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).

[0057a] In one aspect, the present disclosure refers to a use of a tetravalent dengue vaccine composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4 in the manufacture of a medicament for preventing dengue disease in a subject or subject population, wherein preventing dengue disease comprises that one dose of the dengue vaccine composition is to be administered to the subject or subject population, wherein the administration of the one dose results in a reduction of risk of dengue disease after said one dose represented by a combined vaccine efficacy against all four dengue virus serotypes of at least 60%, and wherein each of the four live, attenuated dengue virus strains is selected from the following:

(i) a non-chimeric dengue virus comprising all components from the same dengue serotype, and

(ii) a chimeric dengue virus having parts from two dengue serotypes.

[0057b] In another aspect, the present disclosure refers to a use of a tetravalent dengue vaccine composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4 in the manufacture of a medicament for preventing dengue disease in a subject or subject population traveling to a dengue endemic region, wherein preventing dengue disease comprises that one dose of the dengue vaccine composition is to be administered to the subject or subject population, wherein the administration of the one dose results in a reduction of risk of dengue disease after said one dose represented by a combined vaccine efficacy against all four dengue virus serotypes of at least 60%, and

wherein each of the four live, attenuated dengue virus strains is selected from the following:

(i) a non-chimeric dengue virus comprising all components from the same dengue serotype, and

(ii) a chimeric dengue virus having parts from two dengue serotypes.

[0057c] In yet another aspect, the present disclosure refers to a use of a tetravalent dengue vaccine composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4 in the manufacture of a medicament for preventing dengue disease in a subject or subject population exposed to a dengue outbreak, wherein preventing dengue disease comprises that one dose of the dengue vaccine composition is to be administered to the subject or subject population, wherein the administration of the one dose results in a reduction of risk of dengue disease after said one dose represented by a combined vaccine efficacy against all four dengue virus serotypes of at least 60%, and wherein each of the four live, attenuated dengue virus strains is selected from the following:

(i) a non-chimeric dengue virus comprising all components from the same dengue serotype, and

(ii) a chimeric dengue virus having parts from two dengue serotypes.

[0057d] In yet another aspect, the present disclosure refers to a use of a tetravalent dengue vaccine composition including four live attenuated dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4 in the manufacture of a medicament for preventing dengue disease in a subject or subject population, wherein preventing dengue disease comprises that one dose of the dengue vaccine composition is to be administered to the subject or subject population, wherein the administration of the one dose results in a reduction of risk of dengue disease within three months after said one dose, wherein the administration of the one dose results in a reduction of risk of dengue disease after said one dose represented by a combined vaccine efficacy against all four dengue virus serotypes of at least 60%, and

wherein each of the four live, attenuated dengue virus strains is selected from the following:

(i) a non-chimeric dengue virus comprising all components from the same dengue serotype, and

(ii) a chimeric dengue virus having parts from two dengue serotypes.

[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] 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 a 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.

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

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

[00176] The genetically modified tetravalent dengue vaccine TDV is based on a molecularly characterized and cloned dengue-2 virus strain (TDV-2). This attenuated TDV-2 strain was generated by cDNA cloning of the attenuated laboratory-derived DEN-2 PDK-53 virus strain that was originally isolated at Mahidol University, Bangkok, Thailand (Kinney et al. (1997) Virology 230(2): 300-308). DEN-2 PDK-53 was generated by 53 serial passages in primary dog kidney (PDK) cells at 32°C (Bhamarapravati et al. (1987) Bull. World Health Organ. 65(2): 189-195).

