Hib disease was considerably higher in resource-poor countries, prior to the introduction of the vaccine into their national immunization programs. In 2000, Hib was estimated to have caused two to three million cases of serious disease, notably pneumonia and meningitis, and 386,000 deaths in young children. Hib disease is observed in all parts of the world but is difficult to confirm because it requires prompt laboratory investigation in patients that have not received prior antibiotic treatment.

[0039] Commercially available combined DTaP/IPV/Hib vaccines include Pentacel®, which is based on combined diphtheria toxoid, tetanus toxoid, acellular pertussis (adsorbed), and inactivated poliovirus in combination with a Hib conjugate vaccine.

[0040] Hence, there is a need for a safe and effective method of simultaneously preventing dengue disease and diphtheria, tetanus, pertussis, poliomyelitis and diseases caused by *Haemophilus influenzae* type b.

[0041] Furthermore, it has been reported that elderly subjects infected with dengue virus often present atypically, wherein fever may be the only symptom and are less likely to present with typical symptoms and signs of dengue fever which may be defined as fever plus at least one of the following symptoms: bone pain, myalgia, arthralgia, retro-orbital pain, headache and maculopapular rash (Lee et al. (2013) Am. J. Emerg. Med. 31(5): 783-787). Yet, elderly adults are at increased risk of developing severe dengue and DHF/DSS compared to younger subjects (Liu et al. (2008) Am. J. Infect. Dis. 4(1): 10-17; Lin et al. (2012) Emerg. Infect. Dis. 18(10): 2003-1009; Rowe et al. (2014) PloS Negl. Trop. Dis. 8(4)). Several reasons which may contribute to severe disease in the elderly have been discussed, such as impaired physiological and immune functions, an increased probability of acquiring secondary dengue and an increased prevalence of chronic diseases and other comorbidities in the elderly, i.e. above 60 (see the discussion in Lin et al. (2017) Expert Review of Anti-infective Therapy 15(8): 729-735).

OBJECTS AND SUMMARY

[0042] It is an object of the present disclosure to provide a safe and effective vaccine and corresponding method of inoculation against all serotypes of dengue virus for dengue-endemic and dengue non-endemic populations and for a broad range of ages, in particular for subjects between 2 months and 60 years of age, and independent of previous exposure to dengue virus and corresponding seropositive or seronegative status before vaccination.

[0043] It is an object of the present disclosure to minimize the risk of DHF and DSS caused by infection with DENV-1, DENV-2, DENV-3 or DENV-4, in particular following vaccination in children of young age and individuals of any age who have never been previously exposed to dengue, or who are seronegative to dengue before vaccination.

[0044] It is an object of the disclosure to provide a vaccine and corresponding method of inoculating for controlling a dengue outbreak situation.

[0045] It is an object of the disclosure to provide a vaccine and corresponding method of inoculating which is useful in settings less familiar with clinical management of dengue or in those with fewer resources.

[0046] It is an object of the disclosure to provide a vaccine and corresponding method of inoculating which avoids testing for individual serostatus before individual inoculation or analysis of seroprevalence rates in areas to be mass vaccinated.

[0047] It is an object of the disclosure to provide a vaccine and corresponding method of inoculating which reduces and/or avoids the risk for later antibody dependent enhancement.

[0048] It is an object of the disclosure to provide a vaccine and corresponding method which reduces and/or avoids dengue NS1 toxicosis.

[0049] It is an object of the disclosure to provide a vaccine and corresponding method of inoculating which induces cross reactive multitypic antibody and/or cell mediated immunity.

[0050] It is one object of the present disclosure to provide a safe and effective protection against dengue disease and yellow fever.

[0051] It is another object of the present disclosure to provide a safe and effective protection against dengue disease and hepatitis A.

[0052] It is a further object of the present disclosure to provide a safe and effective protection against dengue disease and HPV-associated cancers or genital warts.

[0053] It is a certain object of the present disclosure to provide a safe and effective protection against dengue disease and measles, mumps and rubella.

[0054] It is another object of the present disclosure to provide safe and effective protection against dengue disease and tetanus, diphtheria, and pertussis.

[0055] It is another object of the present disclosure to provide a safe and effective protection against dengue disease and diphtheria, tetanus, pertussis, poliomyelitis and diseases caused by *Haemophilus influenzae* type b.

[0056] It is another object of the present disclosure to provide safe and effective protection against any combination of the above mentioned diseases.

[0057] It is another object of the present disclosure to provide a vaccine which stimulates a balanced immune response to all four dengue serotypes in a subject, in particular in an elderly subject (i.e. more than 60 years of age).

[0058] The present disclosure is therefore directed in part to a dengue vaccine for a method of mass vaccination without individual testing for serostatus or prior evaluation of seroprevalence rates. This mass vaccination includes endemic regions including outbreak situations as well as in non-endemic regions for travelers.

[0059] In one aspect, the present disclosure provides a unit dose of a dengue vaccine composition comprising: a tetravalent virus composition including four live attenuated dengue virus serotypes wherein the unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises: (i) a chimeric dengue serotype 2/1 strain, in a concentration of at least 3.3 log10 pfu/0.5 ml, (ii) a dengue serotype 2 strain, in a concentration of at least 2.7 log10 pfu/0.5 ml, (iii) a chimeric dengue serotype 2/3 strain, in a concentration of at least 4.0 log10 pfu/0.5 ml, and (IV) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain, in a concentration of at least 4.5 log10 pfu/0.5 ml, wherein upon said reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 ml and based on said total concentration of pfu/0.5 ml, the concentration of (i) in pfu/0.5 ml is at least 1%, the concentration of (ii) in pfu/0.5 ml is less than 10%, the concentration of (iii) in pfu/0.5 ml is at least 10%, and the concentration of (iv) in pfu/0.5 ml is at least 50%, wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and differs in at least three nucleotides from the wild type as follows: a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T) b) NS1-53 Gly-to-Asp (nt-2579 G-to-A) c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing

the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains: - a DENV-2/1 chimera, - a DENV-2/3 chimera and - a DENV-2/4 chimera.

[0059a] In another aspect, the present disclosure provides a vaccine combination comprising a unit dose of a dengue vaccine composition as described herein, and one or more other vaccine(s). In yet another aspect, the present disclosure provides a kit for preparing a reconstituted unit dose comprising the following components: a) one, two or more unit dose(s) as described herein, and preferably, b) a pharmaceutically acceptable diluent for reconstitution, preferably wherein the pharmaceutically acceptable diluent for reconstitution is an aqueous sodium chloride solution including 37 mM sodium chloride.

[0059b] In yet another aspect, the present disclosure provides a use of the unit dose as described herein, in the manufacture of a medicament for preventing dengue disease in a subject. In yet another aspect, the present disclosure provides a unit dose of a dengue vaccine composition which is characterized as safe when to be administered to a subject without the need to determine the occurrence of a previous dengue infection.

