(12) STANDARD PATENT (11) Application No. **AU 2020331884 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Methods for preventing dengue and Hepatitis A

(51) International Patent Classification(s)
A61K 39/295 (2006.01) ***A61K 39/125*** (2006.01)
A61K 39/00 (2006.01) ***A61K 39/29*** (2006.01)
A61K 39/12 (2006.01)

(21) Application No: **2020331884** (22) Date of Filing: **2020.03.04**

(87) WIPO No: **WO21/034349**

(30) Priority Data

(31) Number	(32) Date	(33) Country
PID2019-07241	**2019.08.16**	**ID**
19195692.9	**2019.09.05**	**EP**
16/561,953	**2019.09.05**	**US**
PCT/US2019/049749	**2019.09.05**	**US**

(43) Publication Date: **2021.02.25**
(44) Accepted Journal Date: **2025.01.23**

(71) Applicant(s)
Takeda Vaccines, Inc.

(72) Inventor(s)
WALLACE, Derek

(74) Agent / Attorney
WRAYS PTY LTD, L7 863 Hay St, Perth, WA, 6000, AU

(56) Related Art
JORGE E OSORIO ET AL: LANCET INFECTIOUS DISEASES, vol. 14, no. 9, 1 September 2014 (2014-09-01), US, pages 830 - 838, XP055517052, ISSN: 1473-3099, DOI: 10.1016/S1473-3099(14)70811-4
SARAH L. GEORGE ET AL: JOURNAL OF INFECTIOUS DISEASES. JID, vol. 212, no. 7, 19 March 2015 (2015-03-19), US, pages 1032 - 1041, XP055517050, ISSN: 0022-1899, DOI: 10.1093/infdis/jiv179

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 February 2021 (25.02.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/034349 A1

(51) International Patent Classification:
A61K 39/295 (2006.01) *A61K 39/125* (2006.01)
A61K 39/12 (2006.01) *A61K 39/29* (2006.01)
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2020/020991

(22) International Filing Date:
04 March 2020 (04.03.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PID2019-07241 16 August 2019 (16.08.2019) ID
19195692.9 05 September 2019 (05.09.2019) EP
16/561,953 05 September 2019 (05.09.2019) US
PCT/US2019/049749 05 September 2019 (05.09.2019) US

(71) Applicant: **TAKEDA VACCINES, INC.** [US/US]; 75 Sidney Street, Cambridge, Massachusetts 02139 (US).

(72) Inventor: **WALLACE, Derek**; 20 Adams Street, Brookline, Massachusetts 02446 (US).

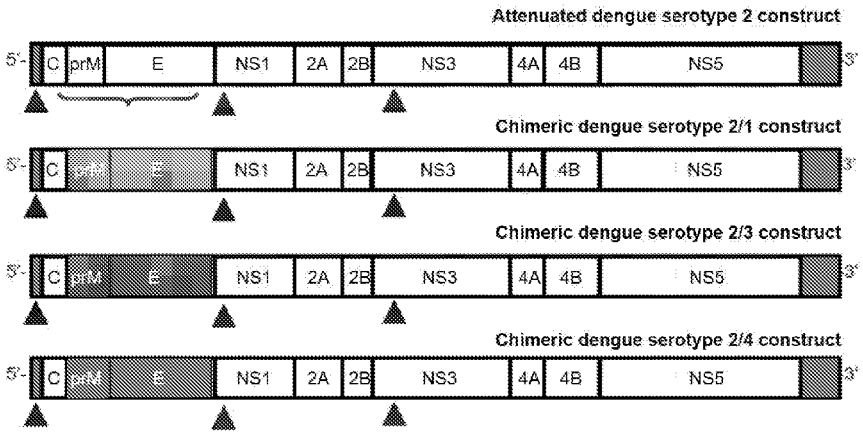
(74) Agent: **GOLDBERG, Joshua B.**; NATH, GOLDBERG & MEYER, 112 S. West Street, Alexandria, Virginia 22314 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) **Title:** METHODS FOR PREVENTING DENGUE AND HEPATITIS A

Figure 1



(57) **Abstract:** The invention relates to a method for preventing dengue disease and hepatitis A in a subject or subject population by simultaneously administering a unit dose of a dengue vaccine composition and a hepatitis A vaccine on the same day. The unit dose of a dengue vaccine composition includes constructs of each dengue serotype, such as TDV-1, TDV-2, TDV-3 and TDV-4, at various concentrations in order to improve protection from dengue infection.

WO 2021/034349 A1

[Continued on next page]

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

METHODS FOR PREVENTING DENGUE AND HEPATITIS A

FIELD OF THE INVENTION

[0001] The present invention relates to a method for administering a unit dose of a dengue vaccine composition to a subject or a subject population simultaneously on the same day with a hepatitis A vaccine. The unit dose according to this invention provides immune responses against all serotypes of dengue virus, i.e. DENV-1, DENV-2, DENV-3 and DENV-4 and against hepatitis A virus.

BACKGROUND OF THE INVENTION

[0002] Vaccines for protection against viral infections have been effectively used to reduce the incidence of human disease. One of the most successful technologies for viral vaccines is to immunize animals or humans with a weakened or attenuated virus strain (a "live attenuated virus"). Due to limited replication after immunization, the attenuated virus strain does not cause disease. However, the limited viral replication is sufficient to express the full repertoire of viral antigens and can generate potent and long-lasting immune responses to the virus. Thus, upon subsequent exposure to a pathogenic virus strain, the immunized individual is protected from the disease. These live attenuated viral vaccines are among the most successful vaccines used in public health.

[0003] Dengue disease is a mosquito-borne disease caused by infection with a dengue virus. Dengue virus infections can lead to debilitating and painful symptoms, including a sudden high fever, headaches, joint and muscle pain, nausea, vomiting and skin rashes. To date, four serotypes of dengue virus have been identified: dengue-l (DENV-l), dengue-2 (DENV-2), dengue-3 (DENV-3) and dengue-4 (DENV-4). Dengue virus serotypes 1-4 can also cause dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). In the most severe cases, DHF and DSS can be life threatening. Dengue viruses cause 50-100 million cases of debilitating dengue fever, 500,000 cases of DHF/DSS, and more than 20,000 deaths each year, a large portion of which are children. All four dengue virus serotypes are endemic throughout the tropical regions of the world and constitute the most significant mosquito-borne viral threat to humans there. Dengue viruses are transmitted to humans primarily by *Aedes aegypti* mosquitoes, but also by *Aedes albopictus* mosquitoes. Infection with one dengue virus serotype results in life-long protection from re-infection by that serotype, but does not prevent secondary infection by one of the other three dengue virus serotypes. In fact, previous infection with one dengue virus serotype may lead to an increased risk of severe disease (DHF/DSS) upon secondary infection with a different serotype.

[0004] To date, only one vaccine, a tetravalent dengue vaccine based on a yellow fever backbone, CYD-TDV (Dengvaxia®, Sanofi Pasteur, Lyon, France), has been licensed in several countries based on the clinical demonstration of an overall vaccine efficacy (VE) against virologically-confirmed dengue (VCD) of 56–61% in children in Asia and Latin America (Capeding MR et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014, 384:1358-65; Villar LA et al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J 2013, 32:1102-9). However, clinical trials have shown that Dengvaxia® can enhance, rather than reduce, the risk of severe disease due to dengue infection in individuals who had not been previously infected by a dengue virus (seronegative populations). Therefore, Dengvaxia® is only recommended for use in individuals who had been previously infected with at least one dengue virus serotype (seropositive populations). More specifically, according to the European Medicine Agencys European Public Assessment report (EPAR) for the product, Dengvaxia® is only for use in people from 9 to 45 years of age

who have been infected with dengue virus before and who live in areas where this infection is endemic. Endemic areas are areas where the disease occurs regularly throughout the year. See also Sridhar S et al. Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. N Engl J Med 2018, 379:327-40; and World Health Organization. Dengue vaccine: WHO position paper – September 2018. Wkly. Epidemiol. Rec. 2018, 93:457-476. S.R. Hadinegoro et al. report in the New England Journal of Medicine, Vol. 373, page 1195, in "Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease" a pooled risk of hospitalization for virologically-confirmed dengue disease among those under the age of 9 years of 1.58 indicating an increased risk for the vaccinated group with respect to severe dengue. This leaves a substantial unmet need for an effective vaccine with a good safety profile in both dengue-naïve and seropositive individuals, including those dengue-naïve populations living in endemic areas, younger individuals who may not have developed any seropositive response to dengue or been exposed to dengue, and travelers and individuals from non-endemic regions. There is also a need for outbreak control or travel vaccination, offering a reduction in the risk of dengue after only one dose.

[0005] One further disadvantage of the only currently approved dengue vaccine, Dengvaxia®, is that it must only be given to people who have had a positive test result showing a previous infection with dengue virus (EPAR), i.e. individuals with known serostatus for dengue. Thus, individuals with unknown serostatus for dengue cannot be vaccinated with Dengvaxia®.

[0006] There is hence a need for a dengue vaccine and corresponding method of inoculation that stimulates an immune response to all dengue serotypes, preferably a balanced immune response to all serotypes, and protects against dengue disease of any severity (including DSS, DHF), both in seronegative and seropositive populations, which is safe for a larger group of ages, in particular also for subjects of 9 years and younger. The development of a safe and effective vaccine capable of protecting all populations, including both seronegative and seropositive populations, and in particular children and young adults and elderly subjects in endemic settings and for the purpose of traveling, represents an important approach to the prevention and control of this global disease.

[0007] There is thus a medical need for a dengue vaccine and corresponding method of inoculation which, as well as being safe and efficacious irrespective of serostatus and in a broad age group. There is a need for a dengue vaccine and corresponding method of inoculation that avoids costly and time consuming serostatus tests or seroprevalence considerations. There is a need for a dengue vaccine and corresponding method of inoculation that can be used in an outbreak situation. Furthermore there is a medical need for a dengue vaccine which as well as being safe and effective can also be administered to individuals with unknown dengue serostatus, children under 9 years and seronegative individuals.

[0008] There is also a need for a vaccine that is administered in fewer doses than the current Dengvaxia® dosing schedule of 3 doses, 6 months apart, such as a vaccine that can be administered in only two doses or one dose to be efficacious.

[0009] The above objects are commensurate with the research priorities provided by the WHO in the Dengue Vaccine: WHO position paper – September 2018 (Wkly. Epidemiol. Rec. 2018, 93:457-476).

[0010] Hepatitis A is a liver disease caused by the hepatitis A virus (HAV). The virus is primarily spread when an uninfected (and unvaccinated) person ingests food or water that is contaminated with the feces of an infected person. The disease is closely associated with unsafe water or food, inadequate sanitation and poor personal hygiene. The virus can also be transmitted through close physical contact with an infectious person. Unlike hepatitis B and C, hepatitis A infection does not cause chronic liver disease and is rarely fatal, but it can cause debilitating

symptoms and fulminant hepatitis (acute liver failure), which is often fatal. Hepatitis A occurs sporadically and in epidemics worldwide, with a tendency for cyclic recurrences.

[0011] The hepatitis A virus is one of the most frequent causes of foodborne infection. Epidemics related to contaminated food or water can erupt explosively, such as the epidemic in Shanghai in 1988 that affected about 300,000 people. Hepatitis A viruses persist in the environment and can withstand food-production processes routinely used to inactivate and/or control bacterial pathogens. The disease can lead to significant economic and social consequences in communities. It can take weeks or months for people recovering from the illness to return to work, school, or daily life. The impact on food establishments identified with the virus, and local productivity in general, can be substantial. In developing countries with poor sanitary conditions and hygienic practices, most children (90%) have been infected with the hepatitis A virus before the age of 10 years.

[0012] The number of people traveling internationally has grown substantially in recent decades. According to the United Nations World Tourism Organization (UNWTO), over 1.1 billion tourists travelled abroad in 2014. The risk of becoming ill during international travel depends on many factors, such as the region of the world visited, the length of the trip, and the diversity of planned activities. Vaccine recommendations are a prominent part of health preparations before international travel. Vaccination against hepatitis A virus is commonly recommended for travelers to at-risk areas around the world including Asia, Africa, and Latin America.

[0013] For routine hepatitis A vaccination, a two-dose schedule is recommended, particularly in travelers at substantial risk of contracting hepatitis A and in immunocompromised individuals. However, in healthy individuals, comparable effectiveness has been achieved with a single dose. The vaccination schedule for children/adolescents (12 months through 18 years of age) as well as for adults (≥19 years of age) consists of a primary dose administered intramuscularly, and a further booster dose administered intramuscularly 6 to 18 months later.

[0014] Available hepatitis A vaccines include HAVRIX® and VAQTA®.

[0015] Hence, there is a need for a safe and effective method of simultaneously preventing dengue disease and hepatitis A. In particular, there is a need for hepatitis A and dengue vaccines which provide non-inferiority when administered simultaneously to a subject or subject population and a suitable administration schedule for achieving synergy.

[0016] Furthermore, there is a need of effectively and safely preventing dengue disease and hepatitis A in subjects being unaware of their hepatitis A and/or dengue serostatus, in particular in subjects from non-endemic countries which travel into dengue and hepatitis A endemic countries.

OBJECTS AND SUMMARY

[0017] It is an object of the present invention to provide a safe and effective protection against dengue disease and hepatitis A.

[0018] It is an object of the present invention to provide a method of administration for preventing hepatitis A and dengue disease which is useful in typical vaccination settings wherein the subjects are unaware of their serostatus for dengue and/or hepatitis A and a corresponding serotest is unavailable, unpractical or unreliable.

[0019] It is an object of the present invention to provide a safe and effective protection against dengue disease and hepatitis A for travelers from hepatitis A and dengue non-endemic countries, in particular for travelers being

vaccinated in travel clinics. In this context it is beneficial if multiple during the same medical appointments are avoided and vaccination can be conducted simultaneously for more than one disease.

[0020] It is an object of the present invention to provide a safe and effective vaccine for preventing hepatitis A and dengue disease in a subject or subject population and a corresponding method of preventing hepatitis A and dengue disease in a subject or a subject population from a dengue-endemic and dengue non-endemic region and for a broad range of ages, in particular for subjects between 2 to 60 years of age, preferably for subjects between 18 and 60 years of age, and independent of previous exposure to any dengue virus serotype and/or to hepatitis A virus and independent of corresponding seropositivity or seronegativity with respect to dengue and/or hepatitis A before vaccination.

[0021] It is an object of the invention to provide vaccines and a corresponding method of preventing hepatitis A and dengue disease which avoids testing for individual dengue and/or hepatitis A serostatus before individual administration of a hepatitis A and a dengue vaccine to a subject or subject population, or analysis of seroprevalence rates of dengue and/or hepatitis A in subjects or subject populations to be vaccinated.

[0022] It is an object of the present invention to provide a dengue vaccine and a hepatitis A vaccine which can be safely co-administered with TDV as travel vaccines before an international travel of a subject to HAV and dengue endemic countries and a method of safely administering these vaccines.

[0023] Therefore, the present invention is directed to a method of preventing dengue disease as well as hepatitis A.

[0024] The present invention is further directed to a method of preventing hepatitis A and dengue disease in a subject or subject population, the method comprising simultaneously on the same day administering a hepatitis A vaccine and a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

[0024a] The present invention is further directed to a method of effective vaccination against hepatitis A and dengue disease in a subject or in a subject population, the method comprising simultaneously on the same day administering the hepatitis A vaccine and a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

[0024b] The present invention is further directed to the use of a hepatitis A vaccine in the manufacture of a medicament for preventing hepatitis A in a subject or subject population and of a unit dose of a dengue vaccine composition in the manufacture of a medicament for preventing dengue disease in the subject or subject population, wherein the prevention of hepatitis A and dengue disease comprises simultaneously on the same day administering the hepatitis A vaccine and the unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

2020331884 23 Dec 2024

[0024c] The present invention is also directed to the use of a unit dose of a dengue vaccine composition in the manufacture of a medicament for preventing dengue disease and hepatitis A in a subject or subject population, wherein the prevention of dengue disease and hepatitis A comprises simultaneously on the same day administering the unit dose of a dengue vaccine composition and a hepatitis A vaccine, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

[0024d] The present invention is further directed to a hepatitis A vaccine and a unit dose of a dengue vaccine composition which are used to prevent hepatitis A and dengue disease in a subject or in a subject population, wherein preventing dengue disease and hepatitis A comprises that the hepatitis A vaccine and the unit dose of the dengue vaccine composition are to be administered to the subject or subject population simultaneously on the same day and wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

[0024e] The present invention is also directed to a unit dose of a dengue vaccine composition which is used to prevent dengue disease and hepatitis A in a subject or in a subject population, wherein preventing dengue disease and hepatitis A comprises that the unit dose of the dengue vaccine composition and a hepatitis A vaccine are to be administered to the subject or subject population simultaneously on the same day and wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

DEFINITIONS

[0025] 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.

[0025a] Throughout the specification and claims, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[0026] 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. optionally after reconstitution. In one embodiment, more than one unit dose of the dengue vaccine composition may be present in a vial so that with the content of one vial more than one subject can be vaccinated.

[0027] A "lyophilized unit dose" or "unit dose in lyophilized form" refers to the unit dose that is obtained by subjecting a given volume of the liquid dengue vaccine composition, such as 0.5 mL, to lyophilization. Thus, the aqueous formulations of the dengue vaccine composition being produced by combining the pharmaceutically acceptable excipients and the dengue virus composition comprising the

2020331884 23 Dec 2024

four dengue virus strains, preferably TDV-1 to TDV-4, is subjected to lyophilization to obtain the lyophilized unit dose.

[0028] A "reconstituted unit dose" or "unit dose in reconstituted form" is obtained from the lyophilized dose by reconstitution with a pharmaceutically acceptable diluent. The diluent does not contain dengue virus. The

[Text continued on page 5]

reconstituted unit dose is a liquid which can be administered to a subject, for example by injection, such as subcutaneous injection.

[0029] As used herein, the term "upon reconstitution with 0.5 mL" is not limiting the reconstitution to be performed using 0.5 mL of the diluent, but refers to the concentration of the dengue viruses that will be present in the reconstituted unit dose when 0.5 mL diluent are used for reconstitution. While using a different volume for reconstitution (e.g. 0.8 mL) will result in a different concentration of dengue viruses in the reconstituted unit dose, the administration of the total volume of the unit dose (e.g. 0.8 mL) will result in the same total amount of dengue virus being administered.

[0030] As used herein, a "concentration of at least X log10 pfu/0.5 mL" refers to the concentration of a dengue serotype in 0.5 mL, but is not limiting the unit dose to be 0.5 mL. If the unit dose has a volume different than 0.5 mL, or is lyophilized from a volume different than 0.5 mL, or is reconstituted with a volume different than 0.5 mL, said concentration will differ from the "concentration of at least X log10 pfu/0.5 mL". However, if the unit dose has a volume of 0.5 mL, or is lyophilized from a volume of 0.5 mL, or is reconstituted with a volume of 0.5 mL, said concentration will be the "concentration of at least X log10 pfu/0.5 mL". Thus, while the concentration may differ, the total amount of virus in the unit dose remains the same.

[0031] As used herein, the term "dengue serotype" refers to a species of dengue virus which is defined by its cell surface antigens and therefore can be distinguished by serological methods known in the art. At present, four serotypes of dengue virus are known, i.e. dengue serotype 1 (DENV-1), dengue serotype 2 (DENV-2), dengue serotype 3 (DENV-3) and dengue serotype 4 (DENV-4).

[0032] As used herein, the term "tetravalent dengue virus composition" refers to a dengue virus composition comprising four different immunogenic components from the four different dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4, preferably comprising four different live, attenuated dengue viruses, each representing one dengue serotype, and which aims to stimulate immune responses to all four dengue serotypes.

[0033] As used herein, the term "live attenuated dengue virus" refers to a viable dengue virus which is mutated to provide reduced virulence. The live attenuated dengue virus can be a dengue virus in which all components are derived from the same dengue serotype or it can be a chimeric dengue virus having parts from two or more dengue serotypes or a mixed chimeric dengue virus having parts from other flaviviruses.

[0034] A "virus strain" and in particular a "dengue virus strain" is a genetic subtype of a virus, in particular of a dengue virus, which is characterized by a specific nucleic acid sequence. A dengue serotype may comprise different strains with different nucleic acid sequences which have the same cell surface antigens. A dengue virus strain can be a dengue virus in which all components are derived from the same dengue serotype or it can be a chimeric dengue virus having parts from two or more dengue serotypes.

[0035] As used herein, "TDV-2" refers to a molecularly characterized and cloned dengue serotype 2 strain derived from the live attenuated DEN-2 PDK-53 virus strain. The PDK-53 strain is described for example in Bhamarapravati et al. (1987) Bulletin of the World Health Organization 65(2): 189-195. In one embodiment, the TDV-2 strain served as a backbone for the chimeric TDV-1, TDV-3 and TDV-4 strains into which parts from the TDV-1, TDV-3 and TDV-4 strains were introduced.

[0036] A "non-chimeric dengue virus" or "non-chimeric dengue serotype strain" or "non-chimeric dengue strain" comprises only parts from one dengue serotype. In particular, a non-chimeric dengue virus does not include parts

from a different flavivirus such as yellow fever virus, Zika virus, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus. TDV-2 is an example of a non-chimeric dengue virus.