[00177] The attenuated DEN-2 PDK-53 strain (the precursor of TDV-2) was derived from the wild type virus strain DEN-2 16681 (SEQ ID NO 11) and differs in nine nucleotides from the wild type as follows (Kinney et al. (1997) Virology 230(2): 300-308):

(i) 5'-noncoding region (NCR)-57 (nt-57 C-to-T): major attenuation locus
(ii) prM-29 Asp-to-Val (nt-524 A-to-T)
(iii) nt-2055 C-to-T (E gene) silent mutation
(iv) NS1-53 Gly-to-Asp (nt-2579 G-to-A): major attenuation locus
(v) NS2A-181 Leu-to-Phe (nt-4018 C-to-T)
(vi) NS3-250 Glu-to-Val (nt-5270 A-to-T): major attenuation locus
(vii) nt-5547 (NS3 gene) T-to-C silent mutation
(viii) NS4A-75 Gly-to-Ala (nt-6599 G-to-C)
* nt-8571 C-to-T (NS5 gene) silent mutation

[00178] The three nucleotide changes located in the 5' noncoding region (NCR) (nucleotide 57) (mutation (i)), the NS-1 (amino acid 828 of SEQ ID NO. 4) (mutation (iv)) and NS-3 genes (amino acid 1725 of SEQ ID NO. 4) (mutation (vi)) form the basis for the attenuation phenotype of the DEN-2 PDK-53 strain (Butrapet et al. (2000) J. Virol. 74(7): 3111-3119) (Table 2). These three mutations are referred to herein as the "attenuating mutations" and are comprised in TDV-1, TDV-2, TDV-3 and TDV-4.

Table 2. Attenuating mutations in the common genetic backbone of all TDV strains

Location of Mutation	**Nucleotide Change in TDV-2**	**Amino Acid Change in TDV-2**
5' Noncoding Region (5'NCR)	57 C to T	Not applicable (silent)
Nonstructural Protein 1 (NS1)	2579 G to A	828 Gly to Asp
Nonstructural Protein 3 (NS3)	5270 A to T	1725 Glu to Val

[00179] In one embodiment, TDV-2 comprises in addition to the three attenuating mutations one or more mutations selected from:

a) a mutation in the prM gene at nucleotide 524 from adenine to thymine resulting in an amino acid change at position 143 from aspartic acid to valine, and/or

b) a silent mutation in the E gene at nucleotide 2055 from cytosine to thymine, and/or

c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymine resulting in an amino acid change at position 1308 from leucine to phenylalanine, and/or

d) a silent mutation in the NS3 gene at nucleotide 5547 from thymine to cytosine, and/or

e) 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

f) a silent mutation in the prM gene at nucleotide 900 from thymine to cytosine.

The silent mutation in the NS5 gene at nucleotide 8571 from cytosine to thymine of DEN-2 PDK-53 is not present in the TDV-2 strain.

[00180] In another embodiment, TDV-2 comprises in addition to the three attenuating mutations one or more mutations selected from:

g) 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 glutamic acid, and/or

h) 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.

[00181] In another embodiment, TDV-2 comprises in addition to the three attenuating mutations the mutations a) and g), preferably the mutations a), g), c), e) and h), more preferably the mutations a), g), c), e), h) and b), even more preferably the mutations a), g), c), e), h), b) and d), and most preferably the mutations a) to h). The nucleotide positions and amino acids positions of TDV-2 refer to the nucleotide sequence as shown in SEQ ID NO. 3 and amino acid sequence as shown in SEQ ID NO. 4.

[00182] The dengue virus structural envelope (E) protein and pre-membrane (prM) protein have been identified as the primary antigens that elicit a neutralizing protective antibody response (Plotkin 2001). For creation of the tetravalent dengue vaccine (TDV), TDV-2 was modified by replacing the nucleic acid sequence encoding the DENV-2 prM and E glycoproteins with the nucleic acid sequence encoding the corresponding wild type prM and E glycoproteins from the DENV-1, DENV-3, and DENV-4 wild type strains DENV-1 16007, DENV-3 16562 or DENV-4 1036 virus, respectively, (see Table 3) using standard molecular genetic engineering methods (Huang et al. (2003) J. Virol. 77(21): 11436-11447).

Table 3.Viral origin of prM/E gene regions of the TDV virus strains

Virus	Strain	Origin	Source	Reference	Nucleotide sequence	Amino acid sequence
DENV-1	16007	Thailand, 1964	DHF/DSS patient	Halstead and Simasthien, 1970	SEQ ID NO. 9	SEQ ID NO. 10
DENV-2	16681	Thailand, 1964	DHF/DSS patient	Halstead and Simasthien, 1970	SEQ ID NO. 11	SEQ ID NO. 12
DENV-3	16562	Philippines, 196	DHF patient	Halstead and Simasthien, 1970	SEQ ID NO. 13	SEQ ID NO. 14
DENV-4	1036	Indonesia, 1976	DF patient	Gubler et al., 1979	SEQ ID NO. 15	SEQ ID NO. 16

[00183] A diagram of the four TDV strains comprised in the dengue vaccine composition is shown in **Figure 1**.