[0059c] In a further aspect, the present disclosure provides a use of a unit dose of a dengue vaccine composition as described herein in the manufacture of a medicament for preventing dengue disease in a subject wherein the use is safe for the subject without the need to determine the occurrence of a previous dengue infection. In a further aspect, the present disclosure provides a use of a dengue vaccine composition as described herein in the medicament for preventing dengue disease in a subject, comprising at least one dose of said unit dose to be administered to the subject that results in the prevention of dengue disease. In a further aspect, the present disclosure provides a use of a unit dose of a dengue vaccine composition as described herein in the manufacture of a medicament for preventing dengue disease in a subject and providing an efficacy against dengue serotype 2 of at least 60%. In a further aspect, the present disclosure provides a use of a unit dose of a dengue vaccine composition as described herein the manufacture of a medicament for preventing dengue disease in a subject comprising said unit dose to be administered to the subject, wherein the use does not require a step of determination whether there was a previous dengue infection in the subject at any time before, during or after said unit dose to be administered, wherein determination of the previous dengue infection in the subject is characterized by a laboratory confirmed history of dengue disease or a validated serological test.

[0059d] The present disclosure is therefore directed to a tetravalent dengue virus composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, wherein preferably the tetravalent dengue virus composition includes a chimeric dengue serotype 2/1 strain and a dengue serotype 2 strain and a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, wherein preferably the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 (represented by SEQ ID NO 11) and differs in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)
b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)
c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

the three chimeric dengue strains being derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,

- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

[0060] The present disclosure is in particular directed to a composition or lyophilized unit dose which upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:

(i) a dengue serotype 1 in a concentration of at least 3.3 log10 pfu/0.5 ml,

(ii) a dengue serotype 2, in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a dengue serotype 3, in a concentration of at least 4.0 log10 pfu/0.5 ml, and

(iv) a dengue serotype 4, in a concentration of at least 4.5 log10 pfu/0.5 ml.

[0061] The present disclosure is also in particular directed to such a composition or unit dose which upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (ii) in pfu/0.5 mL is less than 10%, and the concentration of (iv) in pfu/0.5 mL is at least 50%, and the concentration of (i) in pfu/0.5 mL is at least 1%, and the concentration of (iii) in pfu/0.5 mL is at least 8%, or preferably at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

[0062] The present disclosure is also in particular directed to such method and use wherein upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (ii) in pfu/0.5 mL is less than 2%, the concentration of (iv) in pfu/0.5 mL is at least 50%, the concentration of (i) in pfu/0.5 mL is at least 1%, and the concentration of (iii) in pfu/0.5 mL is at least 6%.

[0063] The present disclosure is therefore directed to a method including and corresponding use of a tetravalent dengue virus composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, the method being directed to inoculating a subject against virologically confirmable dengue disease, wherein in particular the tetravalent dengue virus composition includes a chimeric dengue serotype 2/1 strain and a dengue serotype 2 strain and a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, wherein in particular the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 (represented by SEQ ID NO 11) and differs in at least three nucleotides from the wild type as follows:

d) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

e) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

f) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

the three chimeric dengue strains being derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

[0064] The present disclosure is in particular directed to such method and use wherein the unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:

(i) a dengue serotype 1 in a concentration of at least 3.3 log10 pfu/0.5 ml,

(ii) a dengue serotype 2, in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a dengue serotype 3, in a concentration of at least 4.0 log10 pfu/0.5 ml, and

(iv) a dengue serotype 4, in a concentration of at least 4.5 log10 pfu/0.5 ml.

[0065] The present disclosure is also in particular directed to such method and use wherein upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (ii) in pfu/0.5 mL is less than 10%, and the concentration of (iv) in pfu/0.5 mL is at least 50%, and the concentration of (i) in pfu/0.5 mL is at least 1%, and the concentration of (iii) in pfu/0.5 mL is at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%, and wherein the subject is preferably 2 to 17 years of age or 4 to 16 years of age.

[0066] The present disclosure is also in particular directed to such method and use wherein upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (ii) in pfu/0.5 mL is less than 2%, the concentration of (iv) in pfu/0.5 mL is at least 50%, the concentration of (i) in pfu/0.5 mL is at least 1%, and the concentration of (iii) in pfu/0.5 mL is at least 6%, wherein the subject is 18 to 60 years of age.

[0067] The present disclosure is therefore directed to a method and corresponding use, the method comprising a primary vaccination with only two administrations of the unit dose comprising the steps of:

(A) administering a first unit dose of the tetravalent dengue virus composition to the subject, and

(B) administering a second unit does of the tetravalent dengue virus composition to the subject within 3 months of administration of the first unit dose,

wherein preferably the method and use do not include a step of determination whether there was a previous dengue infection in the subject before administration of the unit dose or wherein the serostatus of the subject is unknown before administration of the unit dose or wherein the method and use do not include a step of determination of a previous dengue infection in the subjects preferably at any time before, during or after the steps of administration or wherein the serostatus of the subject is unknown preferably at any time before, during or after the steps of administration.

[0068] According to one example such a method and use do not include the active surveillance with respect to febrile illness of the subject after the administration of the first- and second-unit dose. During active surveillance any subject with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) will be asked to return to the site for dengue fever evaluation by the Investigator. Subjects/guardians will be contacted at least weekly to ensure robust identification of febrile illness by reminding subjects/guardians of their obligation to return to the site in case of febrile illness. This contact will be implemented through appropriate methods that may differ in each trial site (eg, phone calls, text messaging, home visits, school-based surveillance).

[0069] According to one example such a method and use do not include vaccine immunogenicity analysis including GMTs for dengue neutralizing antibodies.

[0070] According to one example such a method and use do not include a reactogenicity analysis. Such a reactogenicity analysis relates to solicited local AEs (injection site pain, injection site erythema, and injection site swelling) and solicited systemic AEs (child < 6 years: fever, irritability/fussiness, drowsiness and loss of appetite; child ≥ 6 years: asthenia, fever, headache, malaise and myalgia) which will e.g. be assessed for 7 days and 14 days, respectively, following each vaccination (vaccination day included) via collection of diary cards.

[0071] The method according to the disclosure does not require the testing of the serostatus before vaccination and thus allows immediate treatment and outbreak control. According to certain examples the disclosure is directed to

method and use wherein the subject is exposed to a dengue outbreak. In certain such examples the outbreak is due to a dengue serotype 2, and/or due to a serotype 1.

[0072] According to certain examples the method of inoculation against the virologically confirmable dengue disease is due to a dengue serotype 2, and/or due to a dengue serotype 1.

[0073] According to one example the method comprises a primary vaccination consisting of the steps of:

(A) selecting a subject for administration of the unit doses of the tetravalent dengue virus composition in need for protection against dengue infection without determination of a previous dengue infection, and

(B) administering a first unit dose of the tetravalent dengue virus composition to the subject, and

(C) administering a second unit dose of the tetravalent dengue virus composition to the subject within 3 months of administration of the first unit dose.