[0037] A "chimeric dengue virus" or "chimeric dengue serotype strain" or "chimeric dengue strain" comprises parts from at least two different dengue serotypes. As used herein, the chimeric dengue virus does not include parts from a different flavivirus such as yellow fever virus, Zika virus, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus. In particular, the chimeric dengue virus described herein does not include parts from the yellow fever virus. As used herein, a "chimeric dengue serotype 2/1 strain" or "DENV-2/1 chimera" or "TDV-1" refers to a dengue virus chimeric construct which comprises parts from both DENV-2 and DENV-1. In particular, in the chimeric dengue serotype 2/1 strain the prM and E proteins from DENV-1 replace the prM and E proteins from DENV-2 as detailed below. As used herein, a "chimeric dengue serotype 2/3 strain" or "DENV-2/3 chimera" or "TDV-3" refers to a dengue virus chimeric construct which comprises parts from both DENV-2 and DENV-3. In particular, in the chimeric dengue serotype 2/3 strain the prM and E proteins from DENV-3 replace the prM and E proteins from DENV-2 as detailed below. As used herein, a "chimeric dengue serotype 2/4 strain" or "DENV-2/4 chimera" or "TDV-4" refers to a dengue virus chimeric construct which comprises parts from both DENV-2 and DENV-4. In particular, in the chimeric dengue serotype 2/4 strain the prM and E proteins from DENV-4 replace the prM and E proteins from DENV-2 as detailed below. A mixed chimeric dengue virus has parts from other flaviviruses.

[0038] As used herein, "TDV" refers to a tetravalent live attenuated dengue vaccine that comprises a mixture of the four live attenuated dengue virus strains TDV-1, TDV-2, TDV-3 and TDV-4 expressing surface antigens from the four dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4, respectively. In one embodiment (e.g. also in the examples), TDV-1 has the nucleotide sequence according to SEQ ID No. 1 and/or the amino acid sequence according to SEQ ID No. 2. In one embodiment, TDV-2 has the nucleotide sequence according to SEQ ID No. 3 and/or the amino acid sequence according to SEQ ID No. 4. In one embodiment, TDV-3 has the nucleotide sequence according to SEQ ID No. 5 and/or the amino acid sequence according to SEQ ID No. 6. In one embodiment, TDV-4 has the nucleotide sequence according to SEQ ID No. 7 and/or the amino acid sequence according to SEQ ID No. 8.

[0039] As used herein, the term "dengue disease" refers to the disease which is caused by infection with dengue virus. Symptoms of dengue disease include sudden high fever, headaches, joint and muscle pain, nausea, vomiting and skin rashes. The term dengue disease also includes the more severe forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Symptoms of DHF include increased vascular permeability, hypovolemia and abnormal blood clotting mechanisms. Subjects with DHF may present with severe manifestations of plasma leakage and hemorrhage. When a subject with DHF experiences shock he or she will be categorized as having DSS. Symptoms of DSS include bleeding that may appear as tiny spots of blood on the skin and larger patches of blood under the skin. Prolonged shock is the main factor associated with complications including massive gastrointestinal hemorrhage that can lead to death. As used herein, DHF cases are defined as VCD cases meeting WHO 1997 DHF criteria. In the context of preventing dengue disease in elderly subjects, the term "preventing dengue disease" preferably includes preventing DHF and/or DSS. In the context of preventing dengue disease in elderly subjects, the term "preventing dengue disease" preferably includes preventing severe end-organ manifestations of dengue such as hepatomegaly and acute renal failure.

[0040] As used herein, "preventing dengue disease" refers to preventing a subject from developing one or more symptoms of dengue disease because of an infection with a dengue virus. In particular, preventing dengue disease is achieved by vaccinating or inoculating a subject with a dengue vaccine composition, such as the reconstituted unit dose described herein. As used herein, the term "prophylactically treating dengue disease" is equivalent to

"preventing dengue disease". In a particular embodiment, preventing dengue disease includes preventing DHS and/or DSS.

[0041] As used herein, the terms "virologically-confirmed dengue disease", "VCD case", or "VCD fever" refer to febrile illness or illness clinically suspected to be dengue disease with a positive serotype-specific reverse transcriptase polymerase chain reaction (RT-PCR). The term "virologically confirmable dengue" disease refers to a subject having febrile illness or illness clinically suspected to be dengue disease, wherein testing the subject, e.g. using RT-PCR, would confirm the presence of at least one dengue serotype. Severe forms of VCD fever will be identified as follows: Dengue Hemorrhagic Fever (DHF) was defined according to the WHO 1997 criteria. Severe dengue was defined through an assessment of an independent Dengue Case Adjudication Committee which will assess all hospitalized VCD cases (severe / non-severe) based on criteria redefined in a charter. All non-hospitalized cases are considered non-severe.

[0042] As used herein, the term "febrile illness" is defined as temperature ≥38°C on any 2 of 3 consecutive days.

[0043] As used herein, the terms "virologically-confirmed dengue disease with hospitalization", is considered to be a surrogate for severe dengue and the "incidence of virologically-confirmed dengue disease with hospitalization" is used as a safety parameter. As used herein, the "relative risk with respect to virologically-confirmed dengue disease with hospitalization" means the number of events of virologically confirmed dengue disease with hospitalization divided by the number of subjects treated with the unit dose as disclosed herein over the number of events of virologically confirmed dengue disease with hospitalization divided by the number of subjects treated with placebo. If the "relative risk with respect to virologically-confirmed dengue disease with hospitalization" is 1 or lower the vaccine provides for the same or less risk for virologically-confirmed dengue disease with hospitalization as placebo and is considered "safe". In this context the risk of virologically-confirmed dengue disease with hospitalization may be also 0.9 or less, 0.8 or less, 0.7 or less, 0.6 or less, 0.5 or less, 0.4 or less, 0.3 or less, 0.2 or less, or 0.1 or less, in particular when determined from 30 days after a second administration until 12 months after a second administration, in particular when determined in age groups selected from the age group of 4 to 16 year old subjects, the age group of 4 to under 9 year old subjects, the age group of 2 to under 9 year old subjects, the age group of 4 to 5 year old subjects, the age group of 6 to 11 year old subjects, and the age group of 12 to 16 year old subjects.

[0044] As used herein, alternatively a vaccine is considered "safe" when the vaccine efficacy (VE) with respect to virologically-confirmed dengue disease with hospitalization is 0% or higher. This means that the vaccine provides for the same likelihood or less for virologically-confirmed dengue disease with hospitalization as placebo. In particular considered "safe" is the 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 25%, in particular when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects (in particular when measured in age groups selected in particular from the age group of 4 to 16 year old subjects, the age group of 4 to under 9 year old subjects, the age group of 2 to under 9 year old subjects, the age group of 4 to 5 year old subjects, the age group of 6 to 11 year old subjects, and the age group of 12 to 16 year old subjects) being seronegative against all serotypes at baseline or being seropositive against at least one serotype at baseline, in particular when said unit dose or said placebo is administered at least twice within less than 6 months, such as within 3 months, about from first administration or from 30 days after the second or last administration of the administration schedule until at least 12 months, until 12 to 18 months, until 12 months, or until 18 months after the second or last administration of the administration schedule. In particular, the lower bound may be more than 30%, more than 40%, more than 50%, more than 60%, more than 65%, more than 66%, more than 67%, more than

68% more than 70%, or more than 75%. In particular, the 2-sided 95% confidence interval of the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes when comparing seropositive and seronegative subjects provides for lower bounds of the 2-sided confidence interval which are within 10% points or within 15% points or within 20% points. In a particular embodiment "safe" means 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 first administration of the administration schedule until 12 to 18 months after the last administration of the administration schedule.

[0045] If one of the criteria as defined above for the term "safe" is fulfilled, the vaccine is considered safe within the meaning of this invention. In this context, safe in particular refers to a vaccine that is safe for all subjects irrespective of their serostatus at baseline. This means that the vaccine can be administered without the need to determine the occurrence of a previous dengue infection in the subject before administration. Preferably, the vaccine is safe as defined above with respect to all age groups starting from 4 years of age and preferably irrespective of the serostatus, in particular from 4 years of age to 60 years of age, or 4 years of age to 16 years of age. Relevant subgroups in this context are under 9 years of age, from 2 years of age to under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age and 12 to 16 years of age or any age group within 4 to 16 years of age. For further definitions of VE against virologically-confirmed dengue disease with hospitalization reference is made to the disclosure below with respect to certain methods of treatment.

[0046] As used herein, "vaccine efficacy" or "VE" measure the proportionate reduction in cases among vaccinated persons. Vaccine efficacy (VE) is measured by calculating the risk of disease among vaccinated and unvaccinated persons and determining the percentage reduction in risk of disease among vaccinated persons relative to unvaccinated persons. The greater the percentage reduction of illness in the vaccinated group, the greater the vaccine efficacy. For example, a VE of 90% indicates a 90% reduction in disease occurrence among the vaccinated group, or a 90% reduction from the number of cases you would expect if they have not been vaccinated. The vaccine efficiency is calculated by the formula: 100 * (1 – HR), wherein HR is the Hazard Ratio which is defined as the Hazard rate of vaccine (λv) divided by the Hazard rate of placebo (λc), i.e. HR = $\lambda v/\lambda c$. λv denote the hazard rate for the subjects vaccinated with a tetravalent dengue vaccine composition as disclosed herein and λc denote the hazard rate for unvaccinated subjects, i.e. subjects receiving placebo. The hazard rate ratio HR is estimated from a Cox proportional hazard model with study vaccine as a factor, adjusted for age, and stratified by region. As used herein the term"combined vaccine efficacy against all four serotypes" is defined as the vaccine efficacy in relation to the risk of dengue disease irrespective of the serotype being responsible for the virologically-confirmed dengue disease and the subject baseline serostatus. A vaccine is considered "effective" in case the combined vaccine efficacy is above 30%. In this context the combined vaccine efficacy may be also 40% or more, 50% or more, 60% or more, 70% or more, 72% or more, or 80% or more, in particular when determined from 30 days after a second administration until 12 months after a second administration or 18 months after a second vaccination, in particular when determined in age groups selected from the age group of 4 to 16 year old subjects, the age group of 4 to under 9 year old subjects, the age group of 2 to under 9 year old subjects, the age group of 4 to 5 year old subjects, the age group of 6 to 11 year old subjects, and the age group of 12 to 16 year old subjects. In this context, effective in particular refers to a vaccine that is effective for all subjects irrespective of their serostatus at baseline. Preferably, the vaccine is effective with respect to all age groups starting from 4 years of age and preferably irrespective of the serostatus, in particular from 4 years of age to 60 years of age or from 4 years of age to 16 years of age and irrespective of the serostatus. Relevant subgroups in this context are under 9 years of age, from 2 years of age to

under 9 years of age, from 4 years of age to under 9 years of age, 4 to 5 years of age, 6 to 11 years of age and 12 to 16 years of age or any age group within 4 to 16 years of age. In certain embodiments "effective" means 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 administration of the administration schedule until 18 months after the last administration of the administration schedule. Further specific efficacies can be defined. As used herein, "combined vaccine efficacy against all four serotypes in seronegative subjects" refers to the efficacy measured in subjects which are seronegative at baseline. As used herein, "vaccine efficacy against a specific serotype, e.g. serotype 1" refers to the efficacy in relation to a specific serotype being responsible for the virologically-confirmed dengue disease. As used herein, "combined vaccine efficacy against all four serotypes against virologically-confirmed dengue with hospitalization" refers to the efficacy wherein only virologically-confirmed dengue cases with hospitalization are considered. Such vaccine efficacies can be determined with respect to subjects being seronegative or seropositive at baseline and for different age groups.

[0047] As used herein, the "relative risk" means the number of events of virologically confirmed dengue disease divided by the number of subjects treated with the unit dose as disclosed herein over the number of events of virologically confirmed dengue disease divided by the number of subjects treated with placebo. As used herein the term"combined relative risk against all four serotypes" is defined as the relative risk in relation to the risk of dengue disease irrespective of the serotype being responsible for the virologically-confirmed dengue disease and the subject baseline serostatus.

[0048] As used herein, "vaccinating" or "inoculating" refers to the administration of a vaccine to a subject, with the aim to prevent the subject, from developing one or more symptoms of a disease. As used herein, "vaccinating against dengue disease" or "inoculating against dengue disease" refers to the administration of a dengue vaccine composition to a subject, with the aim to prevent the subject, from developing one or more symptoms of dengue disease. In principle the method comprises a primary vaccination and optionally one or more booster vaccinations. The primary vaccination is defined as the primary administration schedule for administering the composition or unit dose as disclosed herein to establish a protective immune response and e.g. consists of two administrations e.g. within three months. Whenever an administration is mentioned within this disclosure such administration refers to the primary vaccination unless it is specified as booster vaccination. The booster vaccination refers to an administration or administration schedule which takes place after the primary vaccination e.g. at least 1 year, or 4 to 4.5 years, or even 5 or 10 years after the last administration, e.g. the second administration, of the primary vaccination schedule. The booster administration attempts at enhancing or reestablishing the immune response of the primary vaccination.

[0049] As used herein, the terms "subject" or "subjects" are limited to human subjects (e.g. infants, children or adults). The terms "elderly subject" or "elderly subjects" refer to subjects with an age of more than 60 years, such as 61 years to 100 years, 61 years to 90 years, 61 years to 80 years, 61 years to 75 years, or 61 years to 70 years.

[0050] As used herein, "subject population" refers to a group of subjects. The subject population may refer to least 40 subjects, at least 50 subjects, at least 60 subjects, at least 100 subjects or at least 1000 subjects and is defined by certain parameters. The parameters that may be used to define a subject population include, but are not limited to, the age of the subjects, whether the subjects are from a dengue endemic region or from a dengue non-endemic region and the serostatus of the subjects.

[0051] As used herein, "endemic region" refers to a region where a disease or infectious agent is constantly present and/or usually prevalent in a population within this region. As used herein, "non-endemic region" refers to a region from which the disease is absent or in which it is usually not prevalent. Accordingly, a "dengue endemic region" refers to geographic areas in which an infection with dengue virus is constantly maintained at a baseline level. A "dengue non-endemic region" is a geographic area in which an infection with dengue virus is not constantly maintained at a baseline level. Accordingly, subject populations or subjects "from a dengue endemic region" or "from a dengue non-endemic region" refer to subject populations or subjects living in geographic areas as defined above. Whether a geographic area or a subject population is dengue-endemic or not can be determined by different calculatory methods such as the ones described in Bhatt et al. (2013) Nature 496 (7446): 504-507 and supplementary material and in Stanaway et al. (2016) Lancet Infect Dis. 16(6): 712-723 and supplementary material. Overviews of dengue endemic regions and dengue epidemiology are regularly published, for example, by the WHO or CDC. Typical dengue-endemic regions are in Latin America, Southeast Asia and the Pacific islands and dengue endemic countries include, but are not limited to, Australia, Brazil, Bangladesh, Colombia, China, Dominican Republic, Indonesia, India, Mexico, Malaysia, Nicaragua, Nigeria, Pakistan, Panama, Philippines, Puerto Rico, Singapore, Sri Lanka, Thailand and Vietnam. The area's force of infection is measured by seroprevalence surveys provided as seroprevalence rate. Areas with very high force of infection are considered to have a seroprevalence rate of more than 80%. As used herein the term "region" when it concerns seroprevalence rates refers to a geographic area where the seroprevalence rate could be determined or is known, e.g. a village, a town, a city, a region, a county, a state, a province or parts of the foregoing or a whole country.

[0052] As used herein, "serostatus" refers to the amount of antibodies a subject has with respect to a certain infectious agent, in particular dengue virus. As used herein, "seronegative" or "seronaïve" means that the subject does not have neutralizing antibodies against any one of dengue serotypes DENV-1, DENV-2, DENV-3 and DENV-4 in the serum. A seronegative or seronaïve subject or subject population is defined by a neutralizing antibody titer of less than 10 for each one of the four dengue serotypes. A subject or subject population having a neutralizing antibody titer of equal to or more than 10 for at least one dengue serotype is defined as being "seropositive" with respect to said dengue serotype. Serostatus at baseline refers to the serostatus before the administration of a dengue vaccine composition as described herein.

[0053] As used herein, a "neutralizing antibody titer" refers to the amount of antibodies in the serum of a subject that neutralize the respective dengue serotype. The neutralizing antibody titer against DENV-1, DENV-2, DENV-3 and DENV-4 is determined in a serum sample of the subject using known methods such as the plaque reduction neutralization test (PRNT) as described in the WHO Guidelines (World Health Organization Department of Immunization Vaccines Biologicals (2007) Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses, WHO/IVB/07.07) or a microneutralization (MNT50) assay as described herein. As used herein, the "ratio of not more than 20 for the neutralizing antibody titer of dengue serotype 2 to the neutralizing antibody titer of dengue serotype 4" means that the neutralizing antibody titer of dengue serotype 2 is divided by the neutralizing antibody titer of dengue serotype 4 and that the ratio obtained hereby is no more than 20. In other words, the neutralizing antibody titer of dengue serotype 2 is not more than 20-times higher than the neutralizing antibody titer of dengue serotype 4 in the subject.

[0054] As used herein, the terms "geometric mean neutralizing antibody titer" and "GMT" refer to the geometric mean value of the titer of neutralizing antibodies against the corresponding dengue serotype in the serum of subjects in a subject population. The geometric mean value is calculated by a well-known formula. As used herein, the "ratio of not more than 20 for the GMT of dengue serotype 2 to the GMT of dengue serotype 4" means that the geometric

mean neutralizing antibody titer of dengue serotype 2 (GMT DENV-2) is divided by the geometric mean neutralizing antibody titer of dengue serotype 4 (GMT DENV-4) and that the ratio obtained hereby is no more than 20. In other words, the geometric mean neutralizing antibody titer of dengue serotype 2 is not more than 20-times higher than the geometric mean neutralizing antibody titer of dengue serotype 4 in the subject population.

[0055] As used herein, an "immune response" refers to a subject's response to the administration of the dengue vaccine. In particular, the immune response includes the formation of neutralizing antibodies to one or more dengue serotypes. It may also include the stimulation of a cell-mediated response or the formation of antibodies to non-structural proteins such as NS1. An immune response is stimulated by the administration of a unit dose of the invention as described herein, if the titer of neutralizing antibodies against at least one dengue virus serotype and preferably against all four dengue virus serotypes is increased after said administration of said unit dose. An immune response is stimulated by the administration of a unit dose of the invention as described herein, if the secretion of interferon gamma by peripheral blood mononuclear cells stimulated with peptides from dengue virus proteins is increased after said administration of said unit dose. An immune response is stimulated by the administration of a unit dose of the invention as described herein, if the titer of antibodies to non-structural proteins such as NS1 is increased after said administration of said unit dose. In a particular embodiment, the administration of a reconstituted unit dose of the present invention as described herein stimulates the formation of neutralizing antibodies to one or more dengue serotypes, a cell-mediated response and the formation of antibodies to non-structural proteins such as NS1.

[0056] As used herein, a "balanced immune response" means that the immune response to the four dengue serotypes is sufficient to provide protection against infection by all four dengue serotypes and preferably the immune response to the four dengue serotypes has a similar strength. In particular, the neutralizing antibody titer against the four dengue serotypes at day 180 or day 365 after administration of a first reconstituted unit dose of the invention as described herein is similar, i.e. it differs by less than factor 30, by less than factor 25 or by less than factor 20.

[0057] The „total concentration in pfu/0.5 ml" which serves as a base value for the calculation of the percentage concentration for each individual component of a tetravalent dengue vaccine is shown for one exemplary tetravalent vaccine composition comprising dengue serotype 1 in a concentration of 3.60 log10pfu/0.5 ml, a dengue serotype 2 concentration of 4.00 log10pfu/0.5 ml, a dengue serotype 3 concentration of 4.60 log10pfu/0.5 ml and a dengue serotype 4 concentration of 5.11 log10pfu/0.5 ml.
Primarily, the logarithmic values of the concentrations are converted into numerical values. The results of this conversion are $4x10^3$ pfu/0.5 ml for serotype 1, $1x10^4$ pfu/0.5ml for serotype 2, $4x10^4$ pfu/0.5 ml for serotype 3 and $1.3x10^5$ pfu/0.5 ml for serotype 4. The total concentration in pfu/0.5 ml is the sum of the preceding numerical values resulting in $1.84 x10^5$ pfu/0.5 ml.

[0058] The "percentage concentration" for each of the serotypes 1, 2, 3 and 4 is obtained by dividing the numerical concentration value (expressed as pfu/0.5 ml) of an individual serotype by the total concentration (expressed in pfu/0.5 ml) and multiplying the result by 100 i.e.:
Percentage concentration of serotype 1 = ($4x10^3$ pfu/0.5 ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 2%
Percentage concentration of serotype 2 = ($1x10^4$ pfu/0.5ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 5%
Percentage concentration of serotype 3 = ($4x10^4$ pfu/0.5 ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 22%
Percentage concentration of serotype 4 = ($1.3x10^5$ pfu/0.5 ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 71%.
The percentage concentrations are rounded to whole numbers.

[0059] As used herein "simultaneous" administration means an administration of at least two different vaccines such as a dengue vaccine and a hepatitis A vaccine on the same day. "On the same day" has the ordinary meaning of within 24 hours, such as e.g. within one calendar day. The simultaneous administration may be administered by the same medical practitioner, such as during the same medical appointment.

[0060] As used herein "sequential" administration means an administration of at least two different vaccines, such as a dengue vaccine and a yellow fever vaccine, or a dengue vaccine and a hepatitis A vaccine on different or subsequent days, such as within 90 days, but in a combined administration schedule.

[0061] As used herein, the term "chronic disease or condition" includes those diseases and conditions which persist in an elderly subject for three months or more. In particular, it includes diabetes, hypertension, allergies, previous strokes, ischemic heart disease, chronic renal impairment and chronic obstructive pulmonary disease.