[00184] The chimeric dengue strains TDV-1, TDV-3 and TDV-4 express the surface antigens prM and E of the DENV-1, DENV-3 or DENV-4 viruses, as depicted in Table 3 respectively, and retain the genetic alterations responsible for the attenuation of TDV-2. Thus, each of the TDV-1, TDV-3 and TDV-4 strains comprises the attenuating mutations described in Table 2.

[00185] In one embodiment, TDV-1 comprises in addition to the three attenuating mutations one or more mutations selected from:

c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymine resulting in an amino acid change at position 1308 from leucine to phenylalanine, and/or

d) a silent mutation in the NS3 gene at nucleotide 5547 from thymine to cytosine, and/or

e) 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

i) a silent mutation in the E gene at nucleotide 1575 from thymine to cytosine, and/or

j) a silent mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotide 453 from adenine to guanine, and/or

k) a mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotides 2381/2382 from thymine-guanine to cytosine-cytosine resulting in an amino acid change at position 762 from valine to alanine.

[00186] In another embodiment, TDV-1 comprises in addition to the three attenuating mutations one or more mutations selected from:

l) 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, and/or

m) a mutation in the NS2B gene at nucleotide 4407 from adenine to thymine resulting in an amino acid change at position 1437 from glutamic acid to aspartic acid, and/or

n) a silent mutation in the NS4B gene at nucleotide 7311 from adenine to guanine.

[00187] In another embodiment, the TDV-1 strain comprises in addition to the three attenuating mutations the mutations l) and m), preferably the mutations l), m), c) and e), even more preferably the mutations l), m), c), e), d) and n), and most preferably the mutations l), m), c), e), d), n), i), j) and k). The nucleotide positions and amino acids positions of TDV-1 refer to the nucleotide sequence as shown in SEQ ID NO. 1 and amino acid sequence as shown in SEQ ID NO. 2.

[00188] In one embodiment, TDV-3 comprises in addition to the three attenuating mutations one or more mutations selected from:

c) a mutation in the NS2A gene at nucleotide 4012 from cytosine to thymine resulting in an amino acid change at position 1306 from leucine to phenylalanine, and/or

d) a silent mutation in the NS3 gene at nucleotide 5541 from thymine to cytosine, and/or

e) a mutation in the NS4A gene at nucleotide 6593 from guanine to cytosine resulting in an amino acid change at position 2166 from glycine to alanine, and/or

j) a silent mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotide 453 from adenine to guanine, and/or

k) a mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotides 2375/2376 from thymine-guanine to cytosine-cytosine resulting in an amino acid change at position 760 from valine to alanine, and/or

o) a silent mutation in the prM gene at nucleotide 552 from cytosine to thymine, and/or

p) a mutation in the E gene at nucleotide 1970 from adenine to thymine resulting in an amino acid change at position 625 from histidine to leucine.

[00189] In another embodiment, TDV-3 comprises in addition to the three attenuating mutations one or more mutations selected from:

q) 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/or

r) a silent mutation in the NS5 gene at nucleotide 7620 from adenine to guanine.

[00190] In another embodiment, TDV-3 comprises in addition to the three attenuating mutations the mutations p) and q), preferably the mutations p), q), c) and e), even more preferably the mutations p), q), c), e), d) and r), and most preferably the mutations p), q), c), e), d), r), j), k) and o). The nucleotide positions and amino acids positions of TDV-3 refer to the nucleotide sequence as shown in SEQ ID NO. 5 and amino acid sequence as shown in SEQ ID NO. 6.