Therefore the method of inoculating is finalized without determination of a previous dengue infection. The method further optionally comprises at least 1 years after the administration of the second unit dose a booster dose of the unit dose.

[0074] According to one example the method and use is directed to a method of inoculating a subject against virologically confirmable dengue disease with a tetravalent dengue virus composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, the dengue serotype 2 strain being derived from the wild type virus strain DEN-2 16681 (represented by SEQ ID NO 11) and differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

the three chimeric dengue strains being derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera,

the method comprises a primary vaccination consisting of the steps of:

(A) selecting a subject for administration of the unit doses of the tetravalent dengue virus composition in need for protection against dengue infection without determination whether there was a previous dengue infection, and

(B) administering a first unit dose of the tetravalent dengue virus composition to the subject, and

(C) administering a second unit dose of the tetravalent dengue virus composition to the subject within 3 months of administration of the first unit dose.

Therefore the method of inoculating is finalized without determination of a previous dengue infection. The method further optionally comprises at least 1 years after the administration of the second unit dose a booster dose of the unit dose.

[0075] According to the disclosure the method and use is applicable to subjects of all kinds of ages. The present disclosure is in particular directed to such a method and use wherein the subject is under 9 years of age, or 4 to 5 years of age, or 6 to 11 years of age, or 12 to 16 years, or 6 to 16 years of age, or 4 to 16 years of age, or 2 to 17 years of age, or 9 years of age, or over 9 years of age, or 9 to 17 years of age, or 18 to 60 years of age, or 18 to 45 years of age, or 46 to 60 years of age, or over 60 years of age.

[0076] In particular the present disclosure is directed to such use wherein the method is safe.

[0077] In particular the present disclosure is directed to such use wherein the method provides a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 65%, when measured against placebo in a subject population of at least 5,000 healthy 4 to 16 year old subjects irrespective of serostatus at baseline from first administration of the administration schedule until 12 to 18 months after the last administration of the administration schedule.

[0078] In particular the present disclosure is directed to such use wherein the method is effective.

[0079] In particular the present disclosure is directed to such use wherein the method provides a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 60%, when measured against placebo in a subject population of at least 5,000 healthy subjects irrespective of serostatus at baseline and 14 to 16 years of age, from the first administration of the administration schedule until 18 months after the last administration of the administration schedule.

[0080] The present disclosure is also directed in part to a reconstituted dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, optionally wherein the dengue virus strains are live, attenuated, and wherein upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent

(i) dengue serotype 1 has a concentration of at least 3.3 log10 pfu/0.5 mL and optionally to 5.0 log10 pfu/0.5 mL,
(ii) dengue serotype 2 has a concentration of at least 2.7 log10 pfu/0.5 mL and optionally to 4.9 log10 pfu/0.5 mL,
(iii) dengue serotype 3 has a concentration of at least 4.0 log10 pfu/0.5 mL and optionally to 5.7 log10 pfu/0.5 mL, and
(iv) dengue serotype 4 has a concentration of at least 4.5 log10 pfu/0.5 mL and optionally to 6.2 log10 pfu/0.5 mL.

[0081] The present disclosure is therefore directed in part to a dengue vaccine composition for use in a method of preventing virologically confirmable dengue disease (VCD) in a subject comprising consecutively administering at least a first and a second unit dose of the dengue vaccine composition to the subject, wherein said first and second unit dose are administered subcutaneously within 3 months and at least 4 weeks apart, optionally at about day 1 and at about day 90, and wherein the dengue vaccine composition is a tetravalent dengue virus composition including four live, attenuated dengue virus strains representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4, wherein the attenuated dengue virus strains comprise chimeric dengue viruses and preferably at least one non-chimeric dengue virus, and wherein the dengue serotype 1 and the dengue serotype 2 are present each in a concentration based on the total concentration in pfu/0.5 mL which is within 5%-points of each other and/or are together less than about 10% of the total concentration in pfu/0.5 mL, in particular wherein the dengue serotype 3 is at least about 10% of the total concentration in pfu/0.5 mL and in particular wherein the dengue serotype 4 is at least about 70% of the total concentration in pfu/0.5 mL.

[0082] The present disclosure is therefore directed in part to a unit dose of a dengue vaccine composition and use thereof, the unit dose comprising:

a tetravalent dengue virus composition including four live attenuated dengue serotypes (e.g. virus strains):

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain),

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain),

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain),

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain),

and one or more pharmaceutically acceptable excipients thereof.

[0083] The present disclosure is further directed in part to a unit dose of a dengue vaccine composition and use thereof, the unit dose comprising:

a tetravalent virus composition including four live attenuated dengue virus strains:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least 3.3 log10 pfu/0.5 ml,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least 4.0 log10 pfu/0.5 ml, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least 4.5 log10 pfu/0.5 ml,

and one or more pharmaceutically acceptable excipients.

[0084] The present disclosure is further directed in part to a unit dose of a dengue vaccine composition and use thereof, the unit dose comprising:

a tetravalent virus composition including four live attenuated dengue virus strains, wherein the unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least 3.3 log10 pfu/0.5 ml,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least 4.0 log10 pfu/0.5 ml, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least 4.5 log10 pfu/0.5 ml.

[0085] The present disclosure is further directed in part to a unit dose of a dengue vaccine composition and use thereof, wherein said unit dose is lyophilized and obtained by lyophilizing 0.5 mL of a solution comprising:

a tetravalent virus composition including four live attenuated dengue virus strains:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of at least 3.3 log10 pfu/0.5 ml,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least 4.0 log10 pfu/0.5 ml, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least 4.5 log10 pfu/0.5 ml,

and one or more pharmaceutically acceptable excipients.

[0086] The present disclosure is in particular directed to such unit dose or composition wherein upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (ii) in pfu/0.5 mL is less than 10%, and the concentration of (iv) in pfu/0.5 mL is at least 50%, and the concentration of (i) in pfu/0.5 mL is at least 1%, and the concentration of (iii) in pfu/0.5 mL is at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%, in particular wherein the concentration of (iii) in pfu/0.5 mL is at least 10%.

[0087] The present disclosure is in particular directed to such unit dose or composition, wherein upon reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said total concentration the concentration of (ii) in pfu/0.5 mL is less than 2%, the concentration of (iv) in pfu/0.5 mL is at least 50%, the concentration of (i) in pfu/0.5 mL is at least 1%, and the concentration of (iii) in pfu/0.5 mL is at least 6%.

[0088] The present disclosure is further directed in part to a kit for preparing a reconstituted unit dose and use thereof, the kit comprising the following components:
a) a lyophilized unit dose of the present disclosure as described herein, and
b) a pharmaceutically acceptable diluent for reconstitution.

[0089] The present disclosure is further directed in part to a container, such as a vial, comprising one to ten unit doses of the present disclosure as described herein.