[0062] As used herein, the term "impaired immune system" means that at least one function of at least one component of the immune system is weaker than in younger subjects, i.e. in subjects with an age of less than 60 years. These functions include a lower antioxidant response of monocytes against oxidative stress induced by dengue virus and lower T cell responses and cytokine production in response to dengue virus infection.

[0063] As used herein, "solicited systemic adverse events" in children under 6 years are defined as fever, irritability/fussiness, drowsiness and loss of appetite that occurred within 14 days after each vaccination, and in children of 6 years or more are defined as fever, headache, asthenia, malaise and myalgia that occurred within 14 days after each vaccination.

[0064] As used herein, "solicited local adverse events" are injection site pain, injection site erythema and injection site swelling that occurred within 7 days after each vaccination.

[0065] As used herein, "unsolicited adverse events" are any adverse events (AEs) that are not solicited local or systemic AEs, as defined above.

[0066] As used herein, a "serious adverse event" or "SAE" is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability / incapacity, is a congenital anomaly / birth defect or is medically important due to other reasons than the above mentioned criteria.

[0067] The relationship of each AE, including solicited systemic AEs (solicited local AEs are considered as related) to trial vaccine(s) will be assessed using the following categories: As used herein, "IP-Related AE" or "vaccine related AE" means that there is suspicion that there is a relationship between the vaccine and the AE (without determining the extent of probability); there is a reasonable possibility that the vaccine contributed to the AE. As used herein, "Non-IP Related" or "non-vaccine related" means that there is no suspicion that there is a relationship between the vaccine and the AE; there are other more likely causes and administration of the vaccine is not suspected to have contributed to the AE.

[0068] As used herein, a subject or subject population being "2 to 60 years of age" "or 18 to 60 years of age" refers to a subject or subject population being 2 to 60 years of age or 18 to 60 years of age on the first day of the administration of the dengue vaccine composition as described herein.

[0069] As used herein "%-points" refers to the difference of two %-values in a %-value. For example two values

in % which are within 5 %-points refers to e.g. one value at 1% and a second value at 6%.

[0070] As used herein, the term "determination of the previous dengue infection in the subject before administration" means that a previous dengue infection has to be assessed before vaccination in that there is a laboratory confirmed history of dengue or through an appropriately validated serological test e.g. by the method as disclosed herein such as the MNT50 test described in Example 2 or any serotesting with adequate performance in terms of specificity and cross reactivity based on the locale disease epidemiology.

[0071] As used herein % w/v refers to % mg/ml wherein e.g. 150 mg/ml are 15% w/v.

[0072] As used herein, the term "hepatitis A virus" may be abbreviated as "HAV".

[0073] As used herein, the term "placebo" may be abbreviated as "Pbo".

[0074] As used herein, "hepatitis A seronegative at baseline" or "hepatitis A naïve (at baseline)" each mean that a subject does not have a predefined amount of anti-hepatitis A antibodies in the serum. Quantitatively, the hepatitis A seronegativity of a subject is defined as an anti-hepatitis A antibody level of < 10 mIU/ml. When anti-hepatitis A antibody levels are determined by ELISA, the lower level of quantification is 12.5 mIU/ml which is effectively the lower anti-HAV antibody level for determining seronegativity. Subjects having anti-hepatitis A antibody levels of ≥ 12.5 mIU/ml are defined as hepatitis A seropositive. An ELISA for determining the anti-hepatitis A antibodies is for example disclosed in Beck et al. J Travel Med 2004; 11:201–207.

[0075] As used herein, "at baseline" refers to the time point of the last measurement of a subject's serostatus prior to the first vaccination.

[0076] As used herein, the unit "mIU/ml" refers to milli-international unit per milliliter. This concentration unit refers to a quantity of anti-hepatitis A antibodies in a subject's serum (e.g. when measured prior or after vaccination). As used herein, the "viral antigen activity of hepatitis A vaccines" of the present invention is expressed in terms of a standard recommendation of the WHO using an enzyme-linked immunosorbent assay (ELISA). According to this recommendation of the WHO (see WHO Information Sheet "Observed Rate of Vaccine Reactions – Hepatitis A Vaccine", published June 2012), the viral antigen activity of a hepatitis A vaccine is expressed in terms of ELISA Units (EL.U.). The viral antigen activity of a hepatitis A vaccine can for example be determined by an ELISA according to Andre FE., Hepburn A. D'Hondt E., "Inactivated candidate vaccines for hepatitis", A. Prog Med Virol 1990; 37:72-95.

[0077] As used herein, the term "CCID" refers to the quantity of virus (e.g. vaccinal virus) infecting 50% of the cell culture. The CCID50 assay is a limit dilution assay with statistical titer calculation (Morrison D et al, J Infect Dis. 2010; 201(3):370-7)).

[0078] "Non-inferiority", as used herein, with respect to a simultaneous on the same day administration of a hepatitis A vaccine and a tetravalent dengue vaccine is in particular concluded, if the seroprotection rate (SPR) difference between the SPR of a subject group receiving HAV and placebo (simultaneously on the same day, i.e. control subject population) and the SPR of a subject group receiving HAV and TDV (simultaneously on the same day) has an upper bound of a two-sided 95% confidence interval which is lower than the non-inferiority margin set at 10%, wherein seroprotection rates are based on measurements on day 30 after the simultaneous administration on day 1, calculated using the Newcombe score method. A non-inferiority clinical study is a study designed to provide a comparison between at least two methods of treatments, in the present case between a simultaneous administration

of a dengue vaccine and a hepatitis A vaccine and a mono-administration of either a dengue vaccine or a hepatitis A vaccine.

[0079] As used herein, the term "seroprotection rate", abbreviated "SPR", is defined by the proportion/percentage of HAV or DEN-naive subjects at baseline who are seroprotected against HAV or DENV, respectively, at day 30 (month 1) after the first vaccination.

[0080] As used herein, the term "control subject population" refers to a group of subjects which does not receive a simultaneous administration of a hepatitis A vaccine and a unit dose of a dengue vaccine composition, but a single verum (such as a hepatitis A vaccine or a unit dose of a dengue vaccine composition) and a placebo on the same day in a clinical study setting as e.g. in a non-inferiority clinical study.

[0081] As used herein, the term "synergism" or "synergy" is defined as an effect of simultaneously on the same day administering the hepatitis A vaccine and the unit dose of the dengue vaccine composition to a subject or subject population, wherein said administering provides a higher anti-hepatitis A antibody concentration and/or a higher mean titer of neutralizing antibodies against each of the dengue virus serotypes than the corresponding simultaneous administration of a hepatitis A vaccine and a placebo on the same day and/or the simultaneous administration of a unit dose of the dengue vaccine composition and a placebo on the same day (mono-administrations). Such higher antibody concentrations after simultaneous administration in comparison to the mono-administrations are signs in favor of the simultaneous administration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0082] Figure 1: Genetic structure of the four dengue strains contained in TDV. The solid red triangles indicate the three attenuating mutations present in the 5'NCR, NS1 and NS3 proteins. The TDV-1, TDV-3 and TDV-4 strains are chimeric viruses where the prM and E genes from dengue serotype 1, 3 and 4, respectively, are inserted into the TDV-2 backbone.

[0083] Figure 2: Schematic drawing illustrating the microneutralization test (MNT) used to determine the titer of neutralizing antibodies.

[0084] Figure 3: Flow diagram of the clinical trial of Example 3.

[0085] Figure 4: Cumulative incidence of A) virologically-confirmed dengue cases and B) hospitalized virologically-confirmed dengue cases over time during Part 1 study period by baseline serostatus (safety set data; data presented truncated at Month 18). Tables show numbers of participants under follow-up at various time points to end of Part 1 study period.

[0086] Figure 5: Study design of phase III study described in example 3.

[0087] Figure 6: Scheme of the trial design of the simultaneous HAV and TDV administration study described in Example 4.

DETAILED DESCRIPTION

Dengue virus strains

[0088] The dengue virus is a single stranded, positive sense RNA virus of the family flaviviridae. The taxonomy is outlined in Table 1. The family flaviviridae includes three genera, flavivirus, hepacivirus and pestivirus. The genus flavivirus contains highly pathogenic and potentially hemorrhagic fever viruses, such as yellow fever virus and dengue virus, encephalitic viruses, such as Japanese encephalitis virus, Murray Valley encephalitis virus and West Nile virus, and a number of less pathogenic viruses.

Table 1. Dengue Virus Taxonomy of the GMO Parental Strain

Family	Flaviviridae
Genus	Flavivirus
Species	Dengue virus
Strains	Dengue Serotype 2 (Strain 16681), Strain DEN-2 PDK-53
GMO parent	TDV-2

[0089] The flavivirus genome comprises in 5' to 3' direction (see **Figure 1**):

- a 5'-noncoding region (5'-NCR),
- a capsid protein (C) encoding region,
- a pre-membrane protein (prM) encoding region,
- an envelope protein (E) encoding region,
- a region encoding nonstructural proteins (NSl, NS2A, NS2B, NS3, NS4A, NS4B, NS5) and
- a 3' noncoding region (3'-NCR).

[0090] The viral structural proteins are C, prM and E, and the nonstructural proteins are NSl to NS5. The structural and nonstructural proteins are translated as a single polyprotein and processed by cellular and viral proteases.

[0091] The unit dose of the invention as described herein comprises a dengue virus composition that comprises four live attenuated dengue virus strains (tetravalent dengue virus composition) representing dengue serotype 1, dengue serotype 2, dengue serotype 3 and dengue serotype 4. Preferably the composition comprises chimeric dengue viruses and optionally at least one non-chimeric dengue virus, in particular a molecularly characterized and cloned dengue serotype 2 strain derived from the live attenuated DEN-2 PDK-53 virus strain (TDV-2), and three chimeric 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).

[0092] 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).

[0093] 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

[0094] 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

[0095] 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 thymidine resulting in an amino acid change at position 143 from asparagine to valine, and/or

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

c) a mutation in the NS2A gene at nucleotide 4018 from cytosine to thymidine 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 thymidine 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 thymidine to cytosine.

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

[0096] 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 glutamine, 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.

[0097] 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.

[0098] 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

[0099] A diagram of the four TDV strains comprised in the dengue vaccine composition is shown in **Figure 1**.

[00100] 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.

[00101] 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 thymidine 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 thymidine 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 thymidine 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 thymidine-guanine to cytosine-cytosine resulting in an amino acid change at position 762 from valine to alanine.

[00102] 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 thymidine resulting in an amino acid change at position 1437 from glutamine to asparagine, and/or

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

[00103] 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.

[00104] 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 thymidine 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 thymidine 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 thymidine-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 thymidine, and/or

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

[00105] 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 thymidine 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.

[00106] 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.

[00107] 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 thymidine 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 thymidine 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 thymidine-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 thymidine 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.

[00108] 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 thymidine, 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 asparagine 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/asparagine mix, and/or

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

aa) a mutation in the NS4A gene at nucleotide 6437 from cytosine to thymidine 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 thymidine to a thymidine/cytosine mix, and/or

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

[00109] In another embodiments, 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 most preferably the mutations s), u), v), c), e), x), y), aa), w), d), z), bb), cc), j), k) and t). The nucleotide positions and amino acids positions of TDV-4 refer to the nucleotide sequence as shown in SEQ ID NO. 7 and amino acid sequence as shown in SEQ ID NO. 8.

[00110] In a preferred embodiment, TDV-1 has the nucleotide sequence of SEQ ID NO. 1, TDV-2 has the nucleotide sequence of SEQ ID NO. 3, TDV-3 has the nucleotide sequence of SEQ ID NO. 5, and/or TDV-4 has the nucleotide sequence of SEQ ID NO. 7. In a further preferred embodiment, TDV-1 has the amino acid sequence of SEQ ID NO. 2, TDV-2 has the amino acid sequence of SEQ ID NO. 4, TDV-3 has the amino acid sequence of SEQ ID NO. 6, and TDV-4 has the amino acid sequence of SEQ ID NO. 8. In a further preferred embodiment, TDV-1 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 2, TDV-2 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 4, TDV-3 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 6, and TDV-4 has a nucleotide sequence encoding the amino acid sequence of SEQ ID NO. 8.

Table 4.Sequences of the TDV virus strains

SEQ ID NO.	dengue virus strain	sequence type
SEQ ID NO. 1	TDV-1	nucleotide sequence
SEQ ID NO. 2	TDV-1	amino acid sequence
SEQ ID NO. 3	TDV-2	nucleotide sequence
SEQ ID NO. 4	TDV-2	amino acid sequence
SEQ ID NO. 5	TDV-3	nucleotide sequence
SEQ ID NO. 6	TDV-3	amino acid sequence
SEQ ID NO. 7	TDV-4	nucleotide sequence
SEQ ID NO. 8	TDV-4	amino acid sequence

[00111] Thus, in a particularly preferred embodiment, the unit dose of the invention as described herein comprises the live attenuated dengue virus strains TDV-1, TDV-2, TDV-3 and TDV-4, wherein TDV-1, TDV-3 and TDV-4 are based on TDV-2 and comprise the prM and E regions of DENV-1, -3 and -4, respectively. In another particularly preferred embodiment, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00112] The E protein of DENV-3 has two fewer amino acids than the E protein of DENV-2. Therefore, the nucleotides and encoded amino acid backbone of TDV-2 starting after the E region of DENV-3 at nucleotide 2374 of SEQ ID NO. 5 and amino acid 760 of SEQ ID NO. 6 are 6 nucleotides less and 2 amino acids less than the original TDV-2 nucleotide and amino acid positions, respectively.

Dengue vaccine composition

[00113] The present invention is in part directed to a unit dose of a dengue vaccine composition as described. The dengue vaccine composition comprises a tetravalent dengue virus composition, also referred to as dengue virus composition, and pharmaceutically acceptable excipients.

Dengue virus composition, virus concentrations and %-concentrations

[00114] The present invention is in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

(i) a dengue serotype 1 preferably in a concentration of at least 3.3 log10 pfu/0.5 mL,
(ii) a dengue serotype 2 preferably in a concentration of at least 2.7 log10 pfu/0.5 mL,
(iii) a dengue serotype3 preferably in a concentration of at least 4.0 log10 pfu/0.5 mL, and
(iv) a dengue serotype 4 preferably strain in a concentration of at least 4.5 log10 pfu/0.5 mL.

[00115] In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

(i) a dengue serotype 1 preferably in a concentration of at least 3.3 log10 pfu/0.5 mL to 3.8 log10 pfu/0.5 mL,
(ii) a dengue serotype 2 preferably in a concentration of at least 2.7 log10 pfu/0.5 mL,
(iii) a dengue serotype 3 preferably in a concentration of at least 4.0 log10 pfu/0.5 mL, and

(iv) a dengue serotype 4 preferably strain in a concentration of at least 4.5 log10 pfu/0.5 ml or 4.6 log10 pfu/0.5 mL, optionally to 6.2 log10 pfu/0.5 ml.

[00116] The present invention is further in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

(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 chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log10 pfu/0.5 mL.

[00117] In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

(i) a chimeric dengue serotype 2/1 strain in a concentration of at least 3.3 log10 pfu/0.5 mL to 3.8 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 chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log10 pfu/0.5 mL or at least 4.6 log10 pfu/0.5 mL to optionally 6.2 log10 pfu/0.5 ml.

[00118] Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4.

[00119] In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/0.5 mL to 5.3 log10 pfu/0.5 mL,
(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/0.5 mL to 5.0 log10 pfu/0.5 mL,
(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/0.5 mL to 6.0 log10 pfu/0.5 mL, and
(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/0.5 mL to 6.5 log10 pfu/0.5 mL.

[00120] In one such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

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

[00121] In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 5.0 log10 pfu/dose,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 4.9 log10 pfu/ dose ,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 5.7 log10 pfu/ dose, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 5.5 log10 pfu/dose .

[00122] In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 4.1 log10 pfu/dose,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 3.6 log10 pfu/dose ,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 4.7 log10 pfu/dose, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 5.3 log10 pfu/dose.

[00123] In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/0.5 mL to 3.6 log10 pfu/0.5 mL,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/0.5 mL to 4.0 log10 pfu/0.5 mL,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/0.5 mL to 4.6 log10 pfu/0.5 mL, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/0.5 ml or 4.6 log10 pfu/0.5 mL to 5.1 log10 pfu/0.5 mL.

[00124] In another embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 4.3 log10 pfu/0.5 mL to 4.4 log10 pfu/0.5 mL,

(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 3.7 log10 pfu/0.5 mL to 3.8 log10 pfu/0.5 mL,

(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.5 log10 pfu/0.5 mL to 5.0 log10 pfu/0.5 mL, and

(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 5.5 log10 pfu/0.5 mL to 5.6 log10 pfu/0.5 mL.

[00125] In a particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 4.4 log10 pfu/0.5 mL,
(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 3.8 log10 pfu/0.5 mL,
(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.5 log10 pfu/0.5 mL, and
(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 5.6 log10 pfu/0.5 mL.

[00126] In another particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:
(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.6 log10 pfu/0.5 mL,
(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 4.0 log10 pfu/0.5 mL,
(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.6 log10 pfu/0.5 mL, and
(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 5.1 log10 pfu/0.5 mL.

[00127] In another preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein the arithmetic sum of all four serotypes is less than 6.7 log10 pfu/0.5 mL, preferably less than 5.5 log10 pfu/0.5 mL. In certain such embodiments, the arithmetic sum of all four serotypes is at least 4.6 log10 pfu/0.5 mL. In a preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains 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.

[00128] Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00129] The present invention is in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue 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/dose,
(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of at least 2.7 log10 pfu/dose,
(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of at least 4.0 log10 pfu/dose, and
(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of at least 4.5 log10 pfu/dose.

[00130] In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 5.3 log10 pfu/dose,

(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 5.0 log10 pfu/dose,

(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 6.0 log10 pfu/dose, and

(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 6.5 log10 pfu/dose.

[00131] In one such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 5.0 log10 pfu/dose,

(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 4.9 log10 pfu/dose,

(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 5.7 log10 pfu/dose, and

(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 6.2 log10 pfu/dose.

[00132] In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 5.0 log10 pfu/dose,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 4.9 log10 pfu/dose,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 5.7 log10 pfu/dose, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 5.5 log10 pfu/dose.

[00133] In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 4.1 log10 pfu/dose,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 3.6 log10 pfu/ dose,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 4.7 log10 pfu/dose, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 5.3 log10 pfu/dose.

[00134] In a further such embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 3.6 log10 pfu/dose,

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 4.0 log10 pfu/dose,

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 4.6 log10 pfu/dose, and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose 4.6 log10 pfu/dose to 5.1 log10 pfu/dose.

[00135] In another embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 4.3 log10 pfu/dose to 4.4 log10 pfu/dose,

(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 3.7 log10 pfu/dose to 3.8 log10 pfu/dose,

(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.5 log10 pfu/dose to 5.0 log10 pfu/dose, and

(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 5.5 log10 pfu/dose to 5.6 log10 pfu/dose.

[00136] In a particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 4.4 log10 pfu/dose,

(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 3.8 log10 pfu/dose,

(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.5 log10 pfu/dose, and

(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 5.6 log10 pfu/dose.

[00137] In another particularly preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein:

(i) the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.6 log10 pfu/dose,

(ii) the dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 4.0 log10 pfu/dose,

(iii) the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.6 log10 pfu/dose, and

(iv) the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 5.1 log10 pfu/dose.

[00138] In another preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein the arithmetic sum of all four serotypes is less than 6.7 log10 pfu/dose, preferably less than 5.5 log10 pfu/dose. In certain such embodiments, the arithmetic sum of all four serotypes is at least 4.6 log10 pfu/dose. in a preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains wherein the

arithmetic sum of all four serotypes is in the range of 4.6 log10 pfu/dose to 6.7 log10 pfu/dose, preferably in the range of 4.6 log10 pfu/dose to 5.5 log10 pfu/dose.

[00139] In one embodiment in the composition (i), (ii), (iii), and (iv) provide a total concentration of pfu/0.5 mL and based on said concentration, the concentration of (iii) at least 10% of the total concentration in pfu/0.5 mL.

[00140] In one embodiment in the composition (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 6%, or at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

[00141] It is preferred that the concentration in the reconstituted unit dose of (iii) in pfu/0.5 mL is at least 10%.

[00142] In one embodiment in the composition (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%.

[00143] Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00144] The concentration of the different dengue viruses is preferably determined by an immuno-focus assay known in the art. For example, the concentration may be determined by an immuno-focus assay wherein serial dilutions of dengue virus are applied to monolayers of adherent cells, such as Vero cells. After a period of time which allows infectious viruses to bind to the cells and to be taken up by the cells, an overlay containing thickening agents, such as agarose or carboxymethylcellulose, is added to prevent diffusion of viruses so that progeny viruses can only infect cells adjacent to the original infected cells. After a period of incubation to allow viral replication, cells are fixed and stained using serotype-specific anti-dengue monoclonal antibodies and a secondary antibody such as an antibody labeled with alkaline phosphatase. The foci are stained by adding a suitable substrate for the enzyme attached to the secondary antibody, such as 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium phosphatase substrate. The number of plaques on the plate corresponds to the plaque forming units of the virus in the solutions applied to the cells. For example, a concentration of 1,000 pfu/µl indicates that 1 µl of the solution applied to the cells contains enough viruses to produce 1,000 plaques in a cell monolayer.

[00145] The dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein a chimeric dengue serotype 2/1 strain, a dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain, and a chimeric dengue serotype 2/4 strain provide a total concentration in pfu/0.5 mL. The term "total concentration in pfu/0.5 mL" or "total concentration in pfu/dose" is the sum of the concentrations of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), dengue serotype 2 (e.g. the dengue serotype 2 strain), the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) and the dengue

serotype 4 (e.g. chimeric dengue serotype 2/4 strain), preferably the sum of the concentrations of TDV-1, TDV-2, TDV-3 and TDV-4, and is defined as 100% of the dengue virus concentration as determined by pfu (plaque forming units) in 0.5 mL or in a dose.