[00191] In one embodiment, TDV-4 comprises in addition to the three attenuating mutations one or more mutations selected from:

c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymine resulting in an amino acid change at position 1308 from leucine to phenylalanine, and/or

d) a silent mutation in the NS3 gene at nucleotide 5547 from thymine to cytosine, and/or

e) 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

j) a silent mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotide 453 from adenine to guanine, and/or

k) a mutation in the junction site between the prM-E gene and the DEN-2 PDK-53 backbone at nucleotides 2381/2382 from thymine-guanine to cytosine-cytosine resulting in an amino acid change at position 762 from valine to alanine, and/or

s) a mutation in the C gene at nucleotide 396 from adenine to cytosine resulting in an amino acid change at position 100 from arginine to serine, and/or

t) a silent mutation in the E gene at nucleotide 1401 from adenine to guanine, and/or

u) a mutation in the E gene at nucleotide 2027 from cytosine to thymine resulting in an amino acid change at position 644 from alanine to valine, and/or

v) a mutation in the E gene at nucleotide 2275 from adenine to cytosine resulting in an amino acid change at position 727 from methionine to leucine.

[00192] In another embodiment, TDV-4 comprises in addition to the three attenuating mutations one or more mutations selected from:

w) a silent mutation in the C gene at nucleotide 225 from adenine to thymine, and/or

x) a mutation in the NS2A gene at nucleotide 3674 from adenine to guanine resulting in an amino acid change at position 1193 from aspartic acid to glycine, and/or

y) 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/arginine mix, and/or

z) a silent mutation in the NS3 gene at nucleotide 5391 from cytosine to thymine, and/or

aa) 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, and/or

bb) a silent mutation in the NS4B gene at nucleotide 7026 from thymine to a thymine/cytosine mix, and/or

cc) a silent mutation in the NS5 gene at nucleotide 9750 from adenine to cytosine.

[00193] In another embodiment, TDV-4 comprises in addition to the three attenuating mutations the mutation s), u) and v), preferably the mutations s), u), v), c), e), x), y) and aa), even more preferably the mutations s), u), v), c), e), x), y), aa) and w), even more preferably the mutations s), u), v), c), e), x), y), aa), w), d), z), bb) and cc), and

- from about 0.05 % (w/v) to about 2 % (w/v) human serum albumin, and
- from about 70 mM to about 140 mM sodium chloride.

10. The unit dose of any one of items 1 to 9, wherein the lyophilized unit dose is prepared from a solution comprising:
- about 15 % (w/v) α,α-trehalose dihydrate,
- about 1 % (w/v) poloxamer 407,
- about 0.1 % (w/v) human serum albumin, and
- and about 100 mM sodium chloride.

11. The unit dose of any one of items 1 to 10, wherein each one of the four live attenuated dengue virus strains has attenuating mutations in the 5'-noncoding region (NCR) at nucleotide 57 from cytosine to thymine, in the NS1 gene at nucleotide 2579 from guanine to adenine resulting in an amino acid change at position 828 from glycine to asparagine, and in the NS3 gene at nucleotide 5270 from adenine to thymine resulting in an amino acid change at position 1725 from glutamine to valine, preferably further comprising one or more of the 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.

12. The unit dose of item 11, wherein (i) further comprises one or more of the 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 glutamic acid to aspartic acid, and
- a silent mutation in the NS4B gene at nucleotide 7311 from adenine to guanine.

13. The unit dose of item 11 or 12, wherein (ii) further comprises one or more of the 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 glutamic acid, 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.

14. The unit dose of any one of items 11 to 13, wherein (iii) further comprises 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.

15. The unit dose of any one of items 11 to 14, wherein (iv) further comprises 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 aspartic acid 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/arginine 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.

16. The unit dose of any one of items 1 to 15, wherein

(i) has the amino acid sequence of SEQ ID NO. 2,

(ii) has the amino acid sequence of SEQ ID NO. 4,

(iii) has the amino acid sequence of SEQ ID NO. 6, and

(iv) has the amino acid sequence of SEQ ID NO. 8.

17. The unit dose of any one of items 1 to 16 reconstituted with 0.3 to 0.8 mL of liquid for reconstitution.

18. The unit dose of item 17 reconstituted with 0.5 mL of liquid for reconstitution.

19. The unit dose of item 17 or 18, wherein the liquid for reconstitution is 37 mM aqueous sodium chloride solution.

20. A kit for preparing a reconstituted unit dose comprising the following components:

a) a unit dose of any one of items 1 to 16, and

b) a pharmaceutically acceptable diluent for reconstitution.

21. The kit of item 20, wherein the pharmaceutically acceptable diluent for reconstitution is 37 mM sodium chloride.

22. Container, such as a vial, comprising one to ten unit doses of any one of items 1 to 19.

23. A method of preventing dengue disease in a subject population comprising administering to the subject population a reconstituted unit dose of any one of items 17 to 19.

24. The method of item 23, wherein the geometric mean neutralizing antibody titers (GMTs) of the subject population 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, 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.

8. The unit dose of any one of items 1 to 7, wherein the arithmetic sum of all four serotypes is in the range of 4.6 log10 pfu/0.5 mL to 6.7 log10 pfu/0.5 mL, preferably in the range of 4.6 log10 pfu/0.5 mL to 5.5 log10 pfu/0.5 mL.

9. The unit dose of any one of items 1 to 8, wherein reconstitution with a pharmaceutically acceptable diluent is made with 0.5 ml of the pharmaceutically acceptable diluent.

10. The unit dose of any one of items 1 to 9, wherein the lyophilized unit dose is prepared from a solution further comprising a non-reducing sugar, a surfactant, a protein and an inorganic salt.

11. The unit dose of item 10, 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.

12. The unit dose of item 10 or 11, wherein the solution comprises:

- from about 10 % (w/v) to about 20 % (w/v) α,α-trehalose dihydrate or an equimolar amount of other forms of α,α-trehalose,
- from about 0.5 % (w/v) to about 1.5 % (w/v) poloxamer 407,
- from about 0.05 % (w/v) to about 2 % (w/v) human serum albumin, and
- from about 70 mM to about 140 mM sodium chloride.

13. The unit dose of any one of items 1 to 12, wherein the lyophilized unit dose is prepared from a solution comprising:

- about 15 % (w/v) α,α-trehalose dihydrate,
- about 1 % (w/v) poloxamer 407,
- about 0.1 % (w/v) human serum albumin, and
- and about 100 mM sodium chloride.

14. The unit dose of any one of items 1 to 13, wherein each one of the four live attenuated dengue virus strains has attenuating mutations in the 5'-noncoding region (NCR) at nucleotide 57 from cytosine to thymine, in the NS1 gene at nucleotide 2579 from guanine to adenine resulting in an amino acid change at position 828 from glycine to asparagine, and in the NS3 gene at nucleotide 5270 from adenine to thymine resulting in an amino acid change at position 1725 from glutamine to valine, preferably further comprising one or more of the 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.

15. The unit dose of item 14, wherein (i) further comprises one or more of the 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 glutamic acid to aspartic acid, and

- a silent mutation in the NS4B gene at nucleotide 7311 from adenine to guanine.

16. The unit dose of item 14 or 15, wherein (ii) further comprises one or more of the 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 glutamic acid, 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.

17. The unit dose of any one of items 14 to 16, wherein (iii) further comprises 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.

18. The unit dose of any one of items 14 to 17, wherein (iv) further comprises 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 aspartic acid 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/arginine 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.

19. The unit dose of any one of items 1 to 18, wherein
(v) has the amino acid sequence of SEQ ID NO. 2,
(vi) has the amino acid sequence of SEQ ID NO. 4,
(vii) has the amino acid sequence of SEQ ID NO. 6, and
(viii) has the amino acid sequence of SEQ ID NO. 8.

20. The unit dose of any one of items 1 to 19 reconstituted with 0.3 to 0.8 mL of liquid for reconstitution.

21. The unit dose of item 20 reconstituted with 0.5 mL of liquid for reconstitution.

22. The unit dose of item 20 or 21, wherein the liquid for reconstitution is 37 mM aqueous sodium chloride solution.

23. A kit for preparing a reconstituted unit dose comprising the following components:
a) a unit dose of any one of items 1 to 19, and
b) a pharmaceutically acceptable diluent for reconstitution.