the manufacture of a combination vaccine according to any one of claims 1 to 23 for the prophylaxis of Hepatitis B viral infections.

26. A method of preparing a combination vaccine composition as claimed in claims 1 to 23 in which the HBsAg is adsorbed to AP wherein the method comprises mixing aluminium phosphate - adsorbed HBsAg with one or more aluminium hydroxide or aluminium phosphate adsorbed antigens.

27. Use of aluminium phosphate as an adjuvant for adsorbing HBsAg characterised in that the use is for the purpose of formulating a stable and effective combined vaccine comprising HBsAg and at least 1 other antigen whereby the stability and /or immunogenicity of the HBsAg component is greater than in the combined vaccine in which the HBsAg component is adsorbed on aluminium hydroxide.

28. Use according to Claim 27 in which the stability of the vaccine is such that the vaccine can be kept at 37° C for 1 week without substantial loss of immunogenicity of the HBsAg.

29. Use according to Claim 27 in which the immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of 200 mIU/ml (one month post dose) or greater is found in human infants after a course of 3 doses of the vaccine given at one month intervals in an appropriate immmunisation schedule.

30. Use according to any one of claims 27 to 29 in which there are at least 2 other antigens in the combined vaccine.

31. A method of preventing hepatitis B infections in humans, which method comprises treating human subjects in need thereof with an effective dose of a vaccine according to any one of claims 1 to 23.

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Abstract

Stable and effective multivalent vaccine compositions comprising Hepatitis B surface antigen (HBsAg) are described wherein the HBsAg component is stable for one week at 37° C and is highly immunogenic, for example when the vaccine is administered to infants. The compositions typically comprise HBsAg adsorbed to aluminium phosphate and other antigens, especially those suitable for use in a paediatric vaccine, adsorbed to aluminium phosphate or aluminium hydroxide. Methods for preparing the vaccines and the use of aluminium phosphate to stabilise HBsAg in a multivalent vaccine formulation are also described.

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COMBINED VACCINES COMPRISING HEPATITIS B SURFACE ~~ANTIGEN AND~~ OTHER ANTIGENS

The present invention relates to novel vaccine formulations, methods for preparing them and to their use in therapy. In particular the present invention relates to novel combination vaccine formulations including a Hepatitis B vaccine component for treating Hepatitis B infections.

Infection with Hepatitis B (HB) virus is a widespread problem but vaccines which have been used for mass immunisation are now available, for example the product 'Engerix-B' (SmithKline Beecham plc). Engerix B has as the Hepatitis B antigenic component Hepatitis B surface antigen (HBsAg) which is obtained by genetic engineering techniques.

However it is often necessary or desirable to administer Hepatitis B vaccine at the same time as other vaccines and this can involve multiple injections. Problems associated with multiple injections include a more complicated administration procedure and a large total injection volume.

There is therefore a need for a combined vaccine comprising a Hepatitis B antigen in combination with other antigens. The other antigens are in particular those capable in a vaccine formulation of preventing Hepatitis A (HA), diphtheria (D), tetanus (T), whole cell pertussis (Pw), acellular pertussis (Pa), Haemophilus influenzae b (Hib) and polio (P).

Aluminium hydroxide (AH) is widely used as an adjuvant in the formulation of vaccines. For example, Engerix B uses Hepatitis B surface antigen (HBsAg) adsorbed to aluminium hydroxide. We have also used AH successfully in the formulation of Hepatitis A vaccine and in the combined vaccines DT, DTPw and DTPa. However, when AH -adsorbed HBsAg is used in combination with other vaccines in a combined formulation there is a substantial decrease of the immune response to HBsAg , resulting in lower or insufficient seroprotection after vaccination. In addition the stability of the HBsAg component of the combined vaccine is poor.

Aluminium phosphate (AP) adsorbed HBsAg has been used in a commercially available monovalent vaccine (HEPPACINE) made by Korean Cheil Sugar Co Ltd. We have found that there is no significant difference in immunogenic properties between an AH- adsorbed HBsAg monovalent vaccine (Engerix B) and an AP-adsorbed HBsAg monovalent vaccine.

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European patent application publication number 0 339 667 discloses a bivalent vaccine comprising HBsAg and a Hepatitis A antigen in which either aluminium hydroxide or aluminium phosphate is used as adjuvant. There appears, however, to be no appreciation of the need to avoid aluminium hydroxide as an adjuvant for a multivalent vaccine comprising HBsAg. Furthermore there appears to be no disclosure in this document or elsewhere of a bivalent or multivalent hepatitis B vaccine in which at least one antigen other than HBsAg is adsorbed on aluminium hydroxide and the HBsAg is adsorbed on aluminium phosphate.

Indeed there appears to be no prior enabling disclosure of a stable and effective multivalent vaccine comprising HBsAg at all.

In one aspect the present invention provides a combined vaccine composition comprising Hepatitis B surface antigen (HBsAg) and a number (n) of other antigens in combination with an adjuvant comprising one or more aluminium salts in which the value of n is 1 or greater and in which the adjuvant used to adsorb the HBsAg is not aluminium hydroxide, with the proviso that when n is 1 the other antigen is not an antigen against Hepatitis A.

Preferably n is 2, 3, 4, 5 or 6.

The advantage of the invention is that no substantial decrease in the immunogenicity of the HBsAg occurs in the combined vaccine formulation. Avoiding the use of AH to adsorb the HBsAg component in the vaccine formulation also gives rise to a product of markedly superior stability. A further advantage of the invention is that the aforesaid problems associated with multiple injections are overcome or at least mitigated and a stable, highly immunogenic combined formulation is provided. The compositions of the invention are particularly suitable for administration to children.

Preferably the HBsAg is adsorbed on AP. In particular we have found in human clinical studies that when AP-adsorbed HBsAg is combined with one or more AH-adsorbed or AP-adsorbed antigens in a combined vaccine no substantial decrease in immunogenicity occurs. The stability of the AP-adsorbed HBsAg in the formulation is also greater than AH-adsorbed HBsAg.

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Accordingly in a further aspect there is provided a vaccine composition according to the invention in which at least one of the antigens other than HBsAg is adsorbed to aluminium phosphate.

In a further preferred aspect at least one of the antigens other than HBsAg is adsorbed to AH.

In a further aspect, the invention provides a combined vaccine comprising Hepatitis B surface antigen (HBsAg) adsorbed to AP and an antigen adsorbed to AP or to AH selected from an antigen providing immunity against one or more of the following viruses: diphtheria (D); tetanus (T); pertussis (P); Inactivated Polio (IPV); Haemophilus influenzae b (Hib); and Hepatitis A (HA).

In a paediatric vaccine other compatible antigens may also be included, eg antigens known to be effective against meningitis B, meningitis A and C, and otitis media.

As used herein the term 'bivalent' is used to refer to a vaccine comprising a combination of two antigens in total (including HBsAg). The term 'multivalent' is applied to a vaccine composition comprising more than two antigens, for example three, four or five or six antigens.

The meaning of the terms 'aluminium phosphate' and 'aluminium hydroxide' as used herein includes all forms of aluminium hydroxide or aluminium phosphate which are suitable for adjuvanting vaccines.

For example, aluminium phosphate can be a precipitate of insoluble aluminium phosphate (amorphous, semi-crystalline or crystalline), which can be optionally but not exclusively prepared by mixing soluble aluminium salts and phosphoric acid salts. "Aluminium hydroxide" can be a precipitate of insoluble (amorphous, semi-crystalline or crystalline) aluminium hydroxide, which can be optionally but not exclusively prepared by neutralizing a solution of aluminium salts. Particularly suitable are the various forms of aluminium hydroxide and aluminium phosphate gels available from commercial sources for example, Alhydrogel (aluminium hydroxide, 3% suspension in water) and Adju-fos (aluminium phosphate, 2% suspension in saline) supplied by Superfos (Vedbaek, 2950 Denmark).

It will be appreciated that for the first time we are able to provide a stable and effective multivalent vaccine composition comprising HBsAg.

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Accordingly, in a further aspect of the invention there is provided a stable and effective combined vaccine composition directed to the prevention of more than two diseases, comprising HBsAg and at least two other antigens.

As regards choice of adjuvant, excellent results are obtained when the HBsAg is adsorbed on AP and at least one of the antigens other than HBsAg is adsorbed to AH. Other suitable adjuvants may, however, be used. For example one or all of the antigens other than HBsAg may be adsorbed to AP.

Preferred stable combination vaccines according to the invention are

Diphtheria-Tetanus- Pertussis-Hepatitis B (DTP-HB)
Diphtheria-Tetanus-Hepatitis B (DT-HB)
DTP - IPV (inactivated polio vaccine) - Hepatitis B

It will be appreciated that for a vaccine containing a Hib component the Hib antigen may be used extemporaneously by formulating the vaccine just prior to administration. In this way the following combined vaccine compositions within the scope of the invention may, for example, be prepared:

Hib-Hepatitis B
DTP-Hib-Hepatitis B
IPV - DTP-Hib-Hepatitis B.

More specifically particular vaccines within the scope of the invention are:

Diphtheria - Tetanus - Pertussis (DTP adsorbed on AH or AP) - Hepatitis B (HBsAg adsorbed on AP)

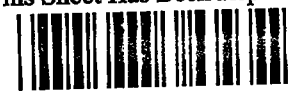
Diphtheria - Tetanus (DT adsorbed on AP or AH) - Hepatitis B (HBsAg adsorbed on AP).

By 'stable' as used herein to describe a vaccine according to the invention is meant a vaccine which can be kept a 37°C for one week without any substantial loss of immunogenicity of the HBsAg component.

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By 'effective' as used herein is meant a vaccine composition, characterised in that the immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of at least 200 mIU/ml, preferably 300 mIU/ml or greater, is found in human infants one month after the third dose of the vaccine when the vaccine is administered at one month intervals in an appropriate vaccination schedule.

In a further aspect the invention provides a multivalent vaccine composition comprising HBsAg and a stabilising adjuvant selected such that the vaccine can be kept at 37° C for one week without any substantial loss in immunogenicity of the HBsAg component. Preferably the multivalent vaccine composition is further characterised by giving rise to a geometric mean titre of at least 200 mIU/ml (one month post third dose), preferably 300 mIU/ml or greater, in human infants when the vaccine is administered at one month intervals in an appropriate vaccination schedule.

As used herein the term 'appropriate vaccination schedule' means a schedule known to those of skill in the art for administering a course of doses of a vaccine, especially for paediatric doses. A schedule of 3, 4 and 5 months may for example be used. This is particularly appropriate for example for DTP - HBsAg containing vaccines according to the invention.

In one aspect the HBsAg can be adsorbed to an aluminium salt other than aluminium hydroxide. Preferably it is adsorbed to AP. The other antigens in the multivalent vaccine formulation may be adsorbed to AP or AH (or both) and are advantageously adsorbed to AH as shown in the examples hereinbelow.

Advantageously the vaccine formulation according to the invention comprises a pertussis vaccine.

The pertussis component is suitably a whole cell pertussis vaccine or an acellular pertussis vaccine containing partially or highly purified antigens.

The above combinations may optionally include a component which is protective against Hepatitis A, i.e. an HAV antigen.

Advantageously the Hepatitis B combination vaccine is a paediatric vaccine.

The preparation of the antigens and adsorption procedure with the adjuvants are well known in the art, see for example as given below.

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The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See for example, Harford *et al.(1983)* in Develop. Biol. Standard 54, page 125 , Gregg *et al.* (1987) in Biotechnology 5, page 479, EP A-0 226 846, EP A- 0 299 108 and references therein.

As used herein the expression 'Hepatitis B surface antigen' or 'HBsAg' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et al, Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. In particular the HBsAg may comprise a polypeptide comprising an amino acid sequence comprising residues 12-52 followed by residues 133-145 followed by residues 175-400 of the L-protein of HBsAg relative to the open reading frame on a Hepatitis B virus of ad serotype (this polypeptide is referred to as L*; see EP 0 414 374). HBsAg within the scope of the invention may also include the preS1-preS2 -S polypeptide described in EP 0 198 474 (Endotronics) or analogues thereof such as those described in EP 0 304 578 (Mc Cormick and Jones). HBsAg as herein described can also refer to mutants, for example the 'escape mutant' described in WO 91/14703 or European Patent Application Publication Number 0 511 855 A1, especially HBsAg wherein the amino acid substitution at position 145 is to arginine from glycine.

Normally the HBsAg will be in particle form. The particles may comprise for example S protein alone or may be composite particles, for example (L*,S) where L* is as defined above and S denotes the S-protein of HBsAg. The said particle is advantageously in the form in which it is expressed in yeast.

Suitable antigens for use in vaccines according to the invention are already commercially available and details may be obtained from the World Health Organisation. For example the IPV component may be the Salk inactivated polio vaccine. The pertussis vaccine may comprise a whole cell product , an acellular product or a recombinantly produced product. In particular the pertussis component can be PT (pertussis toxins) or subfractions thereof, FHA (filamentous haemagglutinin antigen), agglutinogens (fimbrial) and outer membrane proteins, including the 69kDa protein (pertactin, non fimbrial agglutinogen). *References*: Robinson, A., Irons, L. I. & Ashworth, A. E., Vaccines, 3, 1985, 11-22; and

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Brennan, H. J., Li, S. M., Cowell, J. L., Bishen, M. E., Steven, A. C. Novotny., P, Manclarck, C. R., Infection and Immunity, 56, 1988, 3189-3195.

The component affording protection against Hepatitis A is preferably the product known as 'Havrix' (SmithKline Beecham Biologicals) which is a killed attenuated vaccine derived from the HM-175 strain of HAV [see 'Inactivated Candidate Vaccines for Hepatitis A' by F.E. Andre, A. Hepburn and E.D'Hondt (1980), Prog. Med. Virol. Vol 37, pages 72-95 and the product monograph 'Havrix' published by SmithKline Beecham Biologicals (1991).

Flehmig et al (loc cit., pages 56-71) have reviewed the clinical aspects, virology, immunology and epidemiology of Hepatitis A and discussed approaches to the development of vaccines against this common viral infection.

As used herein the expression 'HAV antigen' refers to any antigen capable of stimulating neutralising antibody to HAV in humans. The HAV antigen preferably comprises inactivated attenuated virus particles or may be, for example an HAV capsid or HAV viral protein, which may conveniently be obtained by recombinant DNA technology.

Vaccine preparation is generally described in New Trends and Developments in Vaccines (1978), edited by Voller *et al.*, University Park Press, Baltimore, Maryland U.S.A.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant , adverse side effects in typical vaccinees. Such amount will vary depending on which specific immunogens are employed. Generally it is expected that each dose will comprise 1-1000 μg of total immunogen, preferably 2-100 μg, more preferably 1 - 40 μg, most preferably 1 - 5 μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. A primary vaccination course may include 2 or 3 doses of vaccine, given one to two months apart, following the WHO recommendations for DTP immunization.

The invention thus provides a method of preventing hepatitis B and other infections in humans, especially infants, which method comprises treating a human subject in need thereof with an immunologically effective dose of a vaccine according to any aspect of the invention as hereinabove described.

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In a further aspect of the invention there is provided a vaccine composition according to the invention for use in medicine.

In a further aspect of the invention there is provided the use of HBsAg for the manufacture of a combination vaccine according to the invention for the prophylaxis of Hepatitis B viral infections.

In a further aspect the invention provides the use of AP for the purpose of acting as a stabiliser for, and/or to maintain the efficacy of, HBsAg in a multivalent vaccine according to the invention.

Specifically the invention provides the use of aluminium phosphate for the purpose of preparing a stable combined vaccine comprising HBsAg and at least one other antigen (preferably at least two other antigens) whereby the stability and/or immunogenicity of the HBsAg component is greater than in the corresponding combined vaccine in which the HBsAg component is adsorbed on AH.

More specifically the invention provides the use whereby the vaccine can be kept at 37° C for 1 week (i.e. 7 days) without substantial loss of immunogenicity of the HBsAg.

Also provided is the use whereby the geometric meant titre (GMT) found one month after the third dose of a course of vaccinations given at one month intervals in an appropriate vaccination schedule to human infants is greater than 200, preferably greater than 300, mIU/ml.

In a further aspect of the present invention there is provided a method of manufacture of a combined (i.e. bivalent or multivalent) vaccine effective in preventing hepatitis B infection as illustrated in the examples hereinbelow.

In one preferred aspect the antigens other than HBsAg are all adsorbed on AH. A very effective DTPa - Hepatitis B vaccine can, for example, be made in this way.

In general, the combined vaccine compositions according to any aspect of the invention can be prepared as follows. The required DT, DTPw, DTPa, HA or other components are adsorbed onto a suitable adjuvant, especially AH or AP; HBsAg is adsorbed onto a suitable stabilising adjuvant, selected as hereinabove described,

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especially an aluminium salt other than AH. Preferably it is adsorbed onto AP. After allowing time for complete and stable adsorption of the respective components, the different components are combined under appropriate conditions.

It will be appreciated that certain components, for example the DT, DTPw and DTPa components can be combined separately before adding the adsorbed HBsAg component. Multivalent vaccines comprising HBsAg and other or additional antigens to those mentioned hereinabove may be prepared in a similar manner.

In a preferred aspect there is provided a method of preparing a combined vaccine composition according to the invention wherein the method comprises mixing aluminium phosphate - adsorbed HBsAg with one or more aluminium hydroxide or aluminium phosphate adsorbed antigens.

The following examples illustrate the invention.

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EXAMPLES 1-5

Formulations

Particular formulations according to the present invention were prepared as described below.

Example 1 HBsAg adsorption on $AlPO_4$ as concentrate for formulation of combined vaccines.

A suspension of aluminium phosphate containing 0.03 to 0.3 g aluminium (as aluminium phosphate) in isotonic saline, is mixed with a HBsAg concentrate, containing 10 mg HBsAg protein, in a final volume of 10 to 100 ml. After adjusting the pH to 5 - 6.5 the mixture is left 10 - 24 hrs at room temperature with stirring. Antiseptic is then optionally added (i.e. merthiolate, 1 : 20,000 to 1 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) and the volume is brought to 50 ml with isotonic saline.

Example 2 Formulation of combined Diphtheria-Tetanus-Hepatitis B vaccine.

A concentrate containing 25,000 Lf of diphtheria toxoid and 10,000 Lf of tetanus toxoid adsorbed to 0.35 g Al (as aluminium hydroxide or aluminium phosphate) is prepared in a final volume of 0.15 1 of isotonic saline and adjusted to between pH 6 and 7, as specified by WHO for DT and DTP vaccines. This concentrate is combined with 0.05 1 of the Hepatitis B concentrate of example 1.

This mixture is brought to a final volume of 0.5 1 with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

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D toxoid:	25Lf,
T toxoid:	10 Lf,
HBsAg:	10 µg protein

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 3 Formulation of combined Diphtheria - Tetanus - pertussis (whole cell vaccine) - Hepatitis B vaccine

A concentrate ex Behringwerke containing 7,500 Lf of diphtheria toxoid, 3,250 Lf of Tetanus toxoid and 15,000 capacity units of *B. pertussis* antigen adsorbed to 0.45 mg Al (as aluminium hydroxide and aluminium phosphate) is prepared in a final volume of 0.4 l of isotonic saline and adjusted to pH 6 - 7, as specified by WHO for DTP vaccines. This concentrate is combined with 0.05 l of Hepatitis B concentrate of example 1.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

D toxoid:	7.5Lf,
T toxoid:	3.25 Lf
Pw antigen:	15OU
HBsAg:	10 µg protein.

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 4 Formulation of Diphtheria-Tetanus-Pertussis (acellular component) vaccine.

A concentrate containing 25,000 Lf of diphtheria toxoid and 10,000 Lf of tetanus toxoid adsorbed to 0.35 g Al (as aluminium hydroxide or phosphate gel) is prepared

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in a final volume of 0.15 l of isotonic saline and adjusted to between pH 6 and 7, as specified by WHO for DTP vaccines. 25 mg of inactivated pertussis toxin (DTPa), 25 mg of filamentous hemagglutinin (FHA) and optionally 8 mg of 69kDa outer membrane protein (pertactin), each combined with 0.05 g Al (as aluminium hydroxide or aluminium phosphate) are added. The *B. pertussis* antigens PT, FHA and pertactin can be prepared as described by methods known in the art, for example European patent application 427 462, *PCT application WO 91/12020* or by other procedures giving physiologically acceptable and potent *B. pertussis* antigens.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

D toxoid:	25 Lf,
T toxoid:	10 Lf,
DTd toxoid:	25 µg,
FHA toxoid:	25 µg,
69kDa OMP:	8 µg (optional)

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 5 Formulation of combined Diphtheria - Tetanus - Pertussis (acellular component) - Hepatitis B vaccine

The procedure of example 4 is applied, with the exception that an additional 50 ml of HBsAg adsorbed concentrate as prepared in example. 1 is added to the final mixture.

The resulting mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

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D toxoid:	25 Lf,
T toxoid:	10 Lf
PTd toxoid:	25 µg,
FHA toxoid:	25 µg,
69kDaOMP:	8 µg (optional).

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

EXAMPLES 6-10

Animal and Human Studies

Example 6 Formulation of combined Hepatitis A - Hepatitis B vaccines

An inactivated Hepatitis A virus concentrate (460,000 Elisa units), adsorbed to 0.02 to 0.2 g, preferably 0.04 - 0.1 g aluminium (as aluminium hydroxide) in a final volume of about 125ml was combined to 50 ml of concentrate containing 10 mg HBsAg adsorbed to aluminium phosphate as described in example 1.

The resulting mixture was supplemented with isotonic saline and an amino acid concentrate (Travasol, Baxter-Travenol Inc) to obtain a final volume of 0.5 l containing 1.5 g amino acids. The resulting pH was between 6 and 7.

Our 1 ml dose of this bulk vaccine contains, as active ingredients:

Hepatitis A virus antigen:	800 Elisa units
HBsAg:	20 µg protein

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

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Results:

Clinical studies comparing aluminium hydroxide (AH) and aluminium phosphate (AP) adsorbed HBsAg (Monovalent vaccine)

Initially seronegative healthy adult volunteers were immunised with 3 doses of 20 µg HBsAg protein given at one month interval. Antibody levels were determined in sera obtained one month post 2 and 3 doses using the Ausab (Abbott) test. Responses were defined as subjects with titres significantly above background. Titres were expressed in mIU/ml.

Results are expressed as Geometric Mean Titres (GMT) in mIU/ml.

HBsAg Lot	Adjuvant	N.Subj.	Post 2, month 2		Post 3, month 3	
			GMT	% responders	GMT	% responders
100	AH	43	32	86	141	100
101	AH	45	26	93	198	98
102	AH	46	30	84	147	93
105/P	AP	7	43	83	380	100

HBsAg Lot	Adjuvant	N.Subj.	Post 2, month 2		Post 3, month 3	
			GMT	% responders	GMT	% responders
102	AH	51	14	82	126	98
103	AH	50	15	83	110	98
102	AH	54	17	83	133	96
104/P	AP	54	18	96	270	98
105/P	AP	51	14	90	156	96

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Example 7

Mouse immunogenicity tests and results of accelerated stability tests for combination vaccines comprising HBsAg with aluminium hydroxide (AH) or aluminium phosphate (AP) as adjuvant

Groups of 10 OF1 mice were immunised subcutaneously with 2 doses of 2.5 µg HBsAg (single component or combined) at days 0 and 14. Blood was drawn off at day 21 and titrated for anti-HBsAg using the Ausab (Abbott) test. Antibody titres were calculated in mIU/ml. The number of responding animals was defined as the number of those with antibody levels significantly above background values. The geometric mean titres was also calculated (GMT).

The results of DT-HB, DTPw-HB, DTPa-HB show that AP adsorbed HBsAg performed better than AH adsorbed HBsAg both in terms of number of responding animals and GMTs. The response to AP adsorbed HBsAg in the combination was comparable to that obtained by monovalent HBsAg administration.

Vaccine	4°C		7 days, 37°C		7 days, 45°C	
	N.resp.	GMT	N.resp.	GMT	N.resp	GMT
Engerix B(HB+AH)	7/10	30	9/10	17	6/10	2.7
Engerix B(HB+AH)	9/10	54	8/10	13	5/10	6
HB (AH)	9/10	45	10/10	55	9/10	32
HB (AP)	9/10	54	10/10	50	7/10	6.9
DTPw(AH)HB(AH)	4/10	1.4	nd	nd	nd	nd
DTPw(AH)HB(AP)	9/10	52	8/10	16	8/10	26
DT(AH)HB(AH)	6/10	1.7	nd	nd	nd	nd
DT(AH)HB(AP)	8/10	44	9/10	21	10/10	36
DTPa(AH)HB(AH)	5/10	1.7	nd	nd	nd	nd
DTPa(AH)HB(AP)	10/10	18	8/10	8	9/10	24

nd: not tested

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Example 8

Immunogenicity of HBsAg combined to DTPw in monkeys
Results of aluminium hydroxide (AH) and aluminium phosphate (AP) adsorbed antigen

Cercopithenus aethiops monkeys received two injections of 10 µg HBsAg (alone or combined) at days 0 and 30. Sera were withdrawn at days 30 and 57 and titrated (Ausab, Abbott) for anti-HbsAg. Animals with antibody levels significantly above background (pre-vaccination sera) were considered responders. GMT were calculated in mIU/ml.

Results show AP adsorbed HBsAg performed better than AH adsorbed HBsAg.The response was comparable to that obtained by monovalent HBsAg administration.

	Post 1, day 30		Post 2, day 57	
Vaccine	N. resp.	GMT	N. resp.	GMT
Engerix B (HB)(AH)	4/5	10	5/5	666
DTPw(AH)HB(AH)	4/5	20	5/5	31
DTPw(AH)HB(AP)	5/5	12	5/5	414

Example 9

Clinical studies with combined DTPw vaccines using HBsAg adsorbed to aluminium hydroxide (AH) or aluminium phosphate (AP)

Subjects were immunised with 3 doses of 0.5 ml containing DTPw and 10µg HBsAg protein given at the age of 3, 4 and 5 months. Bleeding was at 6 months and sera were titrated with the Ausab test. Percentage responders (seroconversion) relates to subjects with antibody levels significantly above background. Percentage protection relates to subjects with titres equal to or greater than 10 mIU/ml. GMT in mIU/ml.

Results for DTPw-HB show AP adsorbed HBsAg produced a satisfactory response as opposed to AH adsorbed HBsAg. Seroconversion rates and GMT were comparable to data typically seen with monovalent HBsAg vaccine (Engerix B).

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Vaccine	N. subj.	Bleeding Time	% resp.	% prot.	GMT
DTPw(AH).HB(AH)	32	post 2	nd	nd	nd
		post 3	94	84	38.5
DTPw(AH).HB(AP)	29	post 2	97	97	63
	17	post 3	100	100	.469

Example 10

Immunogenicity and stability of HBsAg adsorbed to aluminium hydroxide (AH) or aluminium phosphate (AP) in a hepatitis A-Hepatitis B combined vaccine

Groups of 10 OF1 mice were immunised subcutaneously with 2 doses of 2.5 µg HBsAg (single component or combined) at days 0 and 14. Blood was drawn at day 21 and titrated for anti-HBsAg as in Example 7.

Results for immunogenicity and stability of HA-HB combined product showed AP adsorbed HBsAg produced higher antibody levels and a more stable form.

Vaccine	Exposure	N. resp.	GMT
HA(AH).HB(AH)	4°C	9/10	41
	1 month, 37°C	6/10	5.6
	1 month, 45°C	5/10	6.4
HA(AH).HB(AP)	4°C	10/10	80
	1 month, 37°C	9/10	45
	1 month, 45°C	8/10	18
Engerix B HB(AH)	4°C	8/10	58

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Example 11: Further Clinical Results in humans

1. Immunogenicity of DTPw - Hepatitis B vaccines in infants

Experiment A

Studies in Slovakia: Schedule: 3-4-5 months. 10μg HBsAg; DTPw ex Behringwerke (DT on AH; Pw on a mixture of AH and AP)

Anti-Hbs titres

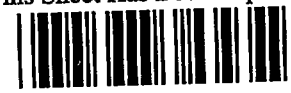
HBsAg adjuvant	Time	N	GMT	SP(%)
Hydroxide	Post II (5 months)	44	45	79.5
Hydroxide	Post III (6 months)	13	34	69.2
Phosphate	Post II (5 months)	32	80	97.0
Phosphate	Post III (6 months)	32	396	100

In this and other examples Post II means after the second dose, post III after the third dose. GMT is always measured one month after the injection time shown in the schedule. SP is the seroprotection rate.

Anti-Diphtheria, Tetanus, B pertussis titres

Post III results	N	GMT	%>0.1 IU/ml	GMT Post/Pre
Anti-Diphtheria	38	2.302	100	37.4
Anti-Tetanus	38	3.281	100	38.4
Anti-B pertussis	38	61	-	7.7

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Experiment B

Study in Greece: Schedule 2-4-6 months (same vaccine as for Experiment A)

Anti-HBs titres (interim results)

HBsAg adjuvant	Time	N	GMT	SP(%)
Hydroxide	Month 7	22	284	90.5
Hydroxide	Month 7	17	193	94.4
Phosphate	Month 7	23	1794	92.0

Experiment C

Study in Slovakia: Schedule 3-4-5 (HBsAg =5µg on aluminium phosphate; DTP ex Behringwerke as for Experiment A)

Anti-HBs titres

Time	N	GMT	SP(%)
Post II	21	94	90.5
Post III	18	311	100

Experiment D

Study in Slovakia: Schedule 3-4-5 months of age (HBsAg =10µg on aluminium phosphate; DTPw ex Behringwerke as for Experiment A)

Anti-HBs titres

Time	N	GMT	SP(%)
Pre	24	0	0
Post II (month 5)	13	259	92.3
Post III (month 6)	10	592	100.0

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Anti-diphtheria antibodies

Timing	N	GMT	SP (%)	GMT Post/Pre
Pre	32	0.054	6.3	1.0
Post II	16	1.094	93.8	20.4
Post III	11	2.314	100.0	43.1

Anti-tetanus antibodies

Timing	N	GMT	SP (%)	GMT Post/Pre
Pre	32	0.083	34.4	1.0
Post II	16	3.146	100.0	37.9
Post III	11	7.989	100.0	96.4

Anti-B pertussis antibodies

Timing	N	GMT	GMT Post/Pre
Pre	32	8	1.0
Post II	16	20	2.7
Post III	11	50	6.6

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2. Immunogenicity of DTPa - Hepatitis B vaccines in infants

Experiment A

Study in Turkey. HBsAg 10μg on AP; DTP (acellular) on AH. Preliminary results

Group 1 (DTPa - Engerix B combination)

Timing	N	S+	%	GMT
Pre	19	0	0	0
Post I	19	4	21.1	24
Post II	19	18	94.7	146
Post III	19	19	100.0	345

Group 2 (DTPa plus Engerix B; separate simultaneous injections)

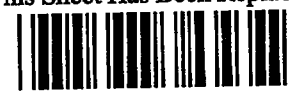
Timing	N	S+	%	GMT
Pre	8	0	0	0
Post I	8	2	25.0	37
Post II	8	5	62.5	33
Post III	7	6	83.7	385

Key: N = number of subjects tested; S+ = number of subjects seropositive at a given blood sampling time; % = seroconversion rate and GMT = geometric mean antibody titre of seroconverters

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CLAIMS

1. A combined vaccine composition comprising Hepatitis B surface antigen (HBsAg) and a number (n) of other antigens in combination with an adjuvant comprising one or more aluminium salts in which the value of n is 1 or greater and in which the adjuvant used to adsorb the HBsAg is not aluminium hydroxide, with the proviso that when n is 1 the other antigen is not an antigen against Hepatitis A.

2. A vaccine composition as claimed in claim 1 in which the HBsAg is adsorbed to aluminium phosphate.

3. A vaccine composition as claimed in claim 2 in which at least one of the other antigens is adsorbed to aluminium phosphate.

4. A vaccine composition as claimed in claim 2 in which at least one of the other antigens is adsorbed to aluminium hydroxide.

5. A vaccine composition as claimed in any preceding claim in which n is 2, 3, 4, 5 or 6.

6. A combined vaccine composition according to claim 5 wherein the antigen adsorbed to aluminium hydroxide or aluminium phosphate is selected from an antigen providing immunity against diphtheria (D); tetanus (T); pertussis (P); Inactivated Polio (IPV); Haemophilus influenzae b (Hib) and Hepatitis A (HA).

7. A stable and effective combined vaccine composition directed to the prevention of more than two diseases comprising HBsAg and at least two other antigens.

8. A vaccine composition according to claim 7 in which the HBsAg is adsorbed to aluminium phosphate.

9. A vaccine composition according to claim 8 in which at least one of the antigens other than HBsAg is adsorbed to aluminium hydroxide.

10. A combined vaccine composition as claimed in any one of claims 7 to 9 which is :



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Diphtheria-Tetanus-Pertussis (DTP) - Hepatitis B; or

Diphtheria - Tetanus (DT) - Hepatitis B ; or

DTP - IPV (inactivated polio vaccine) - Hepatitis B.

11. A combined vaccine composition according to any previous claim in which the stability of the vaccine is such that the vaccine can be kept at 37° C for 1 week without substantial loss of immunogenicity of the HBsAg component.

12. A combined vaccine composition according to any previous claim, characterised in that the immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of 200 mIU/ml (one month post third dose) or greater is found in human infants when a course of the vaccine is given at one month intervals in an appropriate vaccination schedule.

13. A combined vaccine composition as claimed in any preceding claim comprising an antigen component which is protective against Hepatitis A.

14. A combined vaccine composition according to any preceding claim which comprises a pertussis component.

15. A combined vaccine according to claim 14 in which the pertussis component is the whole cell pertussis vaccine or the acellular pertussis vaccine containing partially or highly purified antigens.

16. A multivalent vaccine composition comprising HBsAg and a stabilising adjuvant, the adjuvant being selected such that the vaccine can be kept at 37° C for one week without any substantial loss in immunogenicity of the HBsAg component.

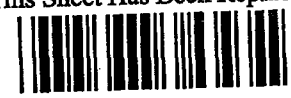
17. A multivalent vaccine composition according to Claim 16 further characterised in that it gives rise to a geometric mean titre of at least 200 mIU/ml (1 month post third dose) when a course of the vaccine is given to human infants at one month intervals in an appropriate vaccination schedule.

18. A multivalent vaccine according to Claim 16 or 17 in which the adjuvant is selected from one or more aluminium salts with the proviso that the HBsAg component is not adsorbed on aluminium hydroxide.

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19. A multivalent vaccine according to Claim 18 in which the HBsAg is adsorbed to aluminium phosphate.

20. A multivalent vaccine according to Claim 18 or 19 in which the antigens present in the vaccine formulation other than HBsAg are adsorbed on aluminium hydroxide.

21. A vaccine formulation according to Claim 20 which is Diphtheria - Tetanus - Perrtussis (acellular) - HBsAg.

22. A multivalent vaccine formulation acording to Claim 18 or 19 in which the antigens present in the vaccine formulation other than HBsAg are adsorbed on aluminium phosphate.

23. A vaccine composition according to Claim 22 which is Diphtheria - Tetanus - Pertussis (whole cell) - HBsAg.

24. A composition as claimed in any one of claims 1 to 23 for use in medicine.

25. Use of HBsAg in the manufacture of a combination vaccine according to any one of claims 1 to 23 for the prophylaxis of Hepatitis B viral infections.

26. A method of preparing a combination vaccine composition as claimed in claims 1 to 23 in which the HBsAg is adsorbed to AP wherein the method comprises mixing aluminium phosphate - adsorbed HBsAg with one or more aluminium hydroxide or aluminium phosphate adsorbed antigens.

27. Use of aluminium phosphate as an adjuvant for adsorbing HBsAg characterised in that the use is for the purpose of formulating a stable and effective combined vaccine comprising HBsAg and at least 1 other antigen whereby the stability and /or immunogenicity of the HBsAg component is greater than in the combined vaccine in which the HBsAg component is adsorbed on aluminium hydroxide.

28. Use according to Claim 27 in which the stability of the vaccine is such that the vaccine can be kept at 37° C for 1 week without substantial loss of immunogenicity of the HBsAg.



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29. Use according to Claim 27 in which the immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of 200 mIU/ml (one month post dose) or greater is found in human infants after a course of 3 doses of the vaccine given at one month intervals in an appropriate immmunisation schedule.

30. Use according to any one of claims 27 to 29 in which there are at least 2 other antigens in the combined vaccine.

31. A method of preventing hepatitis B infections in humans, which method comprises treating human subjects in need thereof with an effective dose of a vaccine according to any one of claims 1 to 23.

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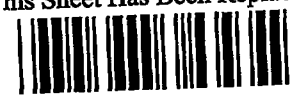
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COMBINED VACCINES COMPRISING HEPATITIS B SURFACE ANTIGEN AND OTHER ANTIGENS

Abstract

Stable and effective multivalent vaccine compositions comprising Hepatitis B surface antigen (HBsAg) are described wherein the HBsAg component is stable for one week at 37° C and is highly immunogenic, for example when the vaccine is administered to infants. The compositions typically comprise HBsAg adsorbed to aluminium phosphate and other antigens, especially those suitable for use in a paediatric vaccine, adsorbed to aluminium phosphate or aluminium hydroxide. Methods for preparing the vaccines and the use of aluminium phosphate to stabilise HBsAg in a multivalent vaccine formulation are also described.

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COMBINED VACCINES COMPRISING HEPATITIS B SURFACE ANTIGEN AND OTHER ANTIGENS

The present invention relates to novel vaccine formulations, methods for preparing them and to their use in therapy. In particular the present invention relates to novel combination vaccine formulations including a Hepatitis B vaccine component for treating Hepatitis B infections.

Infection with Hepatitis B (HB) virus is a widespread problem but vaccines which have been used for mass immunisation are now available, for example the product 'Engerix B' (Registered Trade Mark; SmithKline Beecham plc). Engerix B (Registered Trade Mark) has as the Hepatitis B antigenic component Hepatitis B surface antigen (HBsAg) which is obtained by genetic engineering techniques.

However, it is often necessary or desirable to administer Hepatitis B vaccine at the same time as other vaccines and this can involve multiple injections. Problems associated with multiple injections include a more complicated administration procedure and a large total injection volume.

There is therefore a need for a combined vaccine comprising a Hepatitis B antigen in combination with other antigens. The other antigens are in particular those capable in a vaccine formulation of preventing Hepatitis A (HA), diphtheria (D), tetanus (T), whole cell pertussis (Pw), acellular pertussis (Pa), Haemophilus influenzae b (Hib) and polio (P).

Aluminium hydroxide (AH) is widely used as an adjuvant in the formulation of vaccines. For example, Engerix B (Registered Trade Mark) uses Hepatitis B surface antigen (HBsAg) adsorbed to aluminium hydroxide. We have also used AH successfully in the formulation of Hepatitis A vaccine and in the combined vaccines DT, DTPw and DTPa. However, when AH-absorbed HBsAg is used in combination with other vaccines in a combined formulation there is a substantial decrease of the immune response to HBsAg, resulting in lower or insufficient seroprotection after vaccination. In addition to the stability of the HBsAg component of the combined vaccine is poor.

Aluminium phosphate (AP) adsorbed HBsAg has been used in a commercially available monovalent vaccine (HEPPACINE; Registered Trade Mark) made by Korean Cheil Sugar Co Ltd. We have found that there is no significant difference in immunogenic

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properties between an AH-adsorbed HBsAg monovalent vaccine (Engerix B; Registered Trade Mark) and an AP-adsorbed HBsAg monovalent vaccine.

Couraget et al. (Infection and Immunity, Volume 51, No. 3, March 1986, pages 784-787) describe simultaneous administration of diphtheria-tetanus-pertussis-polio vaccine and hepatitis B vaccine (adjuvanted with aluminium hydroxide) in a simplified immunisation program.

An anonymous research Disclosure entitle 'Polyvalent antigen vaccine for human use' [Research Disclosure (1991) September, No. 329 (32975)] reports the results of toxicity and potency tests on a series of polyvalent antigen comprising HBsAg. Aluminium hydroxide gel was used as adjuvant.

European patent application publication number 0 339 667 discloses a bivalent vaccine comprising HBsAg and a Hepatitis A antigen in which either aluminium hydroxide or aluminium phosphate is used as adjuvant. There appears, however, to be no appreciation of the need to avoid aluminium hydroxide as an adjuvant for a multivalent vaccine comprising HBsAg. Further more there appears to be no disclosure in this document or elsewhere of a bivalent or multivalent hepatitis B vaccine in which at least one antigen other than HBsAg is adsorbed on aluminium hydroxide and the HBsAg is adsorbed on aluminium phosphate.

In one aspect the present invention provides a combined vaccine composition comprising Hepatitis B surface antigen (HBsAg) and a number (n) of other antigens in combination with an adjuvant comprising one or more aluminium salts in which the value of n is 1 or greater and in which the adjuvant used to adsorb the HBsAg is aluminium phosphate, with the proviso that when n is 1 the other antigen is not an antigen against Hepatitis A.

Preferably n is 2,3,4,5 or 6.

The advantage of the invention is that no substantial decrease in the immunogenicity of the HBsAg occurs in the combined vaccine formulation. Avoiding the use of AH to adsorb the HBsAg component in the vaccine formulation also gives rise to a product of markedly superior stability. A further advantage of the invention is that the aforesaid problems associated with multiple injections are overcome or at least mitigated and a stable, highly immunogenic combined formulation is provided. The compositions of the invention are particularly suitable for administration to children.

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In particular we have found in human clinical studies that when AP-adsorbed HBsAg is combined with one or more AH-adsorbed or AP-adsorbed antigens in a combined vaccine no substantial decrease in immunogenicity occurs. The stability of the AP-adsorbed HBsAg in the formulation is also greater than AH-adsorbed HBsAg.

Accordingly in a further aspect there is provided a vaccine composition according to the invention in which at least one of the antigens other than HBsAg is adsorbed to aluminium phosphate.

In a further preferred aspect at least one of the antigens other than HBsAg is adsorbed to AH.

In a further aspect, the invention provides a combined vaccine comprising Hepatitis B surface antigen (HBsAg) adsorbed to AP and an antigen adsorbed to AP or to AH selected from an antigen providing immunity against one or more of the following diphtheria (D); tetanus (T); pertussis (P); Inactivated (IPV); Haemophilus influenzae b (Hib).

In a paediatric vaccine other compatible antigens may also be included, eg antigens known to be effective against meningitis b, meningitis A and C, and otitis media.

As used herein the term 'bivalent' is used to refer to a vaccine comprising a combination of two antigens in total (including HBsAg). The term 'multivalent' is applied to a vaccine composition comprising more than two antigens, for example three, four or five or six antigens.

The meaning of the term 'aluminium phosphate' and aluminium hydroxide' as used herein includes all forms of aluminium hydroxide or aluminium phosphate which are suitable for adjuvanting vaccines.

For example, aluminium phosphate can be a precipitate of insoluble aluminium phosphate (amorphous, semi-crystalline or crystalline), which can be optionally but not exclusively prepared by mixing soluble aluminium salts and phosphoric acid salts. "Aluminium hydroxide" can be a precipitate of insoluble (amorphous, semicrystalline or crystalline) aluminium hydroxide, which can be optionally but not exclusively prepared by neutralizing a solution of aluminium salts. Particularly suitable are the various forms of aluminium hydroxide and aluminium phosphate gels available from commercial

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sources for example, Alhydrogel (aluminium hydroxide, 3% suspension in water) and Adju-for (aluminium phosphate, 2% suspension in saline) supplied by Superfos (Vedbaek, 2950 Denmark).

As regards choice of adjuvant excellent results are obtained when the HBsAg is adsorbed on AP and at least one of the antigens other than HBsAg is adsorbed to AH. Other suitable adjuvants may, however, be used. For example one or all of the antigens other than HBsAg may be adsorbed to AP.

Preferred stable combination vaccines according to the invention are

Diphtheria-Tetanus-Pertussis-Hepatitis B (DTP-HB)
Diphtheria-Tetanus-Hepatitis B (DT-HB)
DTP-IPV (inactivated polio vaccine) - Hepatitis B

It will be appreciated that for a vaccine containing a Hib component the Hib antigen may be used extemporaneously by formulating the vaccine just prior to administration. In this way the following combined vaccine compositions within the scope of the invention may, for example, be prepared:

Hib-Hepatitis B
DTP-Hib-Hepatitis B
IPV-DTP-Hib-Hepatitis B

More specifically particular vaccines within the scope of the invention are:

Diphtheria-Tetanus-Pertussis (DTP adsorbed on AH or AP) - Hepatitis B (HBsAg adsorbed on AP)

Diphtheria-Tetanus (DT adsorbed on AP or AH) - Hepatitis B (HBsAg adsorbed on AP)>

By 'stable' as used herein to describe a vaccine according to the invention is meant a vaccine which can be kept at 37°C for one week without any substantial loss of immunogenicity of the HBsAg component.

By 'effective' as used herein is meant a vaccine composition characterised in that the

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immunogenicity of the HBsAg in the combined vaccine is such that a geometric mean titre of at least 200 mIU/ml, preferably 300mIU/ml or greater, is found in human infants one month after the third dose of the vaccine when the vaccine is administered at one month intervals in an appropriate vaccination schedule.

In a further aspect the multivalent vaccine composition according to the invention comprises HBsAg and a stabilising adjuvant selected such that the vaccine can be kept at 37°C for one week without any substantial loss in immunogenicity of the HBsAg component. Preferably the multivalent vaccine composition is further characterised by giving rise to a geometric mean titre of at least 200 mIU/ml (one month post third dose), preferably 300 mIU/ml or greater, in human infants when the vaccine is administered at one month intervals in an appropriate vaccination schedule.

As used herein the term 'appropriate vaccination schedule' means a schedule known to those of skill in the art for administering a course of doses of a vaccine, especially for paediatric doses. A schedule of 3,4 and 5 months may for example be used. This is particularly appropriate for example for DTP - HBsAg containing vaccines according to the invention.

The other antigens in the multivalent vaccine formulation may be adsorbed to AP or AH (or both) and are advantageously adsorbed to AH as shown in the examples hereinbelow.

Advantageously the vaccine formulation according to the invention comprises a pertussis vaccine.

The pertussis component is suitably a whole cell pertussis vaccine or an acellular pertussis vaccine containing partially or highly purified antigens.

The above combinations may optionally include a component which is protective against Hepatitis A, i.e. an HAV antigen.

Advantageously the Hepatitis B combination vaccine is a paediatric vaccine.

The preparation of the antigens and adsorption procedure with the adjuvants are well known in the art, see for example as given below.

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The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See for example, Harford *et al.(1983)* in Develop. Biol. Standard 54, page 125 , Gregg *et al.* (1987) in Biotechnology 5, page 479, EP A-0 226 846, EP A- 0 299 108 and references therein.

As used herein the expression 'Hepatitis B surface antigen' or 'HBsAg' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et al, Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. In particular the HBsAg may comprise a polypeptide comprising an amino acid sequence comprising residues 12-52 followed by residues 133-145 followed by residues 175-400 of the L-protein of HBsAg relative to the open reading frame on a Hepatitis B virus of ad serotype (this polypeptide is referred to as L*; see EP 0 414 374). HBsAg within the scope of the invention may also include the preS1-preS2 -S polypeptide described in EP 0 198 474 (Endotronics) or analogues thereof such as those described in EP 0 304 578 (Mc Cormick and Jones). HBsAg as herein described can also refer to mutants, for example the 'escape mutant' described in WO 91/14703 or European Patent Application Publication Number 0 511 855 A1, especially HBsAg wherein the amino acid substitution at position 145 is to arginine from glycine.

Normally the HBsAg will be in particle form. The particles may comprise for example S protein alone or may be composite particles, for example (L*,S) where L* is as defined above and S denotes the S-protein of HBsAg. The said particle is advantageously in the form in which it is expressed in yeast.

Suitable antigens for use in vaccines according to the invention are already commercially available and details may be obtained from the World Health Organisation. For example the IPV component may be the Salk inactivated polio vaccine. The pertussis vaccine may comprise a whole cell product , an acellular product or a recombinantly produced product. In particular the pertussis component can be PT (pertussis toxins) or subfractions thereof, FHA (filamentous haemagglutinin antigen), agglutinogens (fimbrial) and outer membrane proteins, including the 69kDa protein (pertactin, non fimbrial agglutinogen). *References*: Robinson, A., Irons, L. I. & Ashworth, A. E., Vaccines, 3, 1985, 11-22; and

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Brennan, H. J., Li, S. M., Cowell, J. L., Bishen, M. E., Steven, A. C. Novotny, P, Manclarck, C. R., Infection and Immunity, 56, 1988, 3189-3195.

The component affording protection against Hepatitis A is preferably the product known as 'Havrix' (Registered Trade Mark, SmithKline Beecham Biologicals) which is a killed attenuated vaccine derived from the HM-175 strain of HAV [see 'Inactivated Candidate Vaccines for Hepatitis A' by F.E. Andre, A. Hepburn and E.D Hondt (1980), Prog. Med. Virol. Vol 37, pages 72-95 and the product monograph 'Havrix' published by SmithKline Beecham Biologicals (1991).

Flehmig et al (loc cit., pages 56-71) have reviewed the clinical aspects, virology, immunology and epidemiology of Hepatitis A and discussed approaches to the development of vaccines against this common viral infection.

As used herein the expression 'HAV antigen' refers to any antigen capable of stimulating neutralising antibody to HAV in humans. The HAV antigen preferably comprises inactivated attenuated virus particles or may be, for example an HAV capsid or HAV viral protein, which may conveniently be obtained by recombinant DNA technology.

Vaccine preparation is generally described in New Trends and Developments in Vaccines (1978), edited by Voller *et al.*, University Park Press, Baltimore, Maryland U.S.A.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant , adverse side effects in typical vaccinees. Such amount will vary depending on which specific immunogens are employed. Generally it is expected that each dose will comprise 1-1000 µg of total immunogen, preferably 2-100 µg, more preferably 1 - 40 µg, most preferably 1 - 5 µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. A primary vaccination course may include 2 or 3 doses of vaccine, given one to two months apart, following the WHO recommendations for DTP immunization.

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In a further aspect of the invention there is provided a vaccine composition according to the invention for use in medicine.

In a further aspect of the invention there is provided the use of HBsAg for the manufacture of a combination vaccine according to the invention for the prophylaxis of Hepatitis B viral infections.

In a further aspect the invention provides the use of AP for the purpose of acting as a stabiliser for, and/or to maintain the efficacy of, HBsAg in a multivalent vaccine according to the invention.

Specifically the invention provides the use of aluminium phosphate for the purpose of preparing a stable combined vaccine comprising HBsAg and at least one other antigen (preferably at least two other antigens) whereby the stability and/or immunogenicity of the HBsAg component is greater than in the corresponding combined vaccine in which the HBsAg component is adsorbed on AH.

More specifically the invention provides the use whereby the vaccine can be kept at 37° C for 1 week (i.e. 7 days) without substantial loss of immunogenicity of the HBsAg.

Also provided is the use whereby the geometric meant titre (GMT) found one month after the third dose of a course of vaccinations given at one month intervals in an appropriate vaccination schedule to human infants is greater than 200, preferably greater than 300, mIU/ml.

In a further aspect of the present invention there is provided a method of manufacture of a combined (i.e. bivalent or multivalent) vaccine effective in preventing hepatitis B infection as illustrated in the examples hereinbelow.

In one preferred aspect the antigens other than HBsAg are all adsorbed on AH. A very effective DTPa - Hepatitis B vaccine can, for example, be made in this way.

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In general, the combined vaccine compositions according to any aspect of the invention can be prepared as follows. The required DT, DTPw, DTPa, HA or other components are adsorbed onto a suitable adjuvant, especially AH or AP. After allowing time for complete and stable adsorption of the respective components, the different components are combined under appropriate conditions.

It will be appreciated that certain components, for example the DT, DTPw and DTPa components can be combined separately before adding the adsorbed HBsAg component. Multivalent vaccines comprising HBsAg and other or additional antigens to those mentioned hereinabove may be prepared in a similar manner.

In a preferred aspect there is provided a method of preparing a combined vaccine composition according to the invention wherein the method comprises mixing aluminium phosphate - adsorbed HBsAg with one or more aluminium hydroxide or aluminium phosphate adsorbed antigens.

The following examples illustrate the invention.

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EXAMPLES 1-5

Formulations

Particular formulations according to the present invention were prepared as described below.

Example 1 HBsAg adsorption on $AlPO_4$ as concentrate for formulation of combined vaccines.

A suspension of aluminium phosphate containing 0.03 to 0.3 g aluminium (as aluminium phosphate) in isotonic saline, is mixed with a HBsAg concentrate, containing 10 mg HBsAg protein, in a final volume of 10 to 100 ml. After adjusting the pH to 5 - 6.5 the mixture is left 10 - 24 hrs at room temperature with stirring. Antiseptic is then optionally added (i.e. merthiolate, 1 : 20,000 to 1 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) and the volume is brought to 50 ml with isotonic saline.

Example 2 Formulation of combined Diphtheria-Tetanus-Hepatitis B vaccine.

A concentrate containing 25,000 Lf of diphtheria toxoid and 10,000 Lf of tetanus toxoid adsorbed to 0.35 g Al (as aluminium hydroxide or aluminium phosphate) is prepared in a final volume of 0.15 l of isotonic saline and adjusted to between pH 6 and 7, as specified by WHO for DT and DTP vaccines. This concentrate is combined with 0.05 l of the Hepatitis B concentrate of example 1.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

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D toxoid:	25Lf,
T toxoid:	10 Lf,
HBsAg:	10 µg protein

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 3 Formulation of combined Diphtheria - Tetanus - pertussis (whole cell vaccine) - Hepatitis B vaccine

A concentrate ex Behringwerke containing 7,500 Lf of diphtheria toxoid, 3,250 Lf of Tetanus toxoid and 15,000 capacity units of *B. pertussis* antigen adsorbed to 0.45 mg Al (as aluminium hydroxide and aluminium phosphate) is prepared in a final volume of 0.4 l of isotonic saline and adjusted to pH 6 - 7, as specified by WHO for DTP vaccines. This concentrate is combined with 0.05 l of Hepatitis B concentrate of example 1.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (c.c. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

D toxoid:	7.5Lf,
T toxoid:	3.25 Lf
Pw antigen:	15OU
HBsAg:	10 µg protein.

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 4 Formulation of Diphtheria-Tetanus-Pertussis (acellular component) vaccine.

A concentrate containing 25,000 Lf of diphtheria toxoid and 10,000 Lf of tetanus toxoid adsorbed to 0.35 g Al (as aluminium hydroxide or phosphate gel) is prepared

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in a final volume of 0.15 l of isotonic saline and adjusted to between pH 6 and 7, as specified by WHO for DTP vaccines. 25 mg of inactivated pertussis toxin, 25 mg of filamentous hemagglutinin (FHA) and optionally 8 mg of 69kDa outer membrane protein (pertactin), each combined with 0.05 g Al (as aluminium hydroxide or aluminium phosphate) are added. The *B. pertussis* antigens PT, FHA and pertactin can be prepared as described by methods known in the art, for example European patent application 427 462, *PCT application WO 91/12020* or by other procedures giving physiologically acceptable and potent *B. pertussis* antigens.

This mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (e.g. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

D toxoid:	25 Lf,
T toxoid:	10 Lf,
PTd toxoid:	25 µg,
FHA toxoid:	25 µg,
69kDa OMP:	8 µg (optional)

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

Example 5 Formulation of combined Diphtheria - Tetanus - Pertussis (acellular component) - Hepatitis B vaccine

The procedure of example 4 is applied, with the exception that an additional 50 ml of HBsAg adsorbed concentrate as prepared in example. 1 is added to the final mixture.

The resulting mixture is brought to a final volume of 0.5 l with isotonic saline. Antiseptic media (e.g. merthiolate 1 : 20,000 to 0 : 10,000 or 2-phenoxyethanol, 3 to 6 mg/ml) can be optionally added. The final pH is between 6 and 7, as specified by WHO for DT and DTP vaccines.

One 0.5 ml dose of this bulk vaccine contains, as active ingredients:

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D toxoid:	25 Lf.
T toxoid:	10 Lf
PTd toxoid:	25 µg.
FHA toxoid:	25 µg.
69kDaOMP:	8 µg (optional).

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

EXAMPLES 6-10

Animal and Human Studies

Reference Example 6 Formulation of combined Hepatitis A - Hepatitis B vaccines

An inactivated Hepatitis A virus concentrate (460,000 Elisa units), adsorbed to 0.02 to 0.2 g, preferably 0.04 - 0.1 g aluminium (as aluminium hydroxide) in a final volume of about 125ml was combined to 50 ml of concentrate containing 10 mg HBsAg adsorbed to aluminium phosphate as described in example 1.

The resulting mixture was supplemented with isotonic saline and an amino acid concentrate (Travasol, Registered Trade Mark, Baxter-Travenol Inc) to obtain a final volume of 0.5l containing 1.5g amino acids. The resulting pH as between 6 and 7.

Our 1 ml dose of this bulk vaccine contains, as active ingredients:

Hepatitis A virus antigen:	800 Elisa units
HBsAg:	20 µg protein

The procedure can be optionally amended to use higher or lower quantities of the active ingredients.

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Results:

Clinical studies comparing aluminium hydroxide (AH) and aluminium phosphate (AP) adsorbed HBsAg (Monovalent vaccine)

Initially seronegative healthy adult volunteers were immunised with 3 doses of 20 μg HBsAg protein given at one month interval. Antibody levels were determined in sera obtained one month post 2 and 3 doses using the Ausab (Registered Trade Mark, Abbott) test. Responses were defined as subjects with titres significantly above background. Titres were expressed in mIU/ml.

Results are expressed as Geometric Mean Titres (GMT) in mIU/ml.

HBsAg Lot	Adjuvant	N.Subj.	Post 2, month 2		Post 3, month 3	
			GMT	% responders	GMT	% responders
100	AH	43	32	86	141	100
101	AH	45	26	93	198	98
102	AH	46	30	84	147	93
105/P	AP	7	43	83	380	100

HBsAg Lot	Adjuvant	N.Subj.	Post 2, month 2		Post 3, month 3	
			GMT	% responders	GMT	% responders
102	AH	51	14	82	126	98
103	AH	50	15	83	110	98
102	AH	54	17	83	133	96
104/P	AP	54	18	96	270	98
105/P	AP	51	14	90	156	96

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Example 7

Mouse immunogenicity tests and results of accelerated stability tests for combination vaccines comprising HBsAg with aluminium hydroxide (AH) or aluminium phosphate (AP) as adjuvant

Groups of 10 OF1 mice were immunised subcutaneously with 2 doses of 2.5 μg HBsAg (single component or combined) at days 0 and 14. Blood was drawn off at day 21 and titrated for anti-HBsAg using the Ausab (Registered Trade Mark; Abbott) test. Antibody titres were calculated in mIU/ml. The number of responding animals was defined as the number of those with antibody levels significantly above background values. The geometric mean titres was also calculated (GMT).

The results of DT-HB, DTPw-HB, DTPa-HB show that AP adsorbed HBsAg performed better than AH adsorbed HBsAg both in terms of number of responding animals and GMTs. The response to AP adsorbed HBsAg in the combination was comparable to that obtained by monovalent HBsAg administration.

Vaccine	4°C		7 days, 37°C		7 days, 45°C	
	N.resp.	GMT	N.resp.	GMT	N.resp	GMT
Engerix B(HB+AH)	7/10	30	9/10	17	6/10	2.7
Engerix B(HB+AH)	9/10	54	8/10	13	5/10	6
HB (AH)	9/10	45	10/10	55	9/10	32
HB (AP)	9/10	54	10/10	50	7/10	6.9
DTPw(AH)HB(AH)	4/10	1.4	nd	nd	nd	nd
DTPw(AH)HB(AP)	9/10	52	8/10	16	8/10	26
DT(AH)HB(AH)	6/10	1.7	nd	nd	nd	nd
DT(AH)HB(AP)	8/10	44	9/10	21	10/10	36
DTPa(AH)HB(AH)	5/10	1.7	nd	nd	nd	nd
DTPa(AH)HB(AP)	10/10	18	8/10	8	9/10	24

nd: not tested

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Example 8

Immunogenicity of HBsAg combined to DTPw in monkeys
Results of aluminium hydroxide (AH) and aluminium phosphate (AP) adsorbed antigen

Cercopithenus aethiops monkeys received two injections of 10 μg HBsAg (alone or combined) at days 0 and 30. Sera were withdrawn at days 30 and 57 and titrated (Ausab, Registered Trade Mark; Abbott) for anti-HBsAg. Animals with antibody levels significantly above background (pre-vaccination sera) were considered responders. GMT were calculated in mIU/ml.

Results show AP adsorbed HBsAg performed better than AH adsorbed HBsAg.The response was comparable to that obtained by monovalent HBsAg administration.

Vaccine	Post 1, day 30		Post 2, day 57	
	N. resp.	GMT	N. resp.	GMT
Engerix B (HB)(AH)	4/5	10	5/5	666
DTPw(AH)HB(AH)	4/5	20	5/5	31
DTPw(AH)HB(AP)	5/5	12	5/5	414

Example 9

Clinical studies with combined DTPw vaccines using HBsAg adsorbed to aluminium hydroxide (AH) or aluminium phosphate (AP)

Subjects were immunised with 3 doses of 0/5 ml containing DTPw and 10 μg HBsAg protein given at the age of 3, 4 and 5 months. Bleeding was at 6 months and sera were titrated with the Ausab (Registered Trade Mark) test. Percentage responders (seroconversion) relates to subjects with antibody levels significantly above background. Percentage protection relates to subjects with titres equal to or greater than 10 mIU/ml. GMT in mIU/ml.

Results for DTPw-HB show AP adsorbed HBsAg produced a satisfactory response as opposed to AH adsorbed HBsAg. Seroconversion rates and GMT were comparable to data typically seen with monovalent HBsAg vaccine (Engerix B).

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Vaccine	N. subj.	Bleeding Time	% resp.	% prot.	GMT
DTPw(AH).HB(AH)	32	post 2	nd	nd	nd
		post 3	94	84	38.5
DTPw(AH).HB(AP)	29	post 2	97	97	63
	17	post 3	100	100	469

Reference Example 10

Immunogenicity and stability of HBsAg adsorbed to aluminium hydroxide (AH) or aluminium phosphate (AP) in a hepatitis A-Hepatitis B combined vaccine

Groups of 10 OF1 mice were immunised subcutaneously with 2 doses of 2.5 µg HBsAg (single component or combined) at days 0 and 14. Blood was drawn at day 21 and titrated for anti-HBsAg as in Example 7.

Results for immunogenicity and stability of HA-HB combined product showed AP adsorbed HBsAg produced higher antibody levels and a more stable form.

Vaccine	Exposure	N. resp.	GMT
HA(AH).HB(AH)	4°C	9/10	41
	1 month, 37°C	6/10	5.6
	1 month, 45°C	5/10	6.4
HA(AH).HB(AP)	4°C	10/10	80
	1 month, 37°C	9/10	45
	1 month, 45°C	8/10	18
Engerix B HB(AH)	4°C	8/10	58

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Example 11: Further Clinical Results in humans

1. Immunogenicity of DTPw - Hepatitis B vaccines in infants

Experiment A

Studies in Slovakia: Schedule: 3-4-5 months. 10μg HBsAg; DTPw ex Behringwerke (DT on AH; Pw on a mixture of AH and AP)

Anti-Hbs titres

HBsAg adjuvant	Time	N	GMT	SP(%)
Hydroxide	Post II (5 months)	44	45	79.5
Hydroxide	Post III (6 months)	13	34	69.2
Phosphate	Post II (5 months)	32	80	97.0
Phosphate	Post III (6 months)	32	396	100

In this and other examples Post II means after the second dose, post III after the third dose. GMT is always measured one month after the injection time shown in the schedule. SP is the seroprotection rate.

Anti-Diphtheria, Tetanus, B pertussis titres

Post III results	N	GMT	%>0.1 IU/ml	GMT Post/Pre
Anti-Diphtheria	38	2.302	100	37.4
Anti-Tetanus	38	3.281	100	38.4
Anti-B pertussis	38	61	-	7.7

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Experiment B

Study in Greece: Schedule 2-4-6 months (same vaccine as for Experiment A)

Anti-HBs titres (interim results)

HBsAg adjuvant	Time	N	GMT	SP(%)
Hydroxide	Month 7	22	284	90.5
Hydroxide	Month 7	17	193	94.4
Phosphate	Month 7	23	1794	92.0

Experiment C

Study in Slovakia: Schedule 3-4-5 (HBsAg =5µg on aluminium phosphate; DTP ex Behringwerke as for Experiment A)

Anti-HBs titres

Time	N	GMT	SP(%)
Post II	21	94	90.5
Post III	18	311	100

Experiment D

Study in Slovakia: Schedule 3-4-5 months of age (HBsAg =10µg on aluminium phosphate; DTPw ex Behringwerke as for Experiment A)

Anti-HBs titres

Time	N	GMT	SP(%)
Pre	24	0	0
Post II (month 5)	13	259	92.3
Post III (month 6)	10	592	100.0

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Anti-diphtheria antibodies

Timing	N	GMT	SP (%)	GMT Post/Pre
Pre	32	0.054	6.3	1.0
Post II	16	1.094	93.8	20.4
Post III	11	2.314	100.0	43.1

Anti-tetanus antibodies

Timing	N	GMT	SP (%)	GMT Post/Pre
Pre	32	0.083	34.4	1.0
Post II	16	3.146	100.0	37.9
Post III	11	7.989	100.0	96.4

Anti-B pertussis antibodies

Timing	N	GMT	GMT Post/Pre
Pre	32	8	1.0
Post II	16	20	2.7
Post III	11	50	6.6

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2. Immunogenicity of DTPa - Hepatitis B vaccines in infants

Experiment A

Study in Turkey. HBsAg 10µg on AP; DTP (acellular) on AH. Preliminary results

Group 1 (DTPa - Engerix B combination)

Timing	N	S+	%	GMT
Pre	19	0	0	0
Post I	19	4	21.1	24
Post II	19	18	94.7	146
Post III	19	19	100.0	345

Group 2 (DTPa plus Engerix B; separate simultaneous injections)

Timing	N	S+	%	GMT
Pre	8	0	0	0
Post I	8	2	25.0	37
Post II	8	5	62.5	33
Post III	7	6	83.7	- 385

Key: N = number of subjects tested; S+ = number of subjects seropositive at a given blood sampling time; % = seroconversion rate and GMT = geometric mean antibody titre of seroconverters

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CLAIMS

1. A vaccine composition comprising Hepatitis B surface antigen (HBsAg) and a number (n) of other antigens in combination with an adjuvant comprising one or more aluminium salts in which the value of n is 1 or greater and in which the adjuvant used to adsorb the HBsAg is aluminium phosphate (AP), with the proviso that when n is 1 the other antigen is not an antigen against Hepatitis A.

2. A vaccine composition as claimed in Claim 1 in which the said other antigens are selected to provide immunity against one or more of the following diphtheria, tetanus, pertussis, polio, Haemophilus influenzae b, Hepatitis A, meningitis A, meningitis B, meningitis C, and otitis media.

3. A vaccine composition as claimed in Claim 1 or Claim 2 in which at least one of the said other antigens is adsorbed to AP.

4. A vaccine composition as claimed in Claim 1 or Claim 2 in which at at least one of the said other antigens is adsorbed to aluminium hydroxide (AH).

5. A vaccine composition as claimed in any preceding claim in which n is 2, 3, 4, 5 or 6.

6. A vaccine composition according to claim 5 wherein the said other antigens provide immunity against diphtheria (D), tetanus (T) and pertussis (P).

7. A vaccine composition as claimed in claim 6 wherein the pertussis component is whole cell pertussis (Pw) or acellular pertussis antigen (Pa).

8. A vaccine composition as claimed in Claim 6 or Claim 7 additionally comprising an antigen against Haemophilus influenzae b (Hib).

9. A vaccine composition as claimed in any one of Claims 6 to 8 additionally comprising inactivated polio (IPV).

10. A vaccine composition as claimed in any one of claims 6 to 9 additionally comprising an antigen against Hepatitis A.

11. A vaccine composition according to any previous claim in which the stability of the vaccine is such that the vaccine can be kept at 37°C for one week without substantial loss of immunogenicity of the HBsAg component.

12. A vaccine composition according to any previous claim characterised in that the immunogenicity of the HBsAg in the vaccine is such that a geometric mean titre of 200 mIU/ml (one month post third dose) or greater is found in human infants when a course of the vaccine is given at one month intervals in an appropriate vaccination schedule.



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13. A composition as claimed in any one of Claims 1 to 12 for use in medicine.

14. Use of HBsAg in the manufacture of a vaccine composition according to any one of claims 1 to 12 for the prophylaxis of hepatitis B viral infections.

15. A method of preparing a vaccine composition as claimed in any one of claims 1 to 12 wherein the method comprises mixing AP-adsorbed HBsAg with one or more AH- or AP-adsorbed other antigens.

16. Use of aluminium phosphate (AP) as an adjuvant for adsorbing HBsAg, characterised in that the use is for the purpose of formulating a stable and effective combined vaccine comprising HBsAg and a number (n) of other antigens adsorbed on aluminium phosphate or aluminium hydorxide (AH), wherein the value of n is 1 or larger, and whereby the stability and/or immunogenicity of the HBsAg component is greater than in a corresponding combined vaccine in which the HBsAg component is adsorbed on AH, with the proviso that when n is 1 the said antigen is not an antigen against Hepatitis A.

17. Use according to claim 16 in which the stability of the vaccine is such that the vaccine can be kept at 37°C for 1 week without substantial loss of immunogenicity of the HBsAg.

18. Use according to claim 16 or claim 17 in which the immunogenicity in the combined vaccine is such that the geometric mean titre of 200 mIU/ml (one month post third dose) or greater is found in human infants when a course of the vaccine is given at one month intervals in an appropriate vaccination schedule.

19. Use according to any one of claims 16 to 18 in which there are at least 2 other antigens in the combined vaccine.

20. Use according to claim 19 in which the vaccine is a DTP - Hepatitis B vaccine.

21. Use according to claim 19 in which the vaccine is a DTP-Hepatitis B-Hib vaccine.

22. Use according to claim 19 in which the vaccine is a DTP-Hepatitis B - Hib - IPV vaccine.

23. Use according to claim 19 in which the vaccine is a DTP-Hepatitis B - Hib - IPV - Hepatitis A vaccine.

* C L M S *

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24. A vaccine composition according to any one of claims 1 to 13 or a method or use according to any one of claims 14 to 23 in which an acellular pertussis (Pa) component is present in the vaccine composition, said Pa component comprising pertactin.

25. A vaccine composition according to claim 7 in which a 0.5 ml dose of the vaccine comprises the following:

Diphtheria toxoid:	7.5 Lf
Tetanus toxoid:	3.25 Lf
Whole cell pertussis antigen:	15 OU
HBsAg:	10 µg protein

26. A vaccine composition according to claim 7 in which a 0.5ml dose of the vaccine comprises the following:

Diphtheria toxoid:	25Lf
Tetanus toxoid:	10Lf
Inactivated pertussis toxin	25µg
Filamentous hemagglutinin	25µg
Pertactin	8ug

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COMBINED VACCINES COMPRISING HEPATITIS B SURFACE ANTIGEN AND OTHER ANTIGENS

Abstract

Stable and effective multivalent vaccine compositions comprising Hepatitis B surface antigen (HBsAg) are described wherein the HBsAg component is stable for one week at 37° C and is highly immunogenic, for example when the vaccine is administered to infants. The compositions typically comprise HBsAg adsorbed to aluminium phosphate and other antigens, especially those suitable for use in a paediatric vaccine, adsorbed to aluminium phosphate or aluminium hydroxide. Methods for preparing the vaccines and the use of aluminium phosphate to stabilise HBsAg in a multivalent vaccine formulation are also described.