[00146] In one embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), a dengue serotype 2 (e.g. dengue serotype 2 strain), a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain), and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) provide a total concentration in pfu/0.5 mL, wherein based on said total concentration the concentration of a dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 10% of the total concentration, or less than 8%, or less than 6% of the total concentration, and wherein the concentration of a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 50% or at least 60% or at least 65% of the total concentration. In one embodiment, based on said total concentration the concentration of a dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is 0.3 to 10% or 0.5 to 8% of the total concentration and the concentration of a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is 50% to 90% or 60% to 88% of the total concentration. This means that the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) is lower than the concentration of the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain).

[00147] In one such embodiment, the concentration of a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is at least 1% of the total concentration, and/or the concentration of a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 6% of the total concentration, or at least 7% or 8%, 10%, 12%, 14%, 16% or 18% of the total concentration. In one such embodiment, the concentration of a dengue serotype 2 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% or 2% to 6% or 2.0% to 5.0% of the total concentration, and/or the concentration of a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is 6% to 25% or 7% to 25% or 10% to 25% or 18% to 25% of the total concentration. This means that the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) is lower than the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain).

[00148] In a preferred embodiment, the concentration of a dengue serotype 2 strain, such as TDV-2, measured in pfu/0.5 mL is less than 10% of the total concentration, preferably less than 6% or less than 2%, the concentration of a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain), such as TDV-4, measured in pfu/0.5 mL is at least 50% of the total concentration, preferably at least 65%, the concentration of a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), such as TDV-1, measured in pfu/0.5 mL is at least 1% of the total concentration, preferably between 1% and 7% or 2.0% to 5.0%, and the concentration of a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain), such as TDV-3, measured in pfu/0.5 mL is at least 6% of the total concentration, preferably between 6% and 25% or 10% to 25% or 18% to 25%.

[00149] In a further preferred embodiment, a dengue virus composition comprising a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), a dengue serotype 2 (e.g. dengue serotype 2 strain), a dengue serotype 1 (e.g. chimeric dengue serotype 2/3 strain), and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain), such as TDV-1, TDV-2, TDV-3 and TDV-4, is provided, wherein the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is at least 1% of the total concentration, preferably between 1% and 7% or 2.0% and 5.0%, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 10% of the total concentration, preferably less than 6% or less than 2% and the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least

6% of the total concentration, preferably between 6% and 25% or 10% to 25% or 18% to 25%. It is particularly preferred that the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has the highest concentration of all four dengue serotypes.

[00150] In a further preferred embodiment, the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% of the total concentration, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 10% of the total concentration, and the concentration of the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%. In certain such embodiments, 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.

[00151] In a further preferred embodiment the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and the dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-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 certain such embodiments the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 is preferably at least about 10% of the total concentration in pfu/0.5 mL and more preferably the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 is at least about 70% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 each represent lower concentrations than the concentration of serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

[00152] Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00153] According to a further embodiment, the chimeric dengue serotype 2/4 strain, preferably TDV-4, has the highest concentration in the dengue vaccine composition, followed by the chimeric dengue serotype 2/3 strain, preferably TDV-3, followed by the chimeric dengue serotype 2/1 strain, preferably TDV-1, followed by the dengue serotype 2 strain, preferably TDV-2. It is particularly preferred that the dengue serotype 2 strain has the lowest concentration of the four strains present in the dengue vaccine composition.

[00154] Whenever reference is made to a concentration/0.5ml, this does not limit the volume of the unit dose described herein to 0.5ml. 0.5ml is the reference volume for the determination of the concentrations of the virus strains in the composition in pfu/ml. The volume and/or amount per unit dose is described in the respective chapter.

Pharmaceutically acceptable excipients

[00155] The present invention is in part directed to a unit dose of a dengue vaccine composition, wherein the dengue vaccine composition comprises one or more pharmaceutically acceptable excipients. In one embodiment, the dengue vaccine composition comprises a non-reducing sugar, a surfactant, a protein and an inorganic salt. Preferably, the non-reducing sugar is trehalose, the surfactant is poloxamer 407, the protein is human serum albumin and the inorganic salt is sodium chloride.

[00156] In one embodiment, the unit dose of a dengue vaccine composition comprises the following pharmaceutically acceptable excipients:

- 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 140 mM sodium chloride.

[00157] In one embodiment, the unit dose of a dengue vaccine composition comprises the following pharmaceutically acceptable excipients when measured in 0.5 ml:

- from about 10 % w/v to about 20 % w/v α,α-trehalose 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 140 mM sodium chloride, and preferably
- has a pH of 7 to 8.5.

[00158] In one embodiment, the unit dose of a dengue vaccine composition comprises the following pharmaceutically acceptable excipients when measured in 0.5ml:

- from about 143 mg/ml to about 185 mg/ml α,α-trehalose dihydrate or an equimolar amount of other forms of α,α-trehalose,
- from about 9.1 mg/ml to about 12.4 mg/ml poloxamer 407,
- from about 0.88 % mg/ml to about 1.32 mg/ml human serum albumin, and
- from about 70 mM to 140 mM sodium chloride, and preferably
- has a pH of 7 to 8.5.

[00159] In a preferred embodiment, the lyophilized unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients:

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

[00160] In a preferred embodiment, the lyophilized unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients when measured in 0.5ml:

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

[00161] In a preferred embodiment, the lyophilized unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients:

- about 82.9 mg α,α-trehalose dihydrate,
- about 5 mg poloxamer 407,
- about 0.5 mg human serum albumin, and
- about 50 µmoles sodium chloride.

[00162] In a preferred embodiment, the reconstituted unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients:

- about 15 % w/v α,α-trehalose dihydrate,
- about 1 % w/v poloxamer 407,
- about 0.1 % w/v human serum albumin, and
- about 137 mM sodium chloride, and preferably
- has a pH of 7 to 8.5

[00163] In a preferred embodiment, the reconstituted unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients when measured in 0.5 ml:

- about 15 % w/v α,α-trehalose,
- about 1 % w/v poloxamer 407,
- about 0.1 % w/v human serum albumin, and preferably
- about 137 mM sodium chloride and preferably
- has a pH of 7 to 8.5.

[00164] In a preferred embodiment, the reconstituted unit dose of the invention as described herein comprises the following pharmaceutically acceptable excipients:

- about 82.9 mg α,α-trehalose dihydrate,
- about 5 mg poloxamer 407,
- about 0.5 mg human serum albumin, and preferably
- about 68.5 µmoles sodium chloride, and preferably
- has a pH of 7 to 8.5.

[00165] The human serum albumin may be a native or recombinant human serum albumin (rHSA). The poloxamer 407 may be e.g. Pluronic F127.

[00166] In one embodiment, the unit dose further comprises a buffer. The buffer may be phosphate buffered saline (PBS). The buffer may include at least one of sodium chloride (NaCl), monosodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). In a preferred embodiment, the buffer may include disodium hydrogen phosphate (Na_2HPO_4),

potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). The buffer may have a pH in the range of 7.0 to 8.5 at 25°C.

Unit dose

[00167] The present invention is directed in part to a unit dose of a dengue vaccine composition comprising a tetravalent dengue virus composition as described herein and pharmaceutically acceptable excipients as described herein.

[00168] The present invention is directed in part to a unit dose of a dengue vaccine composition as described above e.g. of

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

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) with 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) with 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) with a concentration of at least 4.5 log10 pfu/0.5 mL.

[00169] Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3, and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00170] In one embodiment, the unit dose is lyophilized. In one such embodiment, the lyophilized unit dose is obtained by subjecting a volume of 0.5 mL of the aqueous dengue vaccine composition produced by combining pharmaceutically acceptable excipients as described herein and the dengue vaccine composition as described herein comprising the four dengue virus strains, in particular TDV-1 to TDV-4, to lyophilization. In a preferred embodiment the residual moisture content as determined by Karl Fischer Determination is equal to or less than 5.0%, preferably equal to or less than 3%.

[00171] In another embodiment, the unit dose is reconstituted. The reconstituted unit dose is obtained by subjecting the lyophilized unit dose to reconstitution with a pharmaceutically acceptable diluent, preferably before administration of the dengue vaccine. In one such embodiment, reconstitution will be accomplished by adding a pharmaceutically acceptable diluent, such as water for injection, phosphate buffered saline or an aqueous sodium chloride solution, to the lyophilized unit dose. In one embodiment, an aqueous sodium chloride solution, such as a 37 mM aqueous sodium chloride solution, is added to the lyophilized unit dose for reconstitution. In one such embodiment, the lyophilized unit dose will be reconstituted with 0.3 to 0.8 mL, or 0.4 to 0.7 mL, or 0.5 mL of diluent. In a preferred embodiment, the lyophilized unit dose is reconstituted with 0.3 to 0.8 mL, 0.4 to 0.7 mL or 0.5 mL of 37 mM aqueous sodium chloride solution. In a more preferred embodiment, the lyophilized unit dose is reconstituted with 0.5 mL of 37 mM aqueous sodium chloride solution. The reconstituted unit dose can subsequently be administered subcutaneously.

[00172] It is preferred that the unit dose in lyophilized form is the final product after manufacture of the unit dose and the storage form of the unit dose, wherein the unit dose in reconstituted form is prepared before administration of the unit dose to a subject.

[00173] The present invention is, moreover, directed in part to a unit dose of a dengue vaccine composition 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, such as 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, such as a dengue serotype 2 strain, in a concentration of at least 2.7 log10 pfu/0.5 ml,

(iii) a dengue serotype 3, such as 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.

[00174] In one embodiment, the reconstituted unit dose has a volume of e.g. 0.5 mL, 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 concentration, the concentration of (iii) at least 10% of the total concentration in pfu/0.5 mL.

[00175] In another embodiment the reconstituted unit dose has a volume of e.g. 0.5 mL, 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 6%, or at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

[00176] It is preferred that the concentration in the reconstituted unit dose of (iii) in pfu/0.5 mL is at least 10%.

[00177] In one embodiment the reconstituted unit dose has a volume of e.g. 0.5 mL, 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%.

[00178] In one embodiment, the present invention is directed to a lyophilized unit dose of a dengue vaccine composition comprising upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) with a concentration of at least 3.3 log10 pfu/0.5 mL, a dengue serotype 2 (e.g. dengue serotype 2 strain) with a concentration of at least 2.7 log10 pfu/0.5 mL, a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) with a concentration of at least 4.0 log10 pfu/0.5 mL, and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) with a concentration of at least 4.5 log10 pfu/0.5 mL and pharmaceutically acceptable excipients as described herein, wherein the unit dose is preferably formulated in 0.5 mL

before lyophilization. Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00179] In one such embodiment, the lyophilized unit dose is obtained by lyophilizing 0.5 mL of a dengue vaccine composition comprising a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of 3.3 log10 pfu/dose to 5.0 log10 pfu/0.5 mL, a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of 2.7 log10 pfu/dose to 4.9 log10 pfu/0.5 mL, a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of 4.0 log10 pfu/dose to 5.7 log10 pfu/0.5 mL, and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of 4.5 log10 pfu/dose to 5.5 log10 pfu/0.5 mL and pharmaceutically acceptable excipients as described herein. Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4.

[00180] In one such embodiment, the lyophilized unit dose is obtained by lyophilizing 0.5 mL of a dengue vaccine composition comprising a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) in a concentration of 3.3 log10 pfu/0.5 mL to 3.6 log10 pfu/0.5 mL, a dengue serotype 2 (e.g. dengue serotype 2 strain) in a concentration of 2.7 log10 pfu/0.5 mL to 4.0 log10 pfu/0.5 mL, a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) in a concentration of 4.0 log10 pfu/0.5 mL to 4.6 log10 pfu/0.5 mL, and a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) in a concentration of 4.5 log10 pfu/0.5 mL or 4.6 log10 pfu/0.5 mL to 5.1 log10 pfu/0.5 mL and pharmaceutically acceptable excipients as described herein. Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4.

[00181] In certain embodiments, the lyophilized unit dose refers to 0.5 mL before lyophilization, wherein TDV-2 and TDV-4 are present in certain relative amounts, based on the total concentration of TDV-1, TDV-2, TDV-3 and TDV-4 in pfu/0.5 mL, and the concentration of TDV-2 measured in pfu/0.5 mL is less than 10% or less than 8% or less than 6%, and the concentration of TDV-4 measured in pfu/0.5 mL is at least 50% or at least 65%. In some of these embodiments, the concentration of TDV-1 measured in pfu/0.5 mL is at least 1% and/or the concentration of TDV-3 measured in pfu/0.5 mL is at least 6%, 7%, 8%, 10%, 12%, 14%, 16% or at least 18%.

[00182] In certain embodiments, the reconstituted unit dose has a volume of 0.5 mL and TDV-2 and TDV-4 are present in certain relative amounts, based on the total concentration of TDV-1, TDV-2, TDV-3 and TDV-4 in pfu/0.5 mL, and the concentration of TDV-2 measured in pfu/0.5 mL is less than 10% or less than 8% or less than 6%, and the concentration of TDV-4 measured in pfu/0.5 mL is at least 50% or at least 65%. In some of these embodiments, the concentration of TDV-1 measured in pfu/0.5 mL is at least 1% and/or the concentration of TDV-3 measured in pfu/0.5 mL is at least 6%, 7%, 8%, 10%, 12%, 14%, 16% or at least 18%.

[00183] In a further preferred embodiment, the reconstituted unit dose has a volume of 0.5 mL and comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein the concentration of the dengue serotype 1 (e.g. dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% of the total concentration, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is

less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, the concentration of the dengue serotype 3 (e.g. dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 10% of the total concentration, and the concentration of the dengue serotype 4 (e.g. dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%. In certain such embodiments, 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.

[00184] In a further preferred embodiment, the reconstituted unit dose has a volume of 0.5 mL and comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains, wherein the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and the dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-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 certain such embodiments the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 is preferably at least about 10% of the total concentration in pfu/0.5 mL and more preferably the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 is at least about 70% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 each represent lower concentrations than the concentration of serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

[00185] The lyophilized unit dose reconstituted in 0.5 mL will provide the above concentrations for the four dengue serotypes. While the unit dose of a dengue vaccine composition as described herein refers to the concentrations of the dengue serotypes in 0.5 mL, the lyophilized unit dose can be reconstituted with other volumes of a pharmaceutically acceptable diluent, such as an aqueous sodium chloride solution, without changing the absolute virus amount administered or the ratios of the viruses to one another.

[00186] In certain embodiments, the lyophilized unit dose of the invention is prepared from a solution comprising a non-reducing sugar, a surfactant, a protein and an inorganic salt.

[00187] In certain embodiments, the lyophilized unit dose of the invention is prepared from a solution comprising trehalose, poloxamer 407, human serum albumin and sodium chloride.

[00188] In certain embodiments, the lyophilized unit dose of the invention is prepared from a solution comprising 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 about 70 mM to about 120 mM sodium chloride.

[00189] In preferred embodiments, the lyophilized unit dose of the invention as described herein 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 about 100 mM sodium chloride.

[00190] In one embodiment, the solution from which the lyophilized unit dose is prepared further comprises a buffer. The buffer may be phosphate buffered saline (PBS). The buffer may include at least one of sodium chloride (NaCl), monosodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). In a preferred embodiment, the buffer may include disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and potassium dihydrogen phosphate (KH_2PO_4). The buffer may have a pH in the range of about 7.0 to about 8.5 at 25°C or a pH of about 6.8 to about 7.6 at 25°C, preferably a pH of about 7.2 at 25°C.

[00191] In preferred embodiments, the reconstituted unit dose of the invention as described herein comprising about 15 % w/v α,α-trehalose dihydrate, about 1 % w/v poloxamer 407, about 0.1 % w/v human serum albumin and about 137 mM sodium chloride. The reconstituted unit dose may have a pH of about 7.0 to about 8.5 at 25°C, preferably a pH of about 7.2 at 25°C.

[00192] The unit dose of the invention as described herein activates multiple arms of the immune system – neutralizing antibodies, cellular immunity and anti-NS1 antibodies – in both seronegative and seropositive subject populations or in both seronegative and seropositive subjects. Thus, the unit dose of the invention as described herein protects both dengue seronegative and dengue seropositive subject populations or subjects against dengue disease.

[00193] In one embodiment, one unit dose is present in a container, preferably a vial, and said unit dose is administered to a subject after reconstitution. In one embodiment, more than one unit dose of the dengue vaccine composition may be present in a container, preferably a vial, so that with the content of one container, preferably a vial, more than one subject can be vaccinated. In one embodiment, the container comprising more than one unit doses of the invention as described herein is used for providing the reconstituted unit dose to be used in the methods of the invention as described herein.

[00194] The certain embodiments, the container comprising the unit dose of the invention is part of a kit. Thus, the invention is directed in part to a kit for preparing a reconstituted unit dose comprising a lyophilized unit dose of the present invention as described herein, and a pharmaceutically acceptable diluent for reconstitution.

[00195] In certain embodiments, the diluent for reconstitution provided in a container, preferably a vial, or a pre-filled syringe. In some embodiments, the diluent for reconstitution is selected from water for injection, phosphate buffered saline or an aqueous sodium chloride solution. In a preferred embodiment, the diluent for reconstitution is 30 to 40 mM sodium chloride, such as 37 mM sodium chloride.

[00196] In certain embodiments, the kit may further comprise a hepatitis A vaccine, such as HAVRIX® or VAQTA®. In some embodiments, the hepatitis A vaccine may be in a separate container, such as a vial. In another embodiment, the hepatitis A vaccine and the unit dose of the invention may be in the same container. Thus, the invention is directed in part to a combined dengue/hepatitis A vaccine, wherein the unit dose of the invention as described herein is combined with a hepatitis A vaccine. Such a combined dengue/hepatitis A vaccine comprises the unit dose of the invention as described herein and a hepatitis A vaccine, such as HAVRIX® or VAQTA®, in the same formulation. In certain embodiments, the invention is directed to a kit comprising such a combined dengue/hepatitis A vaccine and a unit dose of the invention as described herein.

Hepatitis A vaccine

[00197] In certain embodiments, the hepatitis A vaccine is an inactivated hepatitis A vaccine.

[00198] In certain embodiments, the hepatitis A vaccine comprises a hepatitis A virus derived from a hepatitis A virus strain HM-175.

[00199] In certain embodiments, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and the inactivated hepatitis A virus is derived from a wild-type hepatitis A virus strain HM-175.

[00200] In certain embodiments, the inactivated hepatitis A virus is adsorbed on a carrier aluminum. In some of these embodiments, the aluminum is aluminum hydroxide or aluminum hydroxyphosphate sulfate.

[00201] In certain embodiments, wherein the hepatitis A vaccine comprises a phosphate-buffered saline solution and excipients dissolved therein in the form of an amino acid and in the form of polysorbate. In such embodiments, the amino acid is present at a concentration of 0.2 to 0.8% w/v and/or the polysorbate is present at a concentration of 0.01 to 0.09 mg/ml.

[00202] In certain embodiments, the hepatitis A vaccine includes a hepatitis A virus expressing a viral antigen in a concentration ranging from 500 ELISA Units (EL.U.) to 2000 ELISA Units (EL.U.), preferably from 700 EL.U. to 1600 EL.U., most preferably from 1300 to 1550 EL.U.. Alternatively, the concentration ranges from 500 EL.U. to 900 EL.U.. In a further embodiment, the concentration ranges from 200 to 400 EL.U.

[00203] In certain embodiments, the hepatitis A vaccine is included in a liquid 1 ml dose or in a 0.5 ml dose.

[00204] An example of such an hepatitis A vaccine is HAVRIX®, from GlaxoSmithKline, which is a sterile suspension of inactivated virus for intramuscular administration. HAVRIX® makes use of the hepatitis A virus strain HM-175 which is derived from a wild-type hepatitis A virus (HAV) HM-175 of which the complete nucleotide sequence is disclosed in Cohen et al., Journal of Virology, Vol. 61, No. 1, published Jan. 1987, p. 50 to 59 (in particular, the entire sequence of the wild-type hepatitis A virus HM-175 is provided in Figure 1 of said publication).

[00205] The virus (strain HM175) is propagated in MRC-5 human diploid cells. After removal of the cell culture medium, the cells are lysed to form a suspension. This suspension is purified through ultrafiltration and gel permeation chromatography procedures. Treatment of this lysate with formalin ensures viral inactivation. Viral antigen activity is referenced to a standard using an enzyme linked immunosorbent assay (ELISA), and is therefore expressed in terms of ELISA Units (EL.U.). Each 1-mL dose for adults (≥ 18 years of age) of vaccine contains 1440 EL.U. of viral antigen, adsorbed on 0.5 mg of aluminum as aluminum hydroxide. Each 0.5-mL dose for children and adolescents (12 months through 18 years of age) of vaccine contains 720 EL.U. of viral antigen, adsorbed onto 0.25 mg of aluminum as aluminum hydroxide. HAVRIX® contains the following excipients: Amino acid supplement (0.3% w/v) in a phosphate-buffered saline solution and polysorbate 20 (0.05 mg/mL). From the manufacturing process, HAVRIX® also contains residual MRC-5 cellular proteins (not more than 5 µg/mL), formalin (not more than 0.1 mg/mL), and neomycin sulfate (not more than 40 ng/mL), an aminoglycoside antibiotic included in the cell growth media. HAVRIX® is formulated without preservatives.