[0090] The present disclosure is further directed to a method of preventing dengue disease in a subject comprising administering to the subject a reconstituted unit dose of a dengue vaccine composition as described herein, for example by subcutaneous injection.

[0091] The present disclosure is further directed to the use of the reconstituted unit dose of a dengue vaccine composition as described herein for the manufacture of a medicament for preventing dengue disease in a subject, for example by subcutaneous injection.

[0092] The present disclosure is further directed to the reconstituted unit dose of a dengue vaccine composition as described herein for use in a method of preventing dengue disease in a subject , for example by subcutaneous injection.

[0093] The present disclosure is further directed to a method of preventing dengue disease in a subject population, comprising administering to the subject population a reconstituted unit dose of a vaccine composition as described herein, wherein the subject population is seronegative to all dengue serotypes. In said method the geometric mean neutralizing antibody titers (GMTs) when tested in at least 40, or at least 50, or at least 60 subjects at day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, may provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 4 (GMT DENV-2 : GMT DENV-4), and optionally a ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 1 (GMT DENV-2 : GMT DENV-1), and optionally a ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 3 (GMT DENV-2 : GMT DENV-3).

[0094] The present disclosure is further directed to a method of preventing dengue disease in a subject, comprising administering to the subject a reconstituted unit dose of a vaccine composition as described herein, wherein the subject is seronegative to all dengue serotypes. In said method the neutralizing antibody titers when tested in the subject at

day 180 or day 365 after at least a first administration of said unit dose, and optionally a second administration of said unit dose 90 days after said first administration, may provide a ratio of not more than 50, or not more than 40, or nor more than 30, or not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4, and optionally a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 1, and optionally a ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 3.

[0095] In one preferred example, the methods of preventing dengue disease of the present disclosure are not associated with an increased likelihood of solicited systemic adverse events, such as in children under 9 or seronegative individuals.

[0096] The present disclosure is therefore directed in part to a method of preventing dengue disease as well as yellow fever.

[0097] The present disclosure is therefore directed in part to a method of preventing dengue disease as well as hepatitis A.

[0098] The present disclosure is therefore directed in part to a method of preventing dengue disease as well as HPV-associated cancers or genital warts.

[0099] The present disclosure is therefore directed in part to a method of preventing dengue disease as well as measles, mumps and rubella.

[00100] The present disclosure is therefore directed in part to a method of preventing dengue disease as well as tetanus, diphtheria, and pertussis.

[00101] The present disclosure is therefore directed in part to a method of preventing dengue disease as well as diphtheria, tetanus, pertussis, poliomyelitis and diseases caused by *Haemophilus influenzae* type b.

[00102] The present disclosure is therefore also directed in part to a method of prevention any combination of the above mentioned diseases.

[00103] The present disclosure is further directed in part to the use of the reconstituted unit dose of a dengue vaccine composition/ tetravalent dengue virus composition as described herein for the manufacture of a medicament for preventing dengue disease in a subject, for example by subcutaneous injection.

[00104] The present disclosure is further directed in part to the reconstituted unit dose of a dengue vaccine composition/tetravalent dengue virus composition as described herein for use in a method of preventing dengue disease in a subject, as described herein.

DEFINITIONS

[00105] In describing the present invention, the following terms are to be used as indicated below. As used herein, the singular forms "a," "an," and "the" include plural references unless the context clearly indicates otherwise.

[00106] As used herein, the terms "unit dose of a dengue vaccine composition", "unit dose" and "unit dose of the invention as described herein" refer to the amount of a dengue vaccine which is administered to a subject in a single dose. In one embodiment, one unit dose is present in a vial and this unit dose is administered to a subject, e.g.

CLAIMS

1. A unit dose of a dengue vaccine composition comprising:

a tetravalent virus composition including four live attenuated dengue virus serotypes wherein the unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent comprises:

(i) a chimeric dengue serotype 2/1 strain, in a concentration of at least 3.3 log10 pfu/0.5 ml,

(ii) a dengue serotype 2 strain, in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a chimeric dengue serotype 2/3 strain, in a concentration of at least 4.0 log10 pfu/0.5 ml, and

(IV) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain, in a concentration of at least 4.5 log10 pfu/0.5 ml, wherein

upon said reconstitution with a pharmaceutically acceptable diluent (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 ml and based on said total concentration of pfu/0.5 ml

the concentration of (i) in pfu/0.5 ml is at least 1%,

the concentration of (ii) in pfu/0.5 ml is less than 10%,

the concentration of (iii) in pfu/0.5 ml is at least 10%, and

the concentration of (iv) in pfu/0.5 ml is at least 50%,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and differs in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

2. The unit dose according to claim 1, comprising dengue serotype 2 strain in a concentration of 3.8 to 4.9 log10 pfu/dose, and chimeric dengue serotype 2/1 strain in a concentration of 3.3 to 3.6 log10 pfu/dose.

3. The unit dose of claim 1 or 2, wherein the unit dose is lyophilized and comprises a non-reducing sugar, a surfactant, a protein and an inorganic salt, preferably wherein the non-reducing sugar is trehalose, the surfactant is poloxamer 407, the protein is human serum albumin and the inorganic salt is sodium chloride.

4. The unit dose of claim 3, wherein when measured in 0.5 mL comprises:

from 10 % (w/v) to 20 % (w/v), preferably about 15% (w/v) α,α-trehalose dihydrate or an equimolar amount of other forms of α,α-trehalose,

from 0.5 % (w/v) to 1.5 % (w/v) preferably about 1% (w/v), poloxamer 407,

from 0.05 % (w/v) to 2 % (w/v) preferably about 0.1% (w/v), human serum albumin, and

from 70 mM to 140 mM, preferably about 100 mM sodium chloride.

5. The unit dose of any one of claims 1 to 4, wherein the unit dose is lyophilized and comprises a buffer including sodium chloride (NaCl), disodium hydrogen phosphate (Na2HPO4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH2PO4), preferably at a pH in the range of 7.0 to 8.5.

6. The unit dose of any one of claims 1 to 5, wherein each one of the live, attenuated dengue virus strains is characterized by one or more of the additional mutations selected from the list comprising:
a) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymine resulting in an amino acid at position 1308 from leucine to phenylalanine,
b) a silent mutation in the NS3 gene at nucleotide 5547 from thymine to cytosine, and
c) a mutation in the NS4A gene at nucleotide 6599 from guanine to cytosine resulting in an amino acid change at position 2168 from glycine to alanine,

and/or

the chimeric dengue serotype 2/1 strain such as TDV-1, wherein the prM and E from serotype 2 strain are replaced with the corresponding structural proteins from a wild type such as DENV-1 16007, is characterized by one or more of the additional mutations selected from the list comprising:
a mutation in the NS2A gene at nucleotide 3823 from adenine to cytosine resulting in an amino acid change at position 1243 from isoleucine to leucine,
a mutation in the NS2B gene at nucleotide 4407 from adenine to thymine resulting in an amino acid change at position 1437 from glutamine to asparagine, and
a silent mutation in the NS4B gene at nucleotide 7311 from adenine to guanine,

and/or

the dengue serotype 2 strain such as TDV-2, is characterized by one or more of the further mutations selected from the list comprising:
a mutation in the prM gene at nucleotide 592 from adenine to guanine resulting in an amino acid change at position 166 from lysine to glutamine, and
a mutation in the NS5 gene at nucleotide 8803 from adenine to guanine resulting in an amino acid change at position 2903 from isoleucine to valine,

and/or

the chimeric dengue serotype 2/3 strain such as TDV-3, wherein the prM and E from serotype 2 strain are replaced with the corresponding structural proteins from a wild type such as DENV-3 16562, is characterized by one or more of the mutations selected from the list comprising:
a mutation in the E gene at nucleotide 1603 from adenine to thymine resulting in an amino acid change at position 503 from threonine to serine, and
a silent mutation in the NS5 gene at nucleotide 7620 from adenine to guanine,

and/or

the chimeric dengue serotype 2/4 strain such as TDV-4, wherein the prM and E from serotype 2 strain are replaced with the corresponding structural proteins from a wild type such as DENV-4 1036, is characterized by one or more of the mutations selected from the list comprising:
a silent mutation in the C gene at nucleotide 225 from adenine to thymine,
a mutation in the NS2A gene at nucleotide 3674 from adenine to guanine resulting in an amino acid change at position 1193 from asparagine to glycine,
a mutation in the NS2A gene at nucleotide 3773 from adenine to an adenine/guanine mix resulting in an amino acid change at position 1226 from lysine to a lysine/asparagine mix,
a silent mutation in the NS3 gene at nucleotide 5391 from cytosine to thymine,
a mutation in the NS4A gene at nucleotide 6437 from cytosine to thymine resulting in an amino acid change at position 2114 from alanine to valine,
a silent mutation in the NS4B gene at nucleotide 7026 from thymine to a thymine/cytosine mix, and
a silent mutation in the NS5 gene at nucleotide 9750 from adenine to cytosine.

7. The unit dose of any one of claims 1 to 6, wherein
dengue serotype 1 is characterized by a nucleotide sequence of SEQ ID NO. 1, and/or
dengue serotype 2 is characterized by a nucleotide sequence of SEQ ID NO. 3, and/or
dengue serotype 3 is characterized by a nucleotide sequence of SEQ ID NO. 5, and/or
dengue serotype 4 is characterized by a nucleotide sequence of SEQ ID NO. 7,

and/or,

dengue serotype 1 is characterized by amino acid sequence of SEQ ID NO. 2, and/or
dengue serotype 2 is characterized by amino acid sequence of SEQ ID NO. 4, and/or
dengue serotype 3 is characterized by amino acid sequence of SEQ ID NO. 6, and/or
dengue serotype 4 is characterized by amino acid sequence of SEQ ID NO. 8, and/or

dengue serotype 1 is TDV-1, and/or
dengue serotype 2 is TDV-2, and/or
dengue serotype 3 is TDV-3, and/or
dengue serotype 4 is TDV-4.

8. A vaccine combination comprising a unit dose of a dengue vaccine composition according to any one of claims 1 to 7, and one or more other vaccine(s).

9. The vaccine combination of claim 8, wherein the other vaccine is selected from a group consisting of yellow fever vaccine such as YF-17D, a hepatitis A vaccine such as HAVRIX® or VAQTA®, an HPV vaccine preferably a 9vHPV vaccine, such as GARDASIL® 9, an MMR vaccine for such as M-M-R® II, a tetanus, diphtheria, and pertussis (Tdap) vaccine, preferably wherein the Tdap vaccine is a combined tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (adsorbed) vaccine, such as BOOSTRIX®, a DTaP/IPV/Hib vaccine such as Pentacel®, another dengue vaccine such as a tetravalent dengue vaccine based on a yellow fever backbone such as Dengvaxia ®, cholera vaccine, hepatitis E vaccine, Japanese encephalitis vaccine, meningococcal disease vaccine, rabies vaccine, tick-borne encephalitis vaccine, typhoid fever vaccine, or a combination thereof.

10. A kit for preparing a reconstituted unit dose comprising the following components:
a) one, two or more unit dose(s) of any one of claims 1 to 7, and preferably,
b) a pharmaceutically acceptable diluent for reconstitution, preferably wherein the pharmaceutically acceptable diluent for reconstitution is an aqueous sodium chloride solution including 37 mM sodium chloride.

11. A container, such as a vial, comprising one, two or up to ten unit doses of any one of claims 1 to 7.

12. The kit of claim 10, comprising one, two or more dose(s) of one or more additional vaccine(s).

13. The kit of claim 12, wherein the additional vaccine is selected from a group consisting of yellow fever vaccine such as such as YF-17D, a hepatitis A vaccine such as HAVRIX® or VAQTA®, an HPV vaccine preferably a 9vHPV vaccine, such as GARDASIL® 9, an MMR vaccine for such as M-M-R® II, a tetanus, diphtheria, and pertussis (Tdap) vaccine, preferably wherein the Tdap vaccine is a combined tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (adsorbed) vaccine, such as BOOSTRIX®, a DTaP/IPV/Hib vaccine such as Pentacel®, another dengue vaccine such as a tetravalent dengue vaccine based on a yellow fever backbone such as Dengvaxia ®, a cholera vaccine, a hepatitis E vaccine, a Japanese encephalitis vaccine, a meningococcal disease vaccine, a rabies vaccine, a tick-borne encephalitis vaccine, a typhoid fever vaccine, or a combination thereof.

14. Use of the unit dose according to any one of claims 1 to 7, in the manufacture of a medicament for preventing dengue disease in a subject.

15. The use according to claim 14, wherein the subject is seropositive or seronegative at baseline, or the serostatus of the subject at baseline is unknown.

16. The use according to claim 14 or 15, for preventing virologically confirmable dengue disease (VCD), for preventing dengue haemorrhagic fever (DHF) and/or for preventing dengue shock syndrome (DSS).

17. The use according to any one of claims 14 to 16, wherein the use comprises a primary vaccination consisting of the step of:
(A) selecting a subject to be administered the unit doses of dengue virus composition who is in need for protection against dengue infection without determination whether there was a previous dengue infection at any time before, during or after the unit doses of dengue virus composition to be administered or wherein the serostatus of the subject is unknown at any time before, during or after the unit doses of dengue virus composition to be administered comprising that one unit dose is to be administered to the subject.

18. The use according to claim 17, providing at least 80% tetravalent seropositivity preferably in seronegative subjects after unit dose to be administered, such as at day 30.

19. The use according to claim 17 or 18, providing, after the one unit dose to be administered, preferably at least until three months after the one unit dose to be administered, irrespective of the serostatus of the subjects at baseline, a combined vaccine efficacy against all four serotypes in preventing virologically confirmable dengue disease and/or hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 60% when measured against placebo.

20. The use according to any one of claims 17 to 19, wherein one or more booster vaccinations are to be administered after the primary vaccination comprising a second unit dose to be administered after the unit dose of the primary vaccination, preferably at least one year, 5 years or 10 years after the unit dose of the primary vaccination.

21. The use according to any one of claims 14 to 20, wherein the use comprises a primary vaccination consisting of the step of:

(A) selecting a subject for the tetravalent dengue virus composition to be administered who is in need for protection against dengue infection without determination whether there was a previous dengue infection at any time before, during or after the tetravalent dengue virus composition to be administered or wherein the serostatus of the subject is unknown at any time before, during or after the the unit dose to be administered, comprising

(B) a first unit dose of the tetravalent dengue virus composition to be administered to the subject, and

(C) a second unit dose of the tetravalent dengue virus composition to be administered to the subject within 3 months of the first unit dose to be administered.

22. The use according to claim 21, providing at least 80%, or at least 85%, or at least 90% or at least 95% tetravalent seropositivity after the second unit dose to be administered such as at day 120,

and/or

providing, at least 80%, or at least 85%, or at least 90% tetravalent seropositivity after the second unit dose to be administered such as at day 270.

23. The use according to claim 21 or 22 which is safe.

24. The use according to claim 23, providing a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 65%, when measured against placebo in a subject population of at least 5,000 healthy 4 to 16 year old subjects irrespective of serostatus at baseline from the first unit dose to be administered until 12 to 18 months after the second unit dose to be administered.

25. The use according to claim 21 or 22 which is effective.

26. The use according to claim 25, providing a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 60%, when measured against placebo in a subject population of at least 5,000 healthy subjects irrespective of serostatus at baseline and 4 to 16 years of age, from the first unit dose to be administered until 12 to18 months after the unit dose to be administered.

27. The use according to claim 26, providing a vaccine efficacy against serotype 1 in preventing virologically confirmable dengue disease irrespective of the serostatus of the subjects at baseline and 4 to 16 years of age with a 2-sided 95% confidence interval, wherein the lower bound is more than 45% or more than 50% when measured against placebo from the first unit dose to be administered until 12 to 18 months after the second unit dose to be administered.

28. The use according to claim 26, providing a vaccine efficacy against serotype 2 in preventing virologically confirmable dengue disease irrespective of the serostatus of the subjects at baseline and 4 to 16 years of age with a 2-sided 95% confidence interval, wherein the lower bound is more than 60%, or more than 70%, or more than 80%, or more than 85% when measured against placebo from the first unit dose to be administereduntil 12 to 18 months after the second unit dose to be administered.

29. The use according to claim 23 or 25, providing a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease and/or hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 45 %, when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects seronegative against all serotypes at baseline and 4 to 16 years of age, from 30 days after the second unit dose to be administered until 12 to 18 months after the second unit dose to be administered.

30. The use according to claim 29, providing a vaccine efficacy against serotype 1 in preventing virologically confirmable dengue disease and/or hospitalization, in seronegative subjects against all serotypes at baseline, with a 2-sided 95% confidence interval, wherein the lower bound is more than 35 % when measured against placebo from the first unit dose to be administered until 12 or 18 months after the second unit dose to be administered.

31. The use according to claim 29, providing a vaccine efficacy against serotype 2 in preventing virologically confirmable dengue disease, in seronegative subjects against all serotypes at baseline, with a 2-sided 95% confidence interval, wherein the lower bound is more than 60% when measured against placebo from the first unit dose to be administered until 12 or 18 months after the second unit dose to be administered.

32. The use according to any one of claims 14 to 31, wherein the unit dose is to be administered by subcutaneous injection, preferably to the deltoid region of the arm.

33. The use according to any one of claims 14 to 32, wherein the subject is from
a dengue endemic region or a dengue non-endemic region, or
a region wherein the seroprevalence rate is unknown, and/or
wherein the seroprevalence is below 80%, or below 70%, or below 60%.

34. The use according to any one of claims 14 to 33, wherein the subject is exposed to dengue serotype 1, dengue serotype 2, and/or a dengue outbreak, preferably wherein the outbreak is due to a dengue serotype 2, and/or due to a dengue serotype 1, and/or wherein the virologically confirmable dengue disease is due to a dengue serotype 2, and/or a dengue serotype 1.

35. The use according to any one of claims 14 to 34, wherein the subject is under 9 years of age, 4 to 5 years of age, 6 to 11 years of age or 12 to 16 years, or 6 to 16 years of age, or 4 to 16 years of age, or 2 to 17 years of age, or 9 years of age, or over 9 years of age, or 9 to 19 years of age, or 18 to 60 years of age, 18 to 45 years of age, or 46 to 60 years of age, or over 60 years of age.

36. The use according to claim 35, wherein the subject belongs to the elderly population preferably more than 60 years of age, or paediatric population preferably less than 2 years of age, preferably of 2 months to 2 years or 2 months to 1.5 years or 2 months to 1 year.

37. The use according to any one of claims 17 to 36, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially with another vaccine.

38. The use according to claim 37, wherein the other vaccine is to be administered by subcutaneous injection or intramuscular injection, preferably to be administered to the arm such as to the deltoid region of the arm or thigh such as to the anterolateral aspect of the thigh.

39. The use according to claim 37 or 38, wherein the unit dose and the other vaccine are to be administered to different anatomical sites, such as to opposite arms or to arm and thigh.

40. The use according to any one of claims 38 to 39, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with a yellow fever vaccine for preventing yellow fever such as YF-17D, a hepatitis A vaccine for preventing hepatitis A such as HAVRIX® or VAQTA®, an HPV vaccine for preventing HPV-associated cancers or genital warts, preferably wherein the HPV vaccine is a 9vHPV vaccine, such as GARDASIL® 9, an MMR vaccine for preventing measles, mumps and rubella such as M-M-R® II, a tetanus, diphtheria, and pertussis (Tdap) vaccine for preventing tetanus, diphtheria, and pertussis, preferably wherein the Tdap vaccine is a combined tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (adsorbed) vaccine, such as BOOSTRIX®, a DTaP/IPV/Hib vaccine for preventing diphtheria, tetanus, pertussis, poliomyelitis and diseases caused by *Haemophilus influenzae* type b such as Pentacel®, or wherein the unit dose is to be co-administered with cholera vaccine, hepatitis E vaccine, Japanese encephalitis vaccine, meningococcal disease vaccine, rabies vaccine, thick-borne encephalitis vaccine, typhoid fever vaccine, or a combination thereof.

41. The use according to claim 40, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with a yellow fever vaccine for preventing yellow fever such as YF-17D, wherein

- a first dose of the yellow fever vaccine is to be administered on day 0,
- the first unit dose is to be administered after said first dose of the yellow fever vaccine to be administered, such as 3 months later and preferably on day 90, and
- the second unit dose is to be administered after said first unit dose to be administered, such as 3 months later and preferably on day 180,

or

- the first unit dose is to be administered on day 0,
- the second unit dose is to be administered after said first unit dose to be administered, such as 3 months later and preferably on day 90, and
- a first dose of the yellow fever vaccine is to be administered after said second unit dose to be administered, such as 3 months later and preferably on day 180,

or

- the first unit dose and a first dose of the yellow fever vaccine are to be simultaneously administered on day 0,
- the second unit dose is to be administered after said first unit dose to be simultaneously administered and the first dose of the yellow fever vaccine, such as 3 months later and preferably on day 90.

42. The use according to claim 40, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with a hepatitis A vaccine for preventing hepatitis A such as HAVRIX® or VAQTA®, wherein

- the first unit dose and a first dose of the hepatitis A vaccine are to be simultaneously administered on day 0,
- the second unit dose is to be administered after said first unit dose to be simultaneously administered and the first dose of the hepatitis A vaccine, such as 3 months later and preferably on day 90.

43. The use according to claim 40, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with an HPV vaccine for preventing HPV-associated cancers or genital warts, preferably wherein the HPV vaccine is a 9vHPV vaccine, such as GARDASIL® 9 , wherein

- the first unit dose and a first dose of HPV vaccine are to be simultaneously administered on day 0,
- the second unit dose is to be administered after said first unit dose to be simultaneously administered and the first dose of the HPV vaccine such as 3 months later and preferably on day 90,
- a third dose of the HPV vaccine is to be administered after said second unit dose to be simultaneously administered, such as 3 months later and preferably on day 180.

44. The use according to claim 40, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with an MMR vaccine for preventing measles, mumps and rubella such as M-M-R® II , wherein

- the first unit dose and a first dose of the MMR vaccine are to be simultaneously administered on day 0,
- the second unit dose is administered after said first unit dose to be simultaneously administered and the first dose of the MMR vaccine such as 3 months later and preferably on day 90,
- a second dose of the MMR vaccine is to be after said second unit dose to be administered, such as 3 to 6 years after said first unit dose to be simultaneously administered.

45. The use according to claim 40, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with a tetanus, diphtheria, and pertussis (Tdap) vaccine for preventing tetanus, diphtheria, and pertussis, preferably wherein the Tdap vaccine is a combined tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (adsorbed) vaccine, such as BOOSTRIX®, wherein

- the first unit dose and a first dose of the Tdap vaccine are to be simultaneously administered on day 0, and
- the second unit dose is to be administered after said first unit dose to be simultaneously administered and the first dose of the Tdap vaccine, such as 3 months later and preferably on day 90.

46. The use according to claim 40, wherein the unit dose is to be administered concomitantly, simultaneously, or sequentially, with a DTaP/IPV/Hib vaccine for preventing diphtheria, tetanus, pertussis, poliomyelitis and diseases caused by *Haemophilus influenzae* type b such as Pentacel®, wherein

- the first unit dose and a first dose of the DTaP/IPV/Hib vaccine are to be simultaneously administered on day 0,
- a second dose of the DTaP/IPV/Hib vaccine is to be administered after said first unit dose to be simultaneously administered and the first dose of the DTaP/IPV/Hib vaccine such as 2 months later and preferably on day 60,
- the second unit dose is to be administered after said second dose of the DTaP/IPV/Hib vaccine such as 3 months after the first unit dose to be simultaneously administered and the first dose of the DTaP/IPV/Hib vaccine, and preferably on day 90,

- a third dose of the DTaP/IPV/Hib vaccine is to be administered after said second unit dose to be administered such as 4 months after the first unit dose to be simultaneously administered and a first dose of the DTaP/IPV/Hib vaccine, and preferably on day 120,
- a fourth dose of the DTaP/IPV/Hib vaccine is to be administered after said third dose of the DTaP/IPV/Hib vaccine to be administered such as 9 to 12 months later, and preferably on day 390,

or

- a first dose of the DTaP/IPV/Hib vaccine is to be administered on day 0,
- a second dose of the DTaP/IPV/Hib vaccine is to be administered after said first dose of the DTaP/IPV/Hib vaccine to be administered such as 2 months later and preferably on day 60,
- a third dose of the DTaP/IPV/Hib vaccine is to be administered after said second dose of the DTaP/IPV/Hib vaccine to be administered such as 4 months later and preferably on day 120,
- the first unit dose and a fourth dose of the DTaP/IPV/Hib vaccine are to be simultaneously administered after said third dose of the DTaP/IPV/Hib vaccine to be administered such as 9 to 12 months later and preferably on day 390,
- the second unit dose is to be administered after said first unit dose to be simultaneously administered and the fourth dose of the DTaP/IPV/Hib vaccine, such as 3 months later and preferably on day 480.

47. The use according to any one of claims 14 to 36, wherein the use comprises co-vaccination with another dengue vaccine, preferably, wherein the other dengue vaccine is a tetravalent dengue vaccine based on a yellow fever backbone such as Dengvaxia ® preferably, to be administered within 4.5 years after the first dose of the unit dose to be administered.

48. A unit dose of a dengue vaccine composition which is characterized as safe when to be administered to a subject without the need to determine the occurrence of a previous dengue infection.

49. The unit dose according to claim 48, comprising a tetravalent virus composition including four live, attenuated dengue virus serotypes comprising,

(i) a dengue serotype 1, such as a chimeric dengue serotype 2/1 strain,
(ii) a dengue serotype 2, such as a dengue serotype 2 strain,
(iii) a dengue serotype 3, such as a chimeric dengue serotype 2/3 strain, and
(iv) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and is characterized by differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)
b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)
c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

50. The unit dose according to claim 48 or 49, comprising a tetravalent dengue virus composition comprising

a live, attenuated non-chimeric dengue virus strain representing serotype 2 such as TDV-2 characterized by SEQ ID NO: 1 and/or 2, and three live, attenuated chimeric dengue virus strains represented by
DEN 2/1 chimera such as TDV-1 characterized by SEQ ID NO: 3 and/or 4,
DEN 2/3 chimera such as TDV-3 characterized by SEQ ID NO: 5 and/or 6, and
DEN 2/4 chimera such as TDV-4 characterized by SEQ ID NO: 7 and/or 8.

51. Use of a unit dose of a dengue vaccine composition according to any one of claims 1 to 7 in the manufacture of a medicament for preventing dengue disease in a subject wherein the use is safe for the subject without the need to determine the occurrence of a previous dengue infection.

52. The unit dose or uses thereof according to claim 51, wherein the unit dose comprises a tetravalent virus composition including four live, attenuated dengue virus serotypes comprising,

(i) a dengue serotype 1, such as a chimeric dengue serotype 2/1 strain,
(ii) a dengue serotype 2, such as a dengue serotype 2 strain,
(iii) a dengue serotype 3, such as a chimeric dengue serotype 2/3 strain, and
(iv) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and is characterized by differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)
b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)
c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

53. The unit dose or uses thereof according to claim 51 or 52, wherein the unit dose comprises a tetravalent dengue virus composition comprising
a live, attenuated non-chimeric dengue virus strain representing serotype 2 such as TDV-2 characterized by SEQ ID NO: 1 and/or 2, and three live, attenuated chimeric dengue virus strains represented by
DEN 2/1 chimera such as TDV-1 characterized by SEQ ID NO: 3 and/or 4,
DEN 2/3 chimera such as TDV-3 characterized by SEQ ID NO: 5 and/or 6, and
DEN 2/4 chimera such as TDV-4 characterized by SEQ ID NO: 7 and/or 8.

54. Use of a unit dose of a dengue vaccine composition according to any one of claims 1 to 7 in the manufacture of a medicament for preventing dengue disease in a subject, wherein the use is safe for the subject irrespective of the serostatus at baseline with respect to dengue virus.

55. The use according to claim 54, wherein the unit dose comprises a tetravalent virus composition including four live, attenuated dengue virus serotypes comprising,

(i) a dengue serotype 1, such as a chimeric dengue serotype 2/1 strain,
(ii) a dengue serotype 2, such as a dengue serotype 2 strain,
(iii) a dengue serotype 3, such as a chimeric dengue serotype 2/3 strain, and

(iv) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and is characterized by differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

56. The use according to claim 54 or 55, wherein the unit dose comprises a tetravalent dengue virus composition comprising

a live, attenuated non-chimeric dengue virus strain representing serotype 2 such as TDV-2 characterized by SEQ ID NO: 1 and/or 2, and three live, attenuated chimeric dengue virus strains represented by

DEN 2/1 chimera such as TDV-1 characterized by SEQ ID NO: 3 and/or 4,

DEN 2/3 chimera such as TDV-3 characterized by SEQ ID NO: 5 and/or 6, and

DEN 2/4 chimera such as TDV-4 characterized by SEQ ID NO: 7 and/or 8.

57. Use of a unit dose of a dengue vaccine composition according to any one of claims 1 to 7 in the manufacture of a medicament for preventing dengue disease in a subject, comprising at least one dose of said unit dose to be administered to the subject that results in the prevention of dengue disease.

58. The use according to claim 57, wherein the unit dose comprises a tetravalent virus composition including four live, attenuated dengue virus serotypes comprising,

(i) a dengue serotype 1, such as a chimeric dengue serotype 2/1 strain,

(ii) a dengue serotype 2, such as a dengue serotype 2 strain,

(iii) a dengue serotype 3, such as a chimeric dengue serotype 2/3 strain, and

(iv) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and is characterized by differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

59. The use according to claim 57 or 58, wherein the unit dose comprises a tetravalent dengue virus composition comprising

a live, attenuated non-chimeric dengue virus strain representing serotype 2 such as TDV-2 characterized by SEQ ID NO: 1 and/or 2, and three live, attenuated chimeric dengue virus strains represented by

DEN 2/1 chimera such as TDV-1 characterized by SEQ ID NO: 3 and/or 4,

DEN 2/3 chimera such as TDV-3 characterized by SEQ ID NO: 5 and/or 6, and

DEN 2/4 chimera such as TDV-4 characterized by SEQ ID NO: 7 and/or 8.

60. Use of a unit dose of a dengue vaccine composition according to any one of claims 1 to 7 in the manufacture of a medicament for preventing dengue disease in a subject and providing an efficacy against dengue serotype 2 of at least 60%.

61. The use according to claim 60, wherein the unit dose comprises a tetravalent virus composition including four live, attenuated dengue virus serotypes comprising,

(i) a dengue serotype 1, such as a chimeric dengue serotype 2/1 strain,

(ii) a dengue serotype 2, such as a dengue serotype 2 strain,

(iii) a dengue serotype 3, such as a chimeric dengue serotype 2/3 strain, and

(iv) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and is characterized by differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

62. The use according to claim 60 or 61, wherein the unit dose comprises a tetravalent dengue virus composition comprising

a live, attenuated non-chimeric dengue virus strain representing serotype 2 such as TDV-2 characterized by SEQ ID NO: 1 and/or 2, and three live, attenuated chimeric dengue virus strains represented by

DEN 2/1 chimera such as TDV-1 characterized by SEQ ID NO: 3 and/or 4,

DEN 2/3 chimera such as TDV-3 characterized by SEQ ID NO: 5 and/or 6, and

DEN 2/4 chimera such as TDV-4 characterized by SEQ ID NO: 7 and/or 8.

63. Use of a unit dose of a dengue vaccine composition according to any one of claims 1 to 7 in the manufacture of a medicament for preventing dengue disease in a subject comprising said unit dose to be administered to the subject, wherein the use does not require a step of determination whether there was a previous dengue infection in the subject at any time before, during or after said unit dose to be administered, wherein determination of the previous dengue infection in the subject is characterized by a laboratory confirmed history of dengue disease or a validated serological test.

64. The use according to claim 63, wherein the unit dose comprises a tetravalent virus composition including four live, attenuated dengue virus serotypes comprising,

(i) a dengue serotype 1, such as a chimeric dengue serotype 2/1 strain,

(ii) a dengue serotype 2, such as a dengue serotype 2 strain,

(iii) a dengue serotype 3, such as a chimeric dengue serotype 2/3 strain, and

(iv) a dengue serotype 4, such as a chimeric dengue serotype 2/4 strain,

wherein the dengue serotype 2 strain is derived from the wild type virus strain DEN-2 16681 and is characterized by differing in at least three nucleotides from the wild type as follows:

a) 5'-noncoding region (NCR)-57 (nt-57 C-to-T)

b) NS1-53 Gly-to-Asp (nt-2579 G-to-A)

c) NS3-250 Glu-to-Val (nt-5270 A-to-T); and

wherein the three chimeric dengue strains are derived from the serotype 2 strain by replacing the structural proteins prM and E from serotype 2 strain with the corresponding structural proteins from the other dengue serotypes, resulting in the following chimeric dengue strains:

- a DENV-2/1 chimera,
- a DENV-2/3 chimera and
- a DENV-2/4 chimera.

65. The use according to claim 63 or 64, wherein the unit dose comprises a tetravalent dengue virus composition comprising

a live, attenuated non-chimeric dengue virus strain representing serotype 2 such as TDV-2 characterized by SEQ ID NO: 1 and/or 2, and

three live, attenuated chimeric dengue virus strains represented by

DEN 2/1 chimera such as TDV-1 characterized by SEQ ID NO: 3 and/or 4,

DEN 2/3 chimera such as TDV-3 characterized by SEQ ID NO: 5 and/or 6, and

DEN 2/4 chimera such as TDV-4 characterized by SEQ ID NO: 7 and/or 8.