[00206] Another useful hepatitis A vaccine is VAQTA®from Merck Sharp & Dohme Corp., which is an inactivated whole virus vaccine derived from hepatitis A virus grown in cell culture in human MRC-5 diploid fibroblasts. It contains inactivated virus of a strain, which was originally derived by further serial passage of a proven attenuated strain. The virus is grown, harvested, purified by a combination of physical and high performance liquid chromatographic techniques developed at the Merck Research Laboratories, formalin inactivated, and then adsorbed onto amorphous aluminum hydroxyphosphate sulfate. VAQTA® is a sterile suspension for intramuscular injection. One milliliter of the vaccine contains approximately 50 U of hepatitis A virus antigen, which is purified and formulated

without a preservative. Within the limits of current assay variability, the 50 U dose of VAQTA® contains less than 0.1 µg of non-viral protein, less than 4 x 10^{-6} µg of DNA, less than 10^{-4} µg of bovine albumin, and less than 0.8 µg of formaldehyde. Other process chemical residuals are less than 10 parts per billion (ppb), including neomycin. Each 0.5-mL pediatric dose contains 25 U of hepatitis A virus antigen and adsorbed onto approximately 0.225 mg of aluminum provided as amorphous aluminum hydroxyphosphate sulfate, and 35 µg of sodium borate as a pH stabilizer, in 0.9% sodium chloride. Each 1-mL adult dose contains 50 U of hepatitis A virus antigen and adsorbed onto approximately 0.45 mg of aluminum provided as amorphous aluminum hydroxyphosphate sulfate, and 70 µg of sodium borate as a pH stabilizer, in 0.9% sodium chloride.

Yellow Fever vaccine

[00207] YF-VAX®, a yellow fever vaccine from Sanofi, for subcutaneous use, is prepared by culturing the YF-17D strain of yellow fever virus in living avian leukosis virus-free (ALV-free) chicken embryos. The vaccine contains sorbitol and gelatin as a stabilizer and is lyophilized. No preservative is added. YF-VAX is formulated to contain not less than 4.74 $\log_{10}$ pfu per 0.5 mL dose throughout the life of the product.

Combined Vaccine Composition

[00208] The present invention is also directed in part to a combined vaccine composition comprising a hepatitis A antigen as in HAVRIX® or VAQTA®, and a dengue antigen such as the tetravalent dengue vaccine, TDV, as disclosed herein or any other suitable tetravalent live attenuated dengue virus vaccine.

[00209] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

(i) a dengue serotype 1 preferably in a concentration of at least 3.3 log10 pfu/0.5 mL,
(ii) a dengue serotype 2 preferably in a concentration of at least 2.7 log10 pfu/0.5 mL,
(iii) a dengue serotype3 preferably in a concentration of at least 4.0 log10 pfu/0.5 mL, and
(iv) a dengue serotype 4 preferably strain in a concentration of at least 4.5 log10 pfu/0.5 mL.

[00210] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the dengue vaccine composition comprises a tetravalent dengue virus composition including four live attenuated dengue virus strains:

(i) a chimeric dengue serotype 2/1 strain in a concentration of at least 3.3 log10 pfu/0.5 mL to 3.8 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 chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log10 pfu/0.5 mL or at least 4.6 log10 pfu/0.5 mL to optionally 6.2 log10 pfu/0.5 ml.

[00211] Preferably, in said embodiments the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably, TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00212] In certain embodiments, the invention is directed to the combined vaccine composition, wherein upon reconstitution of the dengue vaccine composition 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 (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 6%, at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

[00213] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the dengue vaccine composition comprises one or more pharmaceutically acceptable excipients. In one embodiment, the dengue vaccine composition comprises a non-reducing sugar, a surfactant, a protein and an inorganic salt. Preferably, the non-reducing sugar is trehalose, the surfactant is poloxamer 407, the protein is human serum albumin and the inorganic salt is sodium chloride.

[00214] Furthermore, any vaccine excipients or combinations thereof known to the person skilled in the art, e.g. disclosed in WO 2018/027075 A1, can be used for the combined vaccine composition.

[00215] In one embodiment, the unit dose of a dengue vaccine composition comprises the following pharmaceutically acceptable excipients:

- 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 140 mM sodium chloride.

[00216] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the dengue vaccine composition comprises other dengue vaccines such as Dengvaxia®. Dengvaxia® is a tetravalent dengue vaccine with mixed chimeric dengue viruses based on a yellow fever backbone, CYD-TDV (Dengvaxia®, Sanofi Pasteur, Lyon, France), and has been licensed in several countries based on the clinical demonstration of an overall vaccine efficacy (VE) against virologically-confirmed dengue (VCD) of 56–61% in children in Asia and Latin America (Capeding MR et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014, 384:1358-65; Villar LA et al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J 2013, 32:1102-9). The preparation of these particular strains CYD1, CYD2, CYD3 and CYD4 has been described in detail in international patent applications WO 98/37911, WO 03/101397, WO07/021672, WO 08/007021, WO 08/047023 and WO 08/065315, to which reference may be made for a precise description of the processes for their preparation. The corresponding nucleotide sequences of the prM-E regions of CYD1, CYD2, CYD3 and CYD4 are provided in WO2016034629 and SEQID NOs are set out in Table 16 of this reference.

[00217] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the quantity of a chimeric dengue virus within CYD-TDV comprised in a vaccine composition of the present invention lies within a range of about 10^5 CCID50 to about 10^6 CCID50. The quantity of a live attenuated chimeric dengue virus of each of serotypes 1 to 4 comprised in the CYD dosage form, e.g. Dengvaxia®, is preferably equal.

[00218] In such embodiments, the CYD-TDV is dissolved/dissolvable in a solution containing 0.4% NaCl.

[00219] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the dengue vaccine composition comprises other dengue vaccines such as TV003 or TV005. TV003, developed by the U.S. National Institute of Allergy and Infectious Diseases, comprises vaccine components rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31 and rDEN4Δ30, wherein each of these components is present at a concentration of 3 $\log_{10}$PFU. TV005 is similar to TV003 with the difference that the concentration of rDEN2/4Δ30 in TV005 is 4 $\log_{10}$PFU. The vaccines TV003 and TV005 and their vaccine components as well as their production are described in more detail in WO 2008/022196 A2 and S.S. Whitehead, Expert Rev Vaccines, 2016, 15(4): 509 to 517. Using recombinant DNA technology, two attenuation strategies were utilized for the vaccine components of TV003 or TV005: deletions in the 3′ untranslated region and structural gene chimerization. For example, the component rDEN4Δ30 contains all the structural and non-structural proteins of a wild type DENV-4, but is attenuated by a 30-nucleotide deletion in the 3′ untranslated region (denoted "Δ30"). The other vaccine components are also attenuated due to the 30-nucleotide deletion in the 3′ untranslated region. In addition, rDEN3Δ30/31 includes a 31 nucleotide deletion in the 3′ untranslated region (shown in detail in Fig. 1c and Fig. 13 of WO 2008/022196 A2). The rDEN2/4Δ30 component was created by substituting the prM and E genes of DENV-2 into the rDEN4Δ30 genome. The complete genomic sequences of dengue strains which can be used to produce TV003 or TV005 are available under the Genbank accession numbers in Table A of WO 2008/022196 A1.

[00220] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the hepatitis A vaccine is an inactivated hepatitis A vaccine.

[00221] In certain embodiments, wherein the hepatitis A vaccine comprises a hepatitis A virus derived from a hepatitis A virus strain HM-175.

[00222] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and the inactivated hepatitis A virus is derived from a wild-type hepatitis A virus strain HM-175.

[00223] In certain embodiments, the invention is directed to the combined vaccine composition, the inactivated hepatitis A virus is adsorbed on a carrier aluminum. In some of these embodiments, the aluminum is aluminum hydroxide or aluminum hydroxyphosphate sulfate.

[00224] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the hepatitis A vaccine comprises a phosphate-buffered saline solution and excipients dissolved therein in the form of an amino acid and in and in the form of polysorbate. In such embodiments, the amino acid is present at a concentration of 0.2 to 0.8% w/v and/or the polysorbate is present at a concentration of 0.01 to 0.09 mg/ml.

[00225] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the hepatitis A vaccine includes a hepatitis A virus expressing a viral antigen in a concentration ranging from 500 ELISA Units (EL.U.) to 2000 ELISA Units (EL.U.), preferably from 700 EL.U. to 1600 EL.U., most preferably from 1300 to 1550 EL.U.. Alternatively, the concentration ranges from 500 EL.U. to 900 EL.U.. In a further embodiment, the concentration ranges from 200 to 400 EL.U..

[00226] In certain embodiments, the invention is directed to the combined vaccine composition, wherein the combined vaccine is included in a dose comprising a liquid, wherein the liquid has a volume of 0.5 ml, 1 ml, or 1.5 ml.

[00227] In certain embodiments, the combined vaccine composition is provided in one single vial in a liquid form or in a dehydrated form, such as a lyophilized form.

[00228] In certain embodiments, the combined vaccine composition is obtained from mixing a unit dose of a dengue vaccine composition and a dose of a hepatitis A vaccine in a syringe.

[00229] The invention is also directed in part to a method of administering any of the above combined vaccine compositions to a subject or subject population.

[00230] In certain embodiments, the invention is directed to said methods, wherein the combined vaccine composition is administered subcutaneously or intramuscularly.

Method of preventing dengue disease and hepatitis A, corresponding uses, and corresponding kit

[00231] The present invention is directed to a method of preventing hepatitis A and dengue disease.

[00232] The present invention is directed in part to a method of preventing hepatitis A and dengue disease in a subject or subject population, the method comprising simultaneously on the same day administering a hepatitis A vaccine, such as HAVRIX® or VAQTA®, and a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

[00233] In certain embodiments, the invention is directed to said method, wherein the hepatitis A vaccine, such as HAVRIX®, comprises an inactivated virus. Preferably, the hepatitis A vaccine comprises an inactivated hepatitis A virus and the inactivated hepatitis A virus is derived from a hepatitis A virus strain HM-175.

[00234] In certain embodiments, the hepatitis A vaccine, such as HAVRIX®, is derived from a hepatitis A virus strain HM-175.

[00235] In certain embodiments, the invention is directed to said methods, wherein the hepatitis A vaccine, such as HAVRIX®, which is preferably a virus derived from a hepatitis A virus strain HM-175, is adsorbed on aluminum. According to some of these embodiments, the aluminum is aluminum hydroxide or aluminum hydroxyphosphate sulfate.

[00236] In certain embodiments, the invention is directed to said method, wherein the hepatitis A vaccine, such as HAVRIX®, which is preferably derived from a hepatitis A virus strain HM-175, comprises a phosphate-buffered saline solution and excipients dissolved therein in the form of an amino acid and in and in the form of polysorbate.

[00237] In certain embodiments, the invention is directed to said method, wherein the hepatitis A vaccine, such as HAVRIX®, includes a hepatitis A virus expressing a viral antigen in a concentration ranging from 500 ELISA Units (EL.U.) to 2000 ELISA Units (EL.U.), preferably from 700 EL.U. to 1600 EL.U., most preferably from 1300 to 1550 EL.U.. Alternatively, the concentration ranges from 500 EL.U. to 900 EL.U.. In a further embodiment, the concentration ranges from 200 to 400 EL.U..

[00238] For example, viral antigen activity of a hepatitis A vaccine can be measured according to a method disclosed in Andre FE., Hepburn A., D'Hondt E., "Inactivated candidate vaccines for hepatitis", A. Prog Med Virol 1990; 37:72-95.

[00239] In certain embodiments, the invention is directed to said method, wherein the dengue vaccine composition 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 chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log10 pfu/0.5 mL.

[00240] According to some of these embodiments, upon reconstitution of the dengue vaccine composition 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 (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 6%, at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

[00241] In certain embodiments, the invention is directed to said methods, wherein the subject population or subject is seronegative with respect to all dengue serotypes. According to some of these embodiments, the subject population or subject is seronegative with respect to hepatitis A at baseline.

[00242] In certain embodiments the invention is directed to said methods, wherein the unit dose of the invention as described herein and the hepatitis A vaccine, such as HAVRIX® or VAQTA®, are administered on day 0/1.

[00243] In certain embodiments, the invention is directed to said methods, wherein the unit dose of the invention as described herein is administered by subcutaneous injection and wherein the hepatitis A vaccine, such as HAVRIX® or VAQTA®, is administered by intramuscular injection. According to some embodiments, the injections are administered to the arm, preferably to the deltoid region of the arm. According to some of these embodiments, the subcutaneous injection of the unit dose of the invention as described herein and the intramuscular injection of the hepatitis A vaccine, such as HAVRIX® or VAQTA®, are administered to different anatomical sites, such as to opposite arms.

[00244] In certain embodiments, the invention is directed to said methods, wherein two unit doses of the dengue vaccine composition of the invention as described herein are administered. In some embodiments, the two unit doses of the invention as described herein are administered within 12 month or more, or within 6 month, or within three months, such as at day 0/1 and day 90. According to some of these embodiments, a further third unit dose of the invention as described herein is administered after the second administration. Such a third administration may be administered between 6 to 12 months after the first administration, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months (1 year) after the second administration or even 5 years or longer after the first or second administration and may act as a booster.

[00245] In certain embodiments, the invention is directed to said methods, wherein two unit doses of the invention as described herein and one dose of a hepatitis A vaccine, such as HAVRIX® or VAQTA®, are administered, in particular according to the following schedule

- a first simultaneous administration of the first unit dose and said hepatitis A vaccine on day 0/1, and
- a second administration of the second unit dose after said first simultaneous administration, such as about 3 months later such as on day 90.

[00246] In certain embodiments, the invention is directed to said method, wherein the unit dose of the invention as described herein is administered subcutaneously to a subject or subject population and the hepatitis A vaccine, such as HAVRIX® or VAQTA®, is administered intramuscularly to a subject or subject population, and wherein the subject or the subject population is seronegative with respect to all dengue serotypes. In other embodiments, the subject or subject population is seropositive with respect to at least one dengue serotype.

[00247] In certain embodiments, the invention is directed to said method, wherein the unit dose of the invention as described herein and the hepatitis A vaccine, such as HAVRIX® or VAQTA®, are administered to a subject or subject population from a dengue endemic region. In certain embodiments, the unit dose of the invention as described herein is administered subcutaneously and the hepatitis A vaccine, such as HAVRIX® or VAQTA®, is administered intramuscularly to a subject or subject population from a dengue endemic region.

[00248] In certain embodiments, the invention is directed to said method, wherein the subject or subject population is from a dengue non-endemic region, preferably from a dengue non-endemic and a hepatitis A non-endemic region.

[00249] According to some embodiments, a second dose of a hepatitis A vaccine, such as HAVRIX® or VAQTA®, is administered. The second dose of the hepatitis A vaccine may be administered after the first administration of the hepatitis A vaccine. Such a second administration may act as a booster and may be administered 6 to 12 months or 6 to 18 months, such as 9 months after the first administration of the hepatitis A vaccine, such as on day 270.

[00250] In certain embodiments, the invention is directed to said method, wherein the unit dose of the invention as described herein is administered subcutaneously and wherein the hepatitis A vaccine, such as HAVRIX® or VAQTA®, is administered intramuscularly to a subject or subject population of more than 17 years, or more than 18 years, or 18 to 60 years of age. In further embodiments, the subjects or subject population are adults of more than 21 years, or 21 to 60 years, or 21 to 45 years of age. In some embodiments, the subject or subject population is from a dengue endemic region. In another embodiment, the subject or subject population is from a dengue non-endemic region, preferably from a dengue non-endemic and a hepatitis A non-endemic region. According to certain embodiments, the subject or subject population is seronegative for all four dengue serotypes.

[00251] In certain embodiments, the invention is directed to said method, wherein the method does not include a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition or wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition. According to certain embodiments, the method does not include a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject at any time before, during and after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition or wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown at any time before, during or after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition.

[00252] In certain embodiments, the invention is directed to said method, wherein 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 and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection without determination whether there was a previous dengue infection and/or a previous hepatitis A infection, and

(B) administering simultaneously on the same day a first unit dose of the tetravalent dengue virus composition and a hepatitis A vaccine to the subject, and optionally

(C) administering at least one further unit dose of the tetravalent dengue virus composition to the subject within 3 to 12 months of administration of the first unit dose and optionally

(D) administering at least one further dose of the hepatitis A vaccine to the subject within 6 to 18 months of administration of the first unit dose.

.

[00253] In certain embodiments, the invention is directed to said method, 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 and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection, and

(B) administering simultaneously on the same day a first unit dose of the tetravalent dengue virus composition and a hepatitis A vaccine to the subject, and

(C) administering two further unit doses of the tetravalent dengue virus composition to the subject at about 6 and about 12 months of administration of the first unit dose and administering a further hepatitis A vaccine to the subject at either about 6 or about 12 months of administration of the first unit dose. In some of these embodiments, step (A), the selecting of the subject, is carried out without determination whether there was a previous hepatitis A infection.

[00254] In certain embodiments, the invention is directed to said method, wherein upon reconstitution of the unit dose 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 (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 6%, at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

[00255] In certain embodiments, the method provides compatibility between the dengue vaccine composition and the hepatitis A vaccine. Compatibility means in particular that the immune response after simultaneous administration is not inferior in comparison with a mono-administration of these vaccines.

[00256] In certain embodiments, the method provides synergy between the dengue vaccine composition and the hepatitis A vaccine. Synergy means in particular that the immune response after simultaneous administration is better for one or both vaccines in comparison with a mono administration of these vaccines.

[00257] In certain embodiments, the invention is directed to said method, wherein the method provides non-inferiority in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects divided into one subject population and into one control subject population, wherein the subject population receives simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and the control subject population receives simultaneously on the same day a hepatitis A vaccine and a placebo administration

[00258] In certain embodiments, the invention is directed to said methods, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% on day 30 after an administration (on

day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

[00259] In certain embodiments, the invention is directed to said method, wherein the method provides a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline and seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

a) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, and

b) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

[00260] In certain embodiments, the invention is directed to said method, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% or of at least 99% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline.

[00261] In certain embodiments, the invention is directed to said method, wherein the method provides a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

a) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, wherein the subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline, and

b) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo, wherein the control subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

[00262] In certain embodiments, the invention is directed to said method, wherein the subject or subject population is exposed to a hepatitis A virus outbreak and/or a dengue virus outbreak.

In certain embodiments, the invention is directed to said method, wherein the method provides an anti-hepatitis A virus antibody Geometric Mean Concentration (GMC) of at least 70 mIU/ml or at least 80 mIU/ml or at least 90 mIU/ml on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

[00263] An ELISA for determining the anti-hepatitis A antibodies is for example disclosed in Beck et al. J Travel Med 2004; 11:201–207.

[00264] In certain embodiments, the invention is directed to said method, wherein the simultaneous administration of the hepatitis A vaccine and the unit dose of the dengue vaccine composition to the subject or the subject population does not provide serious adverse events related to the simultaneous administration. Additionally, there are no deaths related to the simultaneous administration.

[00265] In certain embodiments, the invention is directed to said methods, wherein the method provides the Geometric Mean Titer (GMT) of neutralizing antibodies measured by MNT50 of

- at least 110 or at least 140 or at least 150 for dengue serotype 1,
- at least 3000 or at least 3500 or at least 3900 for dengue serotype 2,
- at least 100 or at least 120 or at least 140 for dengue serotype 3, and/or
- at least 80 or at least 110 or at least 140 for dengue serotype 4,

on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

[00266] In some embodiments, the geometric mean neutralizing antibody titers (GMTs) of a subject population or the neutralizing antibody titers of a subject are determined in accordance with a microneutralization test, for example according to the method described in Example 2.

[00267] The present invention is directed in part to a method of preventing hepatitis A and dengue disease in a subject or subject population, the method comprising simultaneously on the same day administering a hepatitis A vaccine, and a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains, wherein the four live, attenuated dengue virus strains are different from the ones used in the unit dose as defined above.

[00268] In one embodiment of the invention, the method is directed to a simultaneous on the same day administration of a hepatitis A vaccine with other dengue vaccines such as Dengvaxia®. Dengvaxia® is a tetravalent dengue vaccine based on a yellow fever backbone, CYD-TDV (Dengvaxia®, Sanofi Pasteur, Lyon, France), and has been licensed in several countries based on the clinical demonstration of an overall vaccine efficacy (VE) against virologically-confirmed dengue (VCD) of 56–61% in children in Asia and Latin America (Capeding MR et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014, 384:1358-65; Villar LA et al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J 2013, 32:1102-9). The preparation of these particular strains CYD1, CYD2, CYD3 and CYD4 has been described in detail in international patent applications WO 98/37911, WO 03/101397, WO07/021672, WO 08/007021, WO 08/047023 and WO 08/065315, to which reference may be made for a precise description of the processes for their preparation. The corresponding nucleotide sequences of the prM-E regions of CYD1, CYD2, CYD3 and CYD4 are provided in WO2016034629 and SEQID NOs are set out in Table 16 of this reference.

[00269] In one such embodiment, the method comprises a vaccination consisting of the steps of:

(A) selecting a subject for administration of the equal doses of the CYD-TDV composition, such as Dengvaxia®, and the hepatitis A vaccine, such as HAVRIX® or VAQTA®, in need for protection against dengue infection and hepatitis A infection, and

(B) administering a first dose of the CYD-TDV composition, such as Dengvaxia®, and the hepatitis A vaccine, such as HAVRIX® or VAQTA® to the subject at month 0,

(C) administering a further dose of the CYD-TDV composition, such as Dengvaxia®, and optionally the hepatitis A vaccine, such as HAVRIX® or VAQTA® to the subject within 3 to 11 months, in particular at about month 6 of the administration of the first CYD-TDV dose, and

(D) administering a final dose of the CYD-TDV, such as Dengvaxia®, and optionally the hepatitis A vaccine, such as HAVRIX® or VAQTA® to the subject at about month 12.

[00270] In certain embodiments, the subject is from 2 to 60 years of age.

[00271] In particular embodiments, the subject is 2 to 18 years of age, or 4 to 16 years of age, or 18 to 60 years of age.

[00272] Preferably, the exact quantity of each component of the CYD-TDV to be administered may vary according to the age and the weight of the subject being vaccinated, the frequency of administration as well as the other ingredients in the composition. The quantity of a chimeric dengue virus within CYD-TDV comprised in a dose of a vaccine composition lies within a range of about 10^5 CCID50 to about 10^6 CCID50 . The quantity of a live attenuated chimeric dengue virus of each of serotypes 1 to 4 comprised in the CYD dosage form, e.g. Dengvaxia®, is preferably equal. Advantageously, a vaccine composition, as described in this section, comprises an effective amount of a dengue antigen as defined herein.

[00273] In certain embodiments, the invention is directed to said method, wherein the dengue vaccine composition comprises other dengue vaccines such as TV003 or TV005. TV003, developed by the U.S. National Institute of Allergy and Infectious Diseases, comprises vaccine components rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31 and rDEN4Δ30, wherein each of these components is present at a concentration of 3 $\log_{10}$PFU. TV005 is similar to TV003 with the difference that the concentration of rDEN2/4Δ30 in TV005 is 4 $\log_{10}$PFU.The vaccines TV003 and TV005 and their vaccine components as well as their production are described in more detail in WO 2008/022196 A2 and S.S. Whitehead, Expert Rev Vaccines, 2016, 15(4): 509 to 517. Using recombinant DNA technology, two

attenuation strategies were utilized for the vaccine components of TV003 or TV005: deletions in the 3' untranslated region and structural gene chimerization. For example, the component rDEN4Δ30 contains all the structural and non-structural proteins of a wild type DENV-4, but is attenuated by a 30-nucleotide deletion in the 3' untranslated region (denoted "Δ30"). The other vaccine components are also attenuated due to the 30-nucleotide deletion in the 3' untranslated region. In addition, rDEN3Δ30/31 includes a 31 nucleotide deletion in the 3' untranslated region (shown in detail in Fig. 1c and Fig. 13 of WO 2008/022196 A2). The rDEN2/4Δ30 component was created by substituting the prM and E genes of DENV-2 into the rDEN4Δ30 genome. The complete genomic sequences of dengue strains which can be used to produce TV003 or TV005 are available under the Genbank accession numbers in Table A of WO 2008/022196 A1.

[00274] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, and Dengvaxia® disclosed herein and the hepatitis A vaccine disclosed herein are simultaneously on the same day administered to the subject or to the subject population.

[00275] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, and the hepatitis A vaccine disclosed herein are simultaneously on the same day administered to the subject or to the subject population on day 0/1 as a first administration and Dengvaxia® disclosed herein is subsequently administered to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration. Alternatively, Dengvaxia® disclosed herein and the hepatitis A vaccine disclosed herein are simultaneously on the same day administered to the subject or to subject population on day 0/1 as a first administration and the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, is administered subsequently to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration.

[00276] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, and TV003 or TV005 disclosed herein and the hepatitis A vaccine disclosed herein are simultaneously on the same day administered to the subject or to the subject population.

[00277] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, and the hepatitis A vaccine disclosed herein are simultaneously on the same day administered to the subject or the subject population on day 0/1 as a first administration and wherein TV003 or TV005 disclosed herein is subsequently administered to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration. Alternatively, TV003 or TV005 disclosed herein and the hepatitis A vaccine disclosed herein are simultaneously on the same day administered to the subject or to the subject population on day 0/1 as a first administration and the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1

strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, is administered subsequently to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as the second administration.

[00278] The above method is also to be considered in the context of a use of the unit dose of dengue vaccine as disclosed herein for such methods of preventing dengue disease and hepatitis A or in the context of the use of the unit dose of dengue vaccine for the manufacture of a medicament for such methods of preventing dengue disease and hepatitis A.

[00279] Furthermore, the present invention is directed to a kit against hepatitis A and dengue disease comprising a box containing at least

(a) a first container holding a hepatitis A vaccine, as defined above such as HAVRIX®, and

(b) a second container holding a unit dose of a dengue vaccine composition as defined above, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

Method of preventing dengue disease and yellow fever and uses

[00280] The present invention is directed in part to a method of preventing dengue disease as well as yellow fever in a subject. Thus, in certain embodiments the invention is directed to a method of preventing dengue disease in a subject, comprising administering to the subject a reconstituted unit dose of the invention as described herein, wherein the method further comprises preventing yellow fever in the subject by concomitant administration of a yellow fever vaccine, in particular YF-17D, to the subject.

[00281] The present invention is directed in part to a method of preventing dengue disease as well as yellow fever in a subject population. Thus, in certain embodiments the invention is directed to a method of preventing dengue disease in a subject population, comprising administering to the subject population a reconstituted unit dose of the invention as described herein, wherein the method further comprises preventing yellow fever in the subject population by concomitant administration of a yellow fever vaccine, in particular YF-17D, to the subject population.

[00282] In certain embodiments the invention is directed to said methods, wherein the unit dose of the invention as described herein and the yellow fever vaccine, in particular YF-17D, are administered simultaneously. In some of these embodiments the simultaneous administration is on day 0 or day 90, preferably on day 0. In other embodiments the administration of the unit dose of the invention as described herein and the yellow fever vaccine, in particular YF-17D, are done sequentially such as wherein the yellow fever vaccine is administered before or after the unit dose of dengue vaccine as described herein, such as within about 6 weeks, or such as within about 4 weeks, or such as within about 2 weeks, or such as about within 1 week.

[00283] In certain embodiments the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered and the yellow fever vaccine, in particular YF-17D, are administered by subcutaneous injection. According to some embodiments, the subcutaneous injections are administered to the arm, preferably to the deltoid region of the arm. According to some of these embodiments the subcutaneous injections of the unit dose of the invention as described herein and yellow fever vaccine, in particular YF-17D, are administered to different anatomical sites, such as to opposite arms, in particular when the vaccines are administered simultaneously.

[00284] In certain embodiments the invention is directed to said methods, wherein two unit doses of the invention as described herein are administered. In some embodiments the two unit doses of the invention as described herein are administered within 12 month or more, or within 6 month, or within three months, such as at day 0/1 and day 90. According to some of these embodiments a further third unit dose of the invention as described herein is administered after the second. Such a third administration may act as a booster and may be administered between 6 to 12 months after the first administration, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months (1 year) after the second administration or even 5 years or longer after the first or second administration .

[00285] In certain embodiments the invention is directed to said methods, wherein two reconstituted unit doses of the invention as described herein and one dose of a yellow fever vaccine, in particular YF-17D, are administered, in particular according to the following schedule

- an administration of said yellow fever vaccine on day 0,
- a first administration of the first reconstituted unit dose after said yellow fever vaccine administration, such as 3 months later and preferably on day 90, and
- a second administration of the second reconstituted unit dose after said first administration of the reconstituted unit dose, such as 3 months later and preferably on day 180.

[00286] In certain embodiments the invention is directed to said methods, wherein two reconstituted unit doses of the invention as described herein and one dose of a yellow fever vaccine, in particular YF-17D, are administered, in particular according to the following schedule

- a first administration of the first reconstituted unit dose on day 0,
- a second administration of the second reconstituted unit dose after said first administration of the reconstituted unit dose, such as 3 months later and preferably on day 90, and
- an administration of said yellow fever vaccine after said second administration of the reconstituted unit dose, such as 3 months later and preferably on day 180.

[00287] In certain embodiments the invention is directed to said methods, wherein two reconstituted unit doses of the invention as described herein and one dose of a yellow fever vaccine, in particular YF-17D, are administered, in particular according to the following schedule

- a simultaneous administration of the first reconstituted unit dose and said yellow fever vaccine on day 0, and
- a second administration of the second reconstituted unit dose after said simultaneous administration, such as 3 months later and preferably on day 90.

[00288] In a preferred embodiment, the yellow fever vaccine and unit dose of the invention as described herein are administered simultaneously on day 0 or simultaneously on day 90.

[00289] In certain embodiments, the invention is directed to said methods, wherein the subject or subject population is seronegative to all dengue serotypes. In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population and the yellow fever vaccine, in particular YF-17D vaccine, is administered subcutaneously to a subject or subject population, and wherein the subject or the subject population is seronegative with respect to all dengue serotypes. In other embodiments, the subject or subject population is seropositive with respect to at least one dengue serotype.

[00290] In certain embodiments, the invention is directed to said methods, wherein the unit dose of the invention as described herein and the yellow fever vaccine, in particular YF-17D, are administered to a subject or subject

population from a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein and the yellow fever vaccine, in particular YF-17D, are administered subcutaneously to a subject or subject population from a dengue endemic region. In other embodiments, the subject or subject population is from a dengue non-endemic region. Such a subject population or such a subject may be vaccinated according to the present invention in the context of traveling to a dengue endemic region and yellow fever endemic region.

[00291] In certain embodiments the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein and of the yellow fever vaccine, in particular YF-17D, are administered subcutaneously to a subject or subject population of more than 17 years, or more than 18 years, or 18 to 60 years of age. In further embodiments, the subjects or subject population are adults of more than 21 years, or 21 to 60 years, or 21 to 45 years of age. In some embodiments, the subject or subject population is from a dengue endemic region. In another embodiment, the subject or subject population is from a dengue non-endemic region, preferably from a dengue non-endemic and yellow fever non-endemic region. According to some of these embodiments, the subject or subject population are seronegative for all four dengue serotypes.

[00292] The above method is also to be considered in the context of a use of the unit dose of dengue vaccine as disclosed herein for such methods or in the context of the use of the unit dose of dengue vaccine for the manufacture of a medicament for such methods.

Method of preventing and uses, method of inoculating against dengue disease and uses

[00293] The present invention is directed in part to a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject. Thus, in certain embodiments the invention is directed to a method of preventing dengue disease in a subject, comprising administering to the subject, a unit dose/tetravalent dengue virus composition, in particular a reconstituted unit dose of the invention as described herein.

[00294] The present invention is directed in part to a method of preventing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Thus, in certain embodiments the invention is directed to a method of preventing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), comprising administering to the subject a reconstituted unit dose/tetravalent dengue virus composition of the invention as described herein.

[00295] The present invention is therefore 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, 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 (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): major attenuation locus
b) NS1-53 Gly-to-Asp (nt-2579 G-to-A): major attenuation locus
c) NS3-250 Glu-to-Val (nt-5270 A-to-T): major attenuation locus; 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.

[00296] Further information regarding the serotypes of the tetravalent composition can be derived from section "Dengue virus strains" above.

[00297] The tetravalent dengue virus composition for such a method may be in the form of a unit dose comprising:

(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.

[00298] The present invention is in particular directed to such a method 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.

[00299] Further information regarding the tetravalent composition or the unit dose can be derived from section "Dengue vaccine composition" and "Unit dose" above.

[00300] The present invention 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.

According to this embodiment the administration of only two doses within 3 months is sufficient to provide effective protection against a subsequent dengue infection.

[00301] Such method preferably 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 second administration of the administration schedule.

[00302] Such method also preferably 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 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 14 to 16 years of age, from 30 days after the second administration of the administration schedule until 18 months after the second administration of the administration schedule.

[00303] According to certain embodiments 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. The method has very high efficacy against dengue serotype 2 and dengue serotype 1 and the highest efficacy against dengue serotype 2.

[00304] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 1, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age from 30 days post second administration until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the lower bound is more than 30%, is more than 35% is more than 40%, is more than 45%, is more than 50%, or is more than 54%.In certain such embodiments the subject population of at least 1,500 is seronegative against all serotypes at base line and the lower bound is more than 35%. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 1 with a 2-sided 95% confidence interval, wherein the lower bounds are within 10 %-points.

[00305] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 1, in preventing virologically confirmable dengue disease, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age from 30 days post second administration until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the vaccine efficacy is more than 40%, is more than 50%, is more than 60%, or is more than 65%. In certain such embodiments the subject population of at least 1,500 is seronegative against all serotypes at base line. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 1 which are within 5 %-points.

[00306] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 2, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age from 30 days post second administration until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the lower bound is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 85%. In certain such embodiments the subject population of at least 1,500, is seronegative against all serotypes. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 2 with a 2-sided 95% confidence interval, wherein the lower bounds are within 5 %-points.

[00307] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 2, in preventing virologically confirmable dengue disease, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age from 30 days post second administration until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the vaccine efficacy is more than 60%, is more than 70%, is more than 80%, or is more than 90%. In certain such embodiments the subject population of at least 1,500 is seronegative against all serotypes at base line. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 2 which are within 5 %-points.

[00308] The efficacy of the method is further described in more detail below in this the section.

[00309] In certain embodiments the unit dose is reconstituted and administered by subcutaneous injection. According to some of these embodiments, the subcutaneous injection is administered to the arm, preferably to the deltoid region of the arm.

[00310] According to one embodiment such a method does 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.

[00311] According to one embodiment such a method does 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.

[00312] The method according to the invention does not require the testing of the serostatus before vaccination and thus allows immediate treatment and outbreak control. According to certain embodiments the use is for a method wherein the subject is exposed to a dengue outbreak. In certain such embodiments the outbreak is due to a dengue serotype 2, and/or due to a serotype 1.

[00313] According to one embodiment such a method the subject is from a region wherein the seroprevalence rate is unknown and/or wherein the seroprevalence rate is below 80%, or below 70%, or below 60%.

[00314] According to one embodiment of such a method the subject is seronegative at baseline and is from a region or travels to a region wherein the seroprevalence rate is high with respect to serotype 1 and/or serotype 2 i.e. 80%, or 90% or above.

[00315] According this embodiment the vaccine and corresponding method is safe for seronegative and seropositive subjects and thus does not require an analysis of the serostatus or a determination of a previous dengue infection or a high seroprevalence rate in the region. Such a method preferably 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, preferably in at least 1,500 healthy 4 to 16 year old subjects seronegative at baseline, from first administration of the administration schedule until 12 to 18 months after the second administration of the administration schedule. Preferably, the 2-sided 95% confidence interval of the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes when comparing seropositive and seronegative subjects provides for lower bounds of the 2-sided confidence interval which are within 10% points or within 15% points or within 20% points. The method is preferably safe with respect to serotype 1 and serotype 2 which may therefore be used in outbreak situations due to serotype 1 and/or serotype 2 or even for seronegative subjects (e.g. travelers) or subjects with unknown serostatus in regions with very high seroprevalence rates (>80%) due to serotype 1 and/or serotype 2.

[00316] The safety of the method is further described in more detail in the section "method of preventing, method of inoculating".

[00317] According to one embodiment such a method does 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).

[00318] According to one embodiment such a method does not include vaccine immunogenicity analysis including GMTs for dengue neutralizing antibodies.

[00319] According to one embodiment such a method does 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.

[00320] According to one embodiment the method does not include an active surveillance, an immunogenicity analysis and a reactogenicity analysis.

[00321] According to such embodiments the vaccine and the corresponding method of inoculation are safe and therefore do not require further steps of surveillance or analysis.

[00322] In view of the above the method according to one embodiment 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.

[00323] Selecting the subject may include all types of considerations but preferably not the determination of a previous dengue infection. The selection may include consideration of the age, health conditions, and threat of infection. The threat of infection includes consideration of the seroprevalence rate in the region in which the subject normally lives or intends to travel, the serotype specific seroprevalence rate and an outbreak situation or serotype specific outbreak situations. The subject may be selected due to its exposure to serotype 1 and/or serotype 2 or due to the fact it requires protection against a specific dengue serotype, i.e. serotype 1 and/or serotype 2.

[00324] According to the invention the method is applicable to subjects of all kinds of ages. According to one embodiment 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 45 years of age, or 46 to 60 years of age, or over 60 years of age.

[00325] In particular the present invention is directed to such a method wherein the method which is safe.

[00326] In particular the present invention is directed to such a method 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 first administration of the administration schedule until 12 to 18 months after the last administration of the administration schedule.

[00327] In particular the present invention is directed to such a method wherein the method which is effective.

[00328] In particular the present invention is directed to such a method 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 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.

[00329] In certain embodiments, the invention is directed to said methods, wherein the subject is seronegative to all dengue serotypes.

[00330] The present invention is directed in part to a method of preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population. Thus, in certain embodiments the invention is directed to a method of preventing dengue disease in a subject population, comprising administering to the subject population a unit dose, in particular a reconstituted unit dose of the invention as described herein.

[00331] The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject population comprising administering to the subject population at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of geometric mean neutralizing antibody titers (GMTs) at day 180 or 365 after administration of said first unit dose to the subject population are achieved. According to some embodiments, the geometric mean neutralizing antibody titer for dengue serotype 2 (GMT DENV-2) and the geometric mean neutralizing antibody titer for dengue serotype 4 (GMT DENV-4) 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 reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of GMT DENV-2: GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of GMT DENV-2 : GMT DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of GMT DENV-2 : GMT DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

[00332] The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject comprising administering to the subject at least a first reconstituted unit dose of the invention as described herein, wherein certain ratios of neutralizing antibody titers at day 180 or 365 after administration of said first unit dose to the subject are achieved. According to some embodiments, the neutralizing antibody titer for dengue serotype 2 and the neutralizing antibody titer for dengue serotype 4 at day 180 or day 365 after at least a first administration of the reconstituted unit dose of the invention as described herein, and optionally a second administration of a reconstituted unit dose of the invention as described herein 90 days after said first administration, provide a ratio of neutralizing antibody titer for DENV-2 : neutralizing

antibody titer for GMT DENV-4 of not more than 50, or not more than 40, or not more than 30, or not more than 20. In some of these embodiments, the ratio of the neutralizing antibody titers of DENV-2 : DENV-1 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose, and/or the ratio of the neutralizing antibody titers of DENV-2 : DENV-3 is not more than 20, or not more than 18, or not more than 15 at day 180 or 365 after administration of said first reconstituted unit dose.

[00333] The geometric mean neutralizing antibody titers (GMTs) of a subject population or the neutralizing antibody titers of a subject are determined in accordance with the microneutralization test disclosed herein, for example according to the method described in Example 2. Without wishing to be bound to any theory, it is presently understood that a method inducing a more balanced immune response due to the administration of the reconstituted unit dose of the invention as described herein, in terms of less differences between the geometric mean neutralizing antibody titers (GMTs) against the four dengue serotypes or the neutralizing antibody titers against the four dengue serotypes, is beneficial to the subject or subject population to be vaccinated. In particular, it is understood that a much greater response to any one of the four serotypes, such as to DENV-2 in comparison to the other serotypes, is less beneficial.

[00334] The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject or subject population wherein the method provides a seropositivity rate in a subject population of at least 50 subjects including the administration of two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population are seronegative to all dengue serotypes at baseline. In certain such embodiments, at least 80% of the subject population are seropositive for all four dengue serotypes at least one month after administration of the first unit dose, such as at day 30, and/or at least 80% of the subject population are seropositive for all four dengue serotypes before or at the time of the administration of the second unit dose, such as at day 90, and/or at least 80%, or at least 85%, or at least 90%, or at least 95% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 120, and/or at least 80%, or at least 85% ,or at least 90% of the subject population are seropositive for all four dengue serotypes after the administration of the second unit dose, such as at day 270.

[00335] The present invention is in part directed to said method for preventing dengue disease (in particular virologically confirmable dengue, VCD) in a subject or subject population wherein the method provides a seropositivity rate in a subject population of at least 100 subjects including administration of two unit doses subcutaneously at day 1 and at day 90, wherein the subjects of the subject population comprises from 20% to 40% subjects who are seronegative to all dengue serotypes and from 60% to 80% subjects who are seropositive to at least one dengue serotype at base line, wherein at day 120 and/or day 270 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 10%-points and/or wherein at day 120 the seropositivity rate for all four dengue serotypes in the seronegative part of the subject population and the seropositivity rate for all four dengue serotypes in the seropositive part of the subject population do not deviate more than 5%-points.

[00336] The present invention is in part directed to a method of preventing virologically confirmable dengue disease in a subject or subject population comprising administering to the subject or subject population a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue serotypes, in particular the virus strains as described herein.

[00337] The present invention is in part directed to a method of preventing virologically confirmable dengue disease with hospitalization in a subject or subject population comprising administering to the subject or subject population a reconstituted unit dose of a tetravalent dengue virus composition including four live, attenuated dengue serotypes, in particular the virus strains as described herein.

[00338] In certain embodiments, the method includes a reconstituted unit dose/tetravalent dengue virus composition of a dengue vaccine composition administered for preventing dengue disease in a subject or a subject population, the reconstituted unit dose comprising: a tetravalent virus composition including four live attenuated dengue virus strains, wherein a unit dose is lyophilized and upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent the reconstituted unit dose is obtained which comprises:

(i) a dengue serotype 1, such as 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, such as a dengue serotype 2 strain, in a concentration of at least 2.7 log10 pfu/0.5 ml,

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

(vi) 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.

[00339] It is preferred that the reconstituted unit dose/tetravalent dengue virus composition is used in the method of preventing dengue disease of the present invention, wherein upon reconstitution of the unit dose 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% and wherein the subject or subject population is of 18 to 60 years of age.

[00340] In another preferred embodiment, the reconstituted unit dose/tetravalent dengue virus composition is used in the method of preventing dengue disease of the present invention, 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% and wherein the subject or subject population is of 2 to 17 years of age.

[00341] In certain embodiments, the invention is directed to said methods, wherein said unit dose comprises a tetravalent dengue virus composition including four live attenuated dengue serotypes, in particular the virus strains described herein wherein the serotypes have certain concentrations as described herein with respect to the virus composition and unit dose such as:

(i) a dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) has a concentration of 3.3 log10 pfu/dose to 5.0 log10 pfu/dose, or 3.3 log10 pfu/0.5 mL to 5.0 log10 pfu/0.5 mL

(ii) a dengue serotype 2 (e.g. dengue serotype 2 strain) has a concentration of 2.7 log10 pfu/dose to 4.9 log10 pfu/0.5 dose, or 2.7 log10 pfu/0.5ml to 4.9 log10 pfu/0.5ml

(iii) a dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) has a concentration of 4.0 log10 pfu/dose to 5.7 log10 pfu/0.5 dose, or 4.0 log10 pfu/0.5 mL to 5.7 log10 pfu/0.5 mL and

(iv) a dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) has a concentration of 4.5 log10 pfu/dose to 5.5 log10 pfu/0.5 dose, or 4.5 log10 pfu/0.5 mL to 5.5 log10 pfu/0.5 mL.

In preferred such embodiments, the subject or subject population is of 2 to 17 years of age, such as 4 to 16 years of age, and preferably less than 9 years of age. In other preferred embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age.

[00342] In certain embodiments, the invention is directed to said methods, wherein said unit dose upon reconstitution with 0.5 mL of a pharmaceutically acceptable diluent has a concentration of 3.3 log10 pfu/0.5 mL to 3.6 log10 pfu/0.5 mL for dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain), has a concentration of 2.7 log10 pfu/0.5 mL to 4.0 log10 pfu/0.5 mL for dengue serotype 2 (e.g. dengue serotype 2 strain), has a concentration of 4.0 log10 pfu/0.5 mL to 4.6 log10 pfu/0.5 mL for dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) and has a concentration of 4.5 log10 pfu/0.5 mL or 4.6 log10 pfu/0.5 mL to 5.1 log10 pfu/0.5 mL for dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain). In preferred such embodiments, the subject or subject population is of 2 to 17 years of age, such as 4 to 16 years of age, and preferably less than 9 years of age. In other preferred embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age.

[00343] In certain embodiments, the invention is directed to said methods, wherein the concentration of the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) measured in pfu/0.5 mL is 1% to 7% of the total concentration, the concentration of the dengue serotype 2 (e.g. dengue serotype 2 strain) measured in pfu/0.5 mL is less than 8% of the total concentration, such as in the range of 1% to 8% of the total concentration, the concentration of the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) measured in pfu/0.5 mL is at least 10% of the total concentration, and the concentration of the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) measured in pfu/0.5 mL is at least 65% of the total concentration, such as in the range of 65% to 80%. In certain such embodiments, 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 Preferably, in said embodiments the subject or subject population is of 2 to 17 years of age, such as 4 to 16 years of age, and even more preferably less than 9 years of age. In other preferred embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age.

[00344] In a further preferred embodiment, the invention is directed to said methods, wherein the dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and the dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-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 certain such embodiments the dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 is preferably at least about 10% of the total concentration in pfu/0.5 mL and more preferably the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 is at least about 70% of the total concentration in pfu/0.5 mL. In certain such embodiments the dengue serotype 4 (e.g. chimeric dengue serotype 2/4 strain) such as TDV-4 represents the highest concentration in the composition of all four serotypes, preferably with at least about 70% of the total concentration in pfu/0.5 mL, dengue serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3 represents the second highest concentration in the composition of all four serotypes, preferably with at least about 10% of the total concentration in pfu/0.5 mL, and dengue serotype 1 (e.g. chimeric dengue serotype 2/1 strain) such as TDV-1 and dengue serotype 2 (e.g. dengue serotype 2 strain) such as TDV-2 each represent lower concentrations than the concentration of serotype 3 (e.g. chimeric dengue serotype 2/3 strain) such as TDV-3, and optionally together represent less than about 10% of the total concentration in pfu/0.5 mL.

[00345] Preferably, the chimeric dengue serotype 2/1 strain is TDV-1, the dengue serotype 2 strain is TDV-2, the chimeric dengue serotype 2/3 strain is TDV-3 and the chimeric dengue serotype 2/4 strain is TDV-4. More preferably,

TDV-1 is characterized by the nucleotide sequence according to SEQ ID No. 1 and the amino acid sequence according to SEQ ID No. 2, TDV-2 is characterized by the nucleotide sequence according to SEQ ID No. 3 and the amino acid sequence according to SEQ ID No. 4, TDV-3 is characterized by the nucleotide sequence according to SEQ ID No. 5 and the amino acid sequence according to SEQ ID No. 6 and TDV-4 is characterized by the nucleotide sequence according to SEQ ID No. 7 and the amino acid sequence according to SEQ ID No. 8.

[00346] In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered by subcutaneous injection. According to some of these embodiments, the subcutaneous injection is administered to the arm, preferably to the deltoid region of the arm.

[00347] In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose is administered to a subject of unknown serostatus and/or wherein no test has been carried out to determine whether the subject is seropositive or seronegative (before) the unit dose as described herein is administered. In certain embodiments, the invention is directed to said methods which do not include a step of determination of a previous dengue infection in the subject or subjects. In certain embodiments, the invention is directed to said methods which do not include the analysis of the seroprevalence rate in the region or is conducted in a region with a seroprevalence of below 80%, below 70% or below 60%. In certain embodiments the invention is directed to a method wherein the serostatus of the subject is unknown. In such embodiments the serostatus is not determined at any time before and after administration in relation to this method. In certain embodiments of the invention the method is used in an outbreak situation. In certain embodiments, the invention is directed to said methods being conducted outside a clinical trial

[00348] In certain embodiments, the invention is directed to said methods, wherein the subject, or subject population is seronegative to all dengue serotypes.

[00349] In certain embodiments, the invention is directed to said methods, wherein two unit doses of the invention as described herein are administered. In some embodiments the two unit doses are administered within 12 months or more, or within six months, or within three months, and optionally at least 4 weeks apart such as at day 0 and day 90 or at day 1 and day 90. According to some of these embodiments, a further third unit dose of the invention as described herein is administered after the second administration. Such a third administration may act as a booster and may be administered between 6 to 12 months after the first administration, such as 12 months after the first administration, or later than 12 month after the first administration, such as 12 months (1 year) after the second administration or even 5 years or longer after the first or second administration.

[00350] In certain embodiments, the method of the invention comprises or consists of a single unit dose of the invention being administered.

[00351] In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population that is seronegative with respect to all dengue serotypes. In other embodiments, the subject or subject population is seropositive with respect to at least one dengue serotype.

[00352] In certain embodiments, the invention is directed to said methods, wherein the unit dose of the invention as described herein is administered to a subject or subject population from a dengue endemic region. In some of these embodiments, the subject or subject population is from Singapore, Dominican Republic, Panama, Philippines, Colombia, Puerto Rico or Thailand, in particular from Singapore, Dominican Republic, Panama, or Philippines. In a

preferred embodiment, the subject or subject population is from Asia Pacific or from Latin America. In some other of these embodiments, the subject or subject population is from Thailand, Sri Lanka, Philippines, Panama, Nicaragua, Dominican Republic, Colombia or Brazil. In other embodiments, the subject, or subject population is from a dengue non-endemic region. Such a subject population or such a subject may be vaccinated according to the present invention in the context of traveling to a dengue endemic region. In certain embodiments, the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject, or subject population that is from a dengue endemic region or a dengue non-endemic region.

[00353] In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to a subject or subject population of 2 to 60 years of age. In some embodiments, the subjects or subject population are adults of more than 17 years, or more than 18 years, or 18 to 60 years. In further specific embodiments, the subjects or subject population are adults of more than 21 years, or 21 to 60 years, or 21 to 45 years of age.

[00354] In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered subcutaneously to children and adolescents of 2 to 17 years of age. In some embodiments, the subjects or subject population are less than 9 years of age, or less than 4 years of age. In some embodiments, the subjects or subject population are from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 9 years of age or from 6 to 9 years of age. In other embodiment, the subject or subject population is 4 to 16 years of age. In some such embodiments, the subject or subject population is 4-5 years of age, 6-11 years of age or 12-16 years of age. Optionally, the subject or subject population is seronegative with respect to all dengue serotypes.

[00355] In certain embodiments, the invention is directed to said methods, wherein the unit dose of the invention as described herein is administered to a pediatric subject or pediatric subject population of 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. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

[00356] In certain embodiments, the invention is directed to said methods, wherein the reconstituted unit dose of the invention as described herein is administered to a pediatric subject or pediatric subject population of 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, preferably by subcutaneous injection. According to some of these embodiments, the pediatric subject or pediatric subject population is seronegative and from a dengue endemic region.

[00357] In a certain embodiments, the invention is directed to said methods, wherein the subject or subject population is 4-5 years of age and from Asia Pacific, 6-11 years of age and from Asia Pacific, or 12-16 years of age and from Asia Pacific. In other embodiments, the subject or subject population is 4-5 years of age and from Latin America, 6-11 years of age and from Latin America, or 12-16 years of age and from Latin America.

[00358] In a certain embodiments, the invention is directed to said methods, wherein the subject or subject population is 4-5 years of age and seropositive for at least 1 dengue serotype, 6-11 years of age and seropositive for at least 1 dengue serotype, or 12-16 years of age and seropositive for at least 1 dengue serotype. In other embodiments, the subject or subject population is 4-5 years of age and seronegative for all dengue serotypes, 6-11 years of age and seronegative for all dengue serotypes, or 12-16 years of age and seronegative for all dengue serotypes.

[00359] In a certain embodiments, the invention is directed to said methods, wherein the subject or subject population is from Asia Pacific or Latin America and seropositive for at least one dengue serotype at baseline. In other embodiments, the subject or subject population is from Asia Pacific or Latin America and seronegative for at all dengue serotype at baseline.

[00360] In certain embodiments, the invention is directed to said methods, wherein the subject or subject population is from Asia Pacific, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the subject or subject population is from Asia Pacific, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In yet other embodiments, the subject or subject population is from Latin America, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the subject or subject population is from America, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age.

[00361] In certain embodiments, the invention is directed to said methods, wherein the subject or subject population had prior vaccination against Yellow Fever. In other embodiments, the subject or subject population had prior vaccination against Japanese Encephalitis. In yet other embodiments, the subject or subject population had no prior vaccination against Yellow Fever. In other embodiments, the subject or subject population had no prior vaccination against Japanese Encephalitis. Prior vaccination indicates a vaccination prior to 30 days after a second administration, such as within 4 months after the first administration, with the reconstituted unit dose as described herein. For example for vaccine efficacy (VE) as determined in Example 6 from 30 days post-second vaccination, a prior vaccination of Yellow Fever is defined as a Yellow Fever vaccination occurring before 30 days post-second vaccination. In certain embodiments, the subject or subject population received Dengvaxia® within the administration regimen as described herein or within 4.5 years after administration of the first dose.

[00362] Particularly unbalanced titers of neutralizing antibodies against the four dengue serotypes are observed in seronegative populations or subjects after administration of the commercially available dengue vaccine. The present invention shows that in particular seronegative subjects show a more balanced immune response to the four dengue serotypes after administration of the reconstituted unit dose of the invention as described herein. It is therefore contemplated that the unit dose of the invention as described herein and methods of the present invention as described herein may provide a more robust immune response in a subject population including both seropositive and seronegative subjects. This balanced response and balanced efficacy and safety is required to allow inoculation without prior serostatus analysis which is a major advantage in vaccination programs and in particular in outbreak situations.

[00363] The present invention is directed in part to a method of preventing virologically confirmable dengue disease in a subject comprising administering to the subject a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, wherein the virus strains are optionally live, attenuated dengue virus strains.

[00364] The present invention is directed in part to a method of preventing virologically confirmable dengue disease in a subject consisting of administering to the subject a tetravalent dengue virus composition including four dengue virus strains representing serotype 1, serotype 2, serotype 3 and serotype 4, wherein the virus strains are optionally live, attenuated dengue virus strains.

[00365] In certain embodiments, the invention is directed to said methods, wherein there is no step of determining the serostatus of the subject at baseline, in other words, said methods do not comprise a determination of a previous dengue infection of the subject at baseline before the administration of the tetravalent dengue virus composition. In particular, such methods are safe and effective. Thus, in certain such embodiments, the subject has not been tested for the presence a previous dengue infection.

[00366] In certain embodiments, the invention is directed to said methods, wherein the vaccine administration is safe irrespective of whether there is a determination that the subject had a previous dengue infection before the administration of the tetravalent dengue virus composition. In particular, such methods are also effective.

[00367] In certain embodiments, the invention is directed to said methods, wherein the method is safe and/or effective.

[00368] In certain embodiments, the invention is directed to said methods, wherein the composition includes at least one chimeric dengue virus. In certain such embodiments, the invention is directed to said methods, wherein the composition includes at least one non-chimeric dengue virus and at least one chimeric dengue virus, in particular 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. The details of the composition are described above.

[00369] Therefore, in certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably 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 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and e.g. 14 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is administered e.g. at least twice within less than 6 months, such as within 3 months, after first administration or 30 days after the second/last administration until at least 12 to 18 months (e.g. at 12 or at 18 months) after the second/last administration. In embodiments, the invention is directed to said methods having a vaccine efficacy, preferably 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 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose or tetravalent dengue virus composition as described herein or placebo is administered at least once, until 15 to 21 months (e.g. 15 or 21 months) after the first administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, more than 40%, more than 50%, more than 55%, more than 60%, more than 65%, more than 70% or more than 72%. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 months, such as on days 0 and 90.

[00370] Therefore, in certain embodiments, the invention is directed to said methods having 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 (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is administered e.g. at least twice within less than 6 months, such as within 3 months, after the first administration until 18 months after the last administration. In these embodiments, the lower bound is e.g. more than 62%, more than 64%, more than 66%, more than 68%, or more than 69%.

[00371] In certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and e.g. 14 to 16 years of age, wherein a reconstituted unit dose or tetravalent dengue virus composition as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, after first administration or 30 days after the second administration/last administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration/last administration. In certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose or tetravalent dengue virus composition as described herein or placebo is administered at least once, until 15 months after the first administration of the administration schedule. In certain such embodiments, the vaccine efficacy is more than 40%, more than 50%, more than 55%, more than 60%, more than 65%, more than 70%, more than 75%, more than 78%, more than 79% or about 80%. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

[00372] Therefore, in certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease of more than 66%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 14 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is administered e.g. at least twice within less than 6 months, such as within 3 months, after the first administration until 18 months after the last administration. In these embodiments, the vaccine efficacy is e.g. more than 68%, more than 70%, more than 72%, or more than 74%.

[00373] In certain embodiments, the invention is directed to said methods having a vaccine efficacy, preferably a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 0%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose or tetravalent dengue virus composition as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 18 months after the second administration. In certain such embodiments, the lower bound is more than 10%, is more than 20%, is more than 30%, is more than 40%, is more than 50%, is more than 55%, is more than 60%, is more than 65%, is more than 70% or is more than 80%, or more than 90%.

[00374] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes in seronegative subjects with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18

months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, or is more than 55%.

[00375] In certain embodiments, the invention is directed to said methods having 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 25%, when measured against placebo in a subject population of at least 1,500 or at least 2,000 or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 30%, is more than 35%, is more than 40%, or is more than 45%.

[00376] In certain embodiments, the invention is directed to said methods having 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 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 40%, is more than 45%, is more than 50%, is more than 60%, or is more than 65%.

[00377] In certain embodiments, the invention is directed to said methods having 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 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline being or seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the difference between the lower bound provided by the seropositive subjects at baseline and the subjects seronegative against all serotypes at baseline is no more than 15%-points.

[00378] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes in seronegative subjects of more than 30%, when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes in seronegative subjects is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%.

[00379] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease, when measured against placebo in a subject population of at least 1,500 or at least 2,000 or at least 5,000 healthy subjects (or at least 10,000, or at

least 15,000 healthy subjects) being seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments the said vaccine efficacy is more than 30%, is more than 40%, is more than 50%, is more than 55%, is more than 60%, or is more than 65%.

[00380] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments the said vaccine efficacy is more than 40%, is more than 50%, is more than 60%, is more than 65%, is more than 70%, or is more than 75%.

[00381] In certain embodiments, the invention is directed to said methods having 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 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline being or seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the difference between the lower bound provided by the seropositive subjects at baseline and the subjects seronegative against all serotypes at baseline is no more than 15%-points, or is no more than 10%-points.

[00382] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 4 to 5 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 45%.

[00383] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 4 to 5 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%.

[00384] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 6 to 11 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, or is more than 70%.

[00385] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 6 to 11 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 75%, or is more than 80%.

[00386] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,000 healthy subjects 12 to 16 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 68%.

[00387] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four dengue serotypes of more than 30%, when measured against placebo in a subject population of at least 1,000 healthy subjects 12 to 16 years of age at the time of randomization and irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the combined vaccine efficacy against all four dengue serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 75%, or is more than 80%.

[00388] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 1 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, or is more than 50%.

[00389] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 1, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 30%, is more than 35% is more than 40%, is more than 45%, is more than 50%, or is more than 54%.In certain such embodiments the subject population of at least 1,500 is seronegative against all serotypes at base line and the lower bound is more than 35%. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 1 with a 2-sided 95% confidence interval, wherein the lower bounds are within 10 %-points.

[00390] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 1 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the vaccine efficacy against dengue serotype 1 is more than 40%, is more than 50%, is more than 60%, is more than 65%, or is more than 70%.

[00391] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 1, in preventing virologically confirmable dengue disease, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 or at 18 months) after the last administration. In certain such embodiments, the vaccine efficacy is more than 40%, is more than 50%, is more than 60%, or is more than 65%. In certain such embodiments the subject population of at least 1,500 is seronegative against all serotypes at base line. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 1 which are within 5 %-points.

[00392] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 2 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50, is more than 60, is more than 70, is more than 80, or is more than 90%.

[00393] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 2, in preventing virologically confirmable dengue disease with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 1,500, or at

least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 85%. In certain such embodiments the subject population of at least 1,500, is seronegative against all serotypes. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 2 with a 2-sided 95% confidence interval, wherein the lower bounds are within 5 %-points.

[00394] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 2 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the vaccine efficacy against dengue serotype 2 is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80, or is more than 90%.

[00395] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against serotype 2, in preventing virologically confirmable dengue disease, when measured against placebo in a subject population of at least 1,500, or at least 2,000, or at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 or at 18 months) after the last administration. In certain such embodiments, the vaccine efficacy is more than 60%, is more than 70%, is more than 80%, or is more than 90%. In certain such embodiments the subject population of at least 1,500 is seronegative against all serotypes at base line. In certain such embodiments the seronegative and seropositive population each provide a vaccine efficacy against serotype 2 which are within 5 %-points.

[00396] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 3 with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%.

[00397] In certain embodiments, the invention is directed to said methods having a vaccine efficacy against dengue serotype 3 of more than 30%, when measured against placebo in a subject population of at least 5,000 healthy subjects, or at least 10,000 healthy subjects, or at least 15,000 healthy subjects irrespective of serostatus at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the vaccine efficacy against dengue serotype 3 is more than 40%, is more than 50%, is more than 55%, or is more than 60%.

[00398] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from first administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration, or from 30 days post last administration until 12 to 18 months (e.g. at 12 or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 10%, is more than 20%, is more than 30%, is more than 40%, is more than 50%, is more than 55%, is more than 60%, is more than 65%, is more than 66%, is more than 67%, is more than 70%, is more than 75%, is more than 77%, or is more than 80%.

[00399] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from first administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration, or from 30 days post last administration until 12 to 18 months (e.g. at 12 or at 18 months) after the last administration. In certain such embodiments, the vaccine efficacy is more than is more than 70%, is more than 75%, is more than 80%, or is more than 82%, or is more than 85%, more than 88%.

[00400] In certain embodiments, the invention is directed to said methods having 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 25%, when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects being seronegative against all serotypes at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, or is more than 75%.

[00401] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 60%, is more than 65%, is more than 66%, is more than 67%, is more than 70%, is more than 75%, is more than 77% or is more than 80%.

[00402] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes of more than 30%, when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects, healthy

subjects being seronegative against all serotypes at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%.

[00403] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the said vaccine efficacy is more than 60%, is more than 65%, is more than 66%, is more than 67%, is more than 70%, is more than 75%, is more than 77%, is more than 80, or is more than 85%.

[00404] In certain embodiments, the invention is directed to said methods having 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 25%, when measured against placebo in a subject population of at least 1,500 or at least 2,000 healthy subjects being seropositive at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, about 30 days after the second administration of the administration schedule until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration of the administration schedule. In certain such embodiments, the lower bound is more than 30%, is more than 40%, is more than 50%, is more than 60%, is more than 70%, or is more than 80%.

[00405] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the lower bound is more than 60%, is more than 65%, is more than 70%, is more than 75%, or is more than 80%.

[00406] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes of more than 30%, when measured against placebo in a subject population at least 1,500 or of at least 2,000 healthy subjects, healthy subjects being seropositive at baseline, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or until 12 to 18 months (e.g. at 12 or at 18 months) after the second administration. In certain such embodiments, the combined vaccine efficacy against virologically-confirmed dengue with hospitalization against all four serotypes is more than 40%, is more than 50%, is more than 60%, is more than 70%, is more than 80%, or is more than 90%.

[00407] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the vaccine efficacy is more than 75%, is more than 70%, is more than 80%, is more than 85%, or is more than 90%.

[00408] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization with a 2-sided 95% confidence interval, wherein the lower bound is more than 25%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline being or seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the difference between the lower bound provided by the seropositive subjects at baseline and the subjects seronegative against all serotypes at baseline is no more than 15%-points.

[00409] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes, in preventing virologically confirmable dengue disease with hospitalization, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) being seropositive at baseline being or seronegative against all serotypes at baseline and 4 to 16 years of age, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is e.g. administered at least twice within less than 6 months, such as within 3 months, from 30 days post last administration until 12 to 18 months (e.g. at 12 months or at 18 months) after the last administration. In certain such embodiments, the difference between the vaccine efficacy provided by the seropositive subjects at baseline and the subjects seronegative against all serotypes at baseline is no more than 10%-points or no more than 5%-points.

[00410] In certain embodiments, the invention is directed to said methods having a relative risk, preferably a combined relative risk against all four serotypes, with a 2-sided 95% confidence interval, wherein the upper bound is less than 0.75, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months after the second administration. In certain such embodiments, the upper bound is less than 0.70, less than 0.65, less than 0.60, less than 0.55, less than 0.50, less than 0.45, less than 0.40, less than 0.35, less than 0.30 or less than 0.28. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

[00411] In certain embodiments, the invention is directed to said methods having a relative risk, preferably a combined relative risk against all four serotypes, of less than 0.70, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second

administration until at least 12 months after the second administration. In certain such embodiments, the relative risk is less than 0.65, less than 0.60, less than 0.55, less than 0.50, less than 0.45, less than 0.40, less than 0.35, less than 0.30, less than 0.25 or less than 0.23. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

[00412] In certain embodiments, the invention is directed to said methods, wherein virologically confirmable dengue disease occurs in less than 2.5% of the subjects, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) irrespective of serostatus at baseline, wherein a reconstituted unit dose/tetravalent dengue virus composition as described herein or placebo is administered at least twice within less than 6 months, such as within 3 months, 30 days after the second administration until at least 12 months or at least 18 months after the second administration. In certain such embodiments, virologically confirmable dengue disease occurs in less than 2.0% of the subjects, less than 1.5% of the subjects, less than 1.0% of the subjects, less than 0.8% of the subjects, or less than 0.6% of the subjects. Preferably said reconstituted unit dose or placebo is administered subcutaneously within about 3 month, such as on days 0 and 90.

[00413] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes with a 2-sided 95% confidence interval, wherein the lower bound is more than 61.0%, or more than 65.0 or more than 70.0% or more than 72.0% when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) from endemic irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 or 13 months after the last administration of the administration schedule.

[00414] In certain embodiments, the invention is directed to said methods having a combined vaccine efficacy against all four serotypes of more than 66 %, or of more than 70%, or of more than 75%, or of more than 77%, or of more than 80.0%, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects) from endemic areas irrespective of serostatus at baseline and being selected from the group consisting of 4 to 16 year old subjects at the time of randomization, wherein said unit dose/tetravalent dengue virus composition or said placebo is administered at least twice within 6 months or less, about 30 days after the last administration of the administration schedule until at least 12 months or 13 month after the last administration of the administration schedule.

[00415] In certain embodiments, the invention is directed to said methods, wherein the combined vaccine efficacy against all four serotypes is measured about 30 days after the last administration of the administration schedule until 12 or 13 months after the last administration of the administration schedule.

[00416] In certain embodiments, the invention is directed to said methods, wherein said unit dose or said placebo is administered at least twice within three months, in particular at about day 1 and about day 90, and wherein the combined vaccine efficacy against all four serotypes is measured 30 days after the second administration until 12 or 13 months after the second administration of the administration schedule.

[00417] In certain embodiments, the invention is directed to said methods, wherein said methods are effective and safe. In some of these embodiments, the subject or subject population is under 9 years of age, under 4 years of age,

or under 2 years of age or from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 9 years of age or from 6 to 9 years of age. Optionally the subject is seronegative with respect to all dengue serotypes.

[00418] In certain embodiments, the invention is directed to said methods, wherein said methods having a relative risk for virologically confirmed dengue with hospitalization of 1 or less, or 0.8 or less, or 0.6 or less, when measured against placebo in a subject population of at least 5,000 healthy subjects (or at least 10,000, or at least 15,000 healthy subjects). In some of these embodiments, the subject or subject population is under 9 years of age, under 4 years of age, or under 2 years of age or from 2 to 9 years of age, or from 2 to 5 years of age, or from 4 to 9 years of age or from 6 to 9 years of age. Optionally the subject is seronegative with respect to all dengue serotypes.

[00419] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are 4 to 16 years of age. In some of such embodiments, the healthy subjects of the subject population are 4 to 5 years of age, 6 to 11 years of age, or 12 to 16 years of age.

[00420] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are defined as being healthy in view of the exclusion criteria specified in Example 6.

[00421] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are from Asia Pacific or Latin America.

[00422] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are seropositive with respect to at least one serotype. In other embodiments, the healthy subjects of the subject population are seronegative with respect to all serotypes.

[00423] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are 4-5 years of age and from Asia Pacific, 6-11 years of age and from Asia Pacific, or 12-16 years of age and from Asia Pacific. In other embodiments, the healthy subjects of the subject population are 4-5 years of age and from Latin America, 6-11 years of age and from Latin America, or 12-16 years of age and from Latin America.

[00424] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are 4-5 years of age and seropositive for at least 1 dengue serotype, 6-11 years of age and seropositive for at least 1 dengue serotype, or 12-16 years of age and seropositive for at least 1 dengue serotype. In other embodiments, the healthy subjects of the subject population are 4-5 years of age and seronegative for all dengue serotypes, 6-11 years of age and seronegative for all dengue serotypes, or 12-16 years of age and seronegative for all dengue serotypes.

[00425] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are from Asia Pacific or Latin America and seropositive for at least one dengue serotype at baseline. In other embodiments, the healthy subjects of the subject population are from Asia Pacific or Latin America and seronegative for at all dengue serotype at baseline.

[00426] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population are from Asia Pacific, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the healthy subjects of the subject population are from Asia Pacific, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or

12-16 years of age. In yet other embodiments, the healthy subjects of the subject population are from Latin America, seropositive for at least one dengue serotype at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age. In other embodiments, the healthy subjects of the subject population are from America, seronegative for all dengue serotypes at baseline and 4-5 years of age, 6-11 years of age, or 12-16 years of age.

[00427] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population had prior vaccination against Yellow Fever. In other embodiments, the healthy subjects of the subject population had no prior vaccination against Yellow Fever. Prior vaccination indicates a vaccination prior to the first vaccination with the reconstituted unit dose as described herein. For example for vaccine efficacy (VE) as determined in Example 6 from 30 days post-second vaccination, a prior vaccination of Yellow Fever is defined as a Yellow Fever vaccination occurring before 30 days post-second vaccination.

[00428] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population had prior vaccination against Japanese Encephalitis. In other embodiments, the healthy subjects of the subject population had no prior vaccination against Japanese Encephalitis.

[00429] In certain embodiments, the invention is directed to said methods, wherein the healthy subjects of the subject population received Dengvaxia® within the administration regimen as described herein or within 4.5 years after administration of the first dose. In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related serious adverse events is less than 0.1%.

[00430] In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related unsolicited adverse events occurring within 4 weeks of administration is less than 2%.

[00431] In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related solicited adverse events occurring within 2 weeks of administration is less than 35%.

[00432] In certain embodiments, the invention is directed to said methods, wherein the occurrence of vaccine related solicited local reactions occurring within 1 weeks of administration is less than 40%.

[00433] In certain embodiments, the invention is directed to said methods, wherein the method does not increase the risk of virologically-confirmed dengue with hospitalization in the individual, such as in a seronegative individual.

[00434] The above methods are also to be considered in the context of a unit dose for use in such methods or in the context of a use of such a unit dose for use in the manufacture of a medicament for such methods.

[00435] In certain embodiments, a tetravalent dengue vaccine such as Dengvaxia® is used for inoculating against dengue disease. Dengvaxia® is a tetravalent dengue vaccine based on a yellow fever backbone, CYD-TDV (Dengvaxia®, Sanofi Pasteur, Lyon, France), and has been licensed in several countries based on the clinical demonstration of an overall vaccine efficacy (VE) against virologically-confirmed dengue (VCD) of 56–61% in children in Asia and Latin America (Capeding MR et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014, 384:1358-65; Villar LA et al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J 2013, 32:1102-9). The preparation of these particular strains CYD1, CYD2, CYD3 and CYD4 has been described in detail in international patent applications WO 98/37911, WO 03/101397, WO07/021672, WO 08/007021, WO 08/047023 and WO 08/065315, to which reference may be made for a precise description of the processes for their preparation. The corresponding nucleotide

sequences of the prM-E regions of CYD1, CYD2, CYD3 and CYD4 are provided in WO2016034629 and SEQID NOs are set out in Table 16 of this reference.

[00436] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, and Dengvaxia® disclosed herein are administered simultaneously on the same day to the subject or to the subject population.

[00437] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, is administered to the subject or to the subject population on day 0/1 as a first administration and Dengvaxia® disclosed herein is subsequently administered to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration. Alternatively, Dengvaxia® disclosed herein is administered to the subject or to the subject population on day 0/1 as a first administration and the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, is administered subsequently to the subject or the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration.

[00438] In certain embodiments, the invention is directed to said method, wherein the dengue vaccine composition comprises other dengue vaccines such as TV003 or TV005. TV003, developed by the U.S. National Institute of Allergy and Infectious Diseases, comprises vaccine components rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31 and rDEN4Δ30, wherein each of these components is present at a concentration of 3 $\log_{10}$PFU. TV005 is similar to TV003 with the difference that the concentration of rDEN2/4Δ30 in TV005 is 4 $\log_{10}$PFU. The vaccines TV003 and TV005 and their vaccine components as well as their production are described in more detail in WO 2008/022196 A2 and S.S. Whitehead, Expert Rev Vaccines, 2016, 15(4): 509 to 517. Using recombinant DNA technology, two attenuation strategies were utilized for the vaccine components of TV003 or TV005: deletions in the 3' untranslated region and structural gene chimerization. For example, the component rDEN4Δ30 contains all the structural and non-structural proteins of a wild type DENV-4, but is attenuated by a 30-nucleotide deletion in the 3' untranslated region (denoted "Δ30"). The other vaccine components are also attenuated due to the 30-nucleotide deletion in the 3' untranslated region. In addition, rDEN3Δ30/31 includes a 31 nucleotide deletion in the 3' untranslated region (shown in detail in Fig. 1c and Fig. 13 of WO 2008/022196 A2). The rDEN2/4Δ30 component was created by substituting the prM and E genes of DENV-2 into the rDEN4Δ30 genome. The complete genomic sequences of dengue strains which can be used to produce TV003 or TV005 are available under the Genbank accession numbers in Table A of WO 2008/022196 A1.

[00439] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, and TV003 or TV005 disclosed herein are administered simultaneously on the same day to the subject or to the subject population.

[00440] In certain embodiments, the invention is directed to said methods, wherein the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a

chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, is administered to the subject or the subject population on day 0/1 as a first administration and wherein TV003 or TV005 disclosed herein is subsequently administered to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration. Alternatively, TV003 or TV005 disclosed herein is administered to the subject or to subject population on day 0/1 as a first administration and the unit dose disclosed herein, which in particular comprises a chimeric dengue serotype 2/1 strain, a live attenuated dengue serotype 2 strain, a chimeric dengue serotype 2/3 strain and a chimeric dengue serotype 2/4 strain, is administered subsequently to the subject or to the subject population within three months from the first administration, such as on day 90 from the first administration, as a second administration.

EXAMPLES

[00441] The following Examples are included to demonstrate certain aspects and embodiments of the invention as described in the claims. It should be appreciated by those of skill in the art, however, that the following description is illustrative only and should not be taken in any way as a restriction of the invention.

Example 1: Preparation of the dengue virus strains.

[00442] The methods used to generate the chimeric dengue strains TDV-1, -3 and -4 were standard molecular cloning and DNA engineering methods and are describeet al. (2003) J. Virology 77(21): 11436-11447. The following well-known methods were used to construct and introduce the prM-E genes of dengue serotypes 1, 3 and 4 into the TDV-2 backbone: Reverse-transcriptase PCR (RT-PCR), PCR, restriction enzyme digestion, DNA fragment ligation, bacterial transformations by electroporation, plasmid DNA preparations, *in vitro* transcription by T7 RNA polymerase, and transfection of Vero cells by electroporation.

[00443] After growing and purifying the different dengue serotypes separately as described in Huang et al. (2013) PLOS Neglected Dis, 7(5):e2243, they are mixed in certain concentrations provided in Example 4. The mixture of dengue serotypes is present in a dengue vaccine composition and combined with a composition of pharmaceutically acceptable excipients resulting in a dengue vaccine composition comprising 15% w/v α,α trehalose dihydrate, 1% w/v poloxamer 407, 0.1% w/v human serum albumin and 100 mM sodium chloride. The dengue vaccine composition is lyophilized and represents a lyophilized unit dose of TDV. The lyophilized unit dose is reconstituted with 37 mM aqueous sodium chloride solution and the reconstituted unit dose comprises 15% w/v α,α trehalose dihydrate, 1% w/v poloxamer 407, 0.1% w/v human serum albumin and 137 mM sodium chloride.

Example 2: Microneutralization test

[00444] Immunogenicity was measured by a microneutralization assay to each one of the four dengue serotypes with titers defined as the dilution resulting in a 50% reduction in plaque values (MNT50). Briefly, on day 1 Vero cells were seeded on 96-well assay plates in DMEM and 10% FBS at a density of 2.5 x 10^5 cells/ml and incubated at 37°C for 24 hours. On day 2 serial dilutions of the heat-inactivated antibody-containing test and control sera samples (dilutions range 1:10 to 1:20480) were prepared and mixed with a constant concentration of dengue viruses, in particular DENV-1 strain 16007, DENV-2 strain 16681, DENV-3 strain 16562 and DENV-4 strain 1036, (target 60-80 pfu/well) in a 96 well microtiter plate and incubated overnight at 2-8°C to enable the neutralization of the virus by the antibodies present in the sera. After the incubation the mixture of virus and antibodies was transferred onto the 96 well plates with Vero cells and the plates were incubated at 37 °C for 90-120 minutes to infect the Vero cells. A 1% methylcellulose overlay in DMEM was applied to the plate to restrict spread of progeny virus and the plate was

incubated for 46-70 hours at 34°C depending on the Dengue serotype:

DENV1 – 66±2 hours
DENV2 - 70±2 hours
DENV3 - 66±2 hours
DENV4 – 46±2 hours

[00445] After the incubation the cells were washed twice with PBS and fixed by adding cold methanol and incubating for 60 minutes at a temperature of ≤ -20°C. After fixing the plates were dried and washed three times with washing buffer (1x PBS, pH 7.4 with 0.5% Tween), before 50 µl of serotype-specific anti-dengue monoclonal antibodies in blocking solution (2.5% nonfat dry milk in PBST) per well were added and incubated with the cells for 18±4 hours at 2-8°C.

[00446] The monoclonal antibodies were made as described in Gentry et al. (1982) Am. J. Trop. Med. Hyg. 31, 548–555; Henchal et al. (1985) Am. J. Trop. Med. Hyg. 34, 162–169; and Henchal et al. (1982) Am. J. Trop. Med. Hyg. 31(4):830-6). Briefly, the anti-DENV-1 HBD was made against dengue 1 strain Hawaii, Envelope, the anti-DENV-2 was made against dengue 2 strain New Guinea C, Envelope, isotype 1, the anti-DENV-3 HBD was made against dengue 3 strain H87, Envelope, isotype 2A, and the anti-DENV-4 HBD was made against dengue 4 strain H241, Envelope, isotype 2A.

[00447] After incubation, the plates were washed three times with washing buffer and 50 µl of a secondary peroxidase labelled goat anti-mouse IgG (H + L) (KPL Cat#074-1806) in blocking solution was added and incubated for 90 to 120 minutes at 37°C. Then the plates were washed three times with washing buffer and 50 µl of precipitant substrate (2-amino-9-ethyl carbazole (AEC) tablet in 2.5 ml DMSO, 47.5 ml 50mM acetate buffer and 250 µl hydrogen peroxide) were added and the mixture was incubated for 20 minutes at room temperature. Finally, the substrate was removed, the plates were rinsed with dH_2O and dried.

[00448] Sample titers are calculated using the linear regression method and reported as MNT50 titers for each sample. Clinical data are reported as a geometric mean titer for all the individual MNT50 titers in each treatment group. Briefly, the number of infectious foci in each well was counted and the titer of neutralizing antibodies was determined by comparing the percent reduction of infectious foci centers in wells containing antibody (test samples) in comparison to wells containing virus alone. The MNT50 was calculated using the following linear regression equation:

MNT50 = 10^[(50-c)/m]) where c = y intercept of regression line and m = slope of regression line

[00449] Each test sample was tested in triplicates and the titer was calculated from the average of the triplicates. A schematic drawing of the steps performed in this test is provided in **Figure 2.**

Example 3: Phase III clinical trial in children

[00450] A Phase III, double-blind, randomized, and placebo-controlled trial in 20100 subjects aged 4 to 16 years living in Thailand, Sri Lanka, Philippines, Panama, Nicaragua, Dominican Republic, Colombia or Brazil was performed evaluating the efficacy, safety and immunogenicity of a tetravalent dengue vaccine referred to hereinafter as TDV (TDV-1, TDV-2, TDV-3 and TDV-4 as described herein). The trial includes 3 parts. Part 1 evaluates vaccine efficacy (VE) and lasts until both of the following 2 criteria are fulfilled: (i) 120 cases of dengue fever are confirmed and (ii) minimum duration of subject follow-up of 12 months post-second vaccination. Part 2 is for an additional 6 months to

evaluate VE and for secondary efficacy analyses. Part 3 will evaluate long-term safety by following participants for side effects and will last an additional 3 years.

[00451] Part 1: Active surveillance for the primary assessment of efficacy in all subjects. During this time subjects were contacted at least weekly to ensure identification of febrile illness that could potentially be due to dengue. This part commenced on the day of vaccination and finished once both of the following 2 criteria were fulfilled: (i) 120 cases of dengue fever are confirmed and (ii) minimum duration of subject follow-up of 12 months post-second vaccination. The end of Part 1 was defined for each subject so that the duration of follow up after the second vaccination was approximately the same for all subjects. Virologically-confirmed cases in Part 1 count towards the primary efficacy objective if occurring at least 30 days post-second vaccination. Part 1 was finished 12 months post-second vaccination

[00452] Part 2: Active surveillance for an additional 6 months for each subject following the completion of Part 1, I, i.e. 18 month post second vaccination. During this time subjects were contacted at least weekly to ensure identification of febrile illness that could potentially be due to dengue. Virologically-confirmed cases in Parts 1 and 2 contribute towards the secondary efficacy objectives.

[00453] Part 3: Modified active surveillance for the assessment of safety in all subjects following the completion of Part 2 and lasting 3 years for each subject. The modified surveillance during Part 3 will maintain at least weekly contacts through Part 3 of the trial, but the intensity of investigation will be modified based on the need for hospitalization. Surveillance will identify febrile illness of any severity that could potentially be due to dengue.

[00454] Criteria for Inclusion include:

- The subject was aged 4 to 16 years inclusive, at the time of randomization.
- Individuals who were in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs) and clinical judgment of the Investigator.
- The subject and/or the subject's parent/guardian signed and dated an assent/written informed consent form where applicable, and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.
- Individuals who can comply with trial procedures and are available for the duration of follow-up.

[00455] Exclusion criteria include:

1. Febrile illness (temperature ≥38°C) or moderate or severe acute illness or infection at the time of randomization.
2. History or any illness that, in the opinion of the Investigator, might interfere with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to:
    a. Known hypersensitivity or allergy to any of the vaccine components.
    b. Female subjects (post-menarche) who are pregnant or breastfeeding.
    c. Individuals with any serious chronic or progressive disease according to judgment of the Investigator (e.g., neoplasm, insulin-dependent diabetes, cardiac, renal or hepatic disease, neurologic or seizure disorder or Guillain-Barré syndrome).
    d. Known or suspected impairment/alteration of immune function, including:
        i. Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/ ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0) (use of

inhaled, intranasal, or topical corticosteroids is allowed).

ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0).

iii. Administration of immunoglobulins and/or any blood products within the 3 months prior to Day 1 (Month 0) or planned administration during the trial.

iv. Receipt of immunostimulants within 60 days prior to Day 1 (Month 0).

v. Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (Month 0).

vi. Human Immunodeficiency Virus (HIV) infection or HIV-related disease.

vii. Genetic immunodeficiency.

3. Receipt of any other vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (Month 0) or planning to receive any vaccine within 28 days after Day 1 (Month 0).
4. Participation in any clinical trial with another investigational product 30 days prior to Day 1 (Month 0) or intent to participate in another clinical trial at any time during the conduct of this trial.
5. Previous participation in any clinical trial of a dengue candidate vaccine, or previous receipt of a dengue vaccine.
6. First degree relatives of individuals involved in trial conduct.
7. Females of childbearing potential who are sexually active, and who have not used any of the acceptable contraceptive method for at least 2 months prior to Day 1 (Month 0).
8. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks post-second vaccination.
9. Deprived of freedom by administrative or court order, or in an emergency setting, or hospitalized involuntarily.
10. Current alcohol abuse or drug addiction that may interfere with the subject's ability to comply with trial procedures.
11. Identified as an employee of the Investigator or trial center, with direct involvement in the proposed trial or other trials under the direction of that Investigator or trial center.

[00456] Eligible subjects were randomized (2:1) into two treatment groups: groups 1 received one subcutaneous (SC) dose of TDV in the upper arm on Day 1 and on Day 90, and group 2 received one subcutaneous dose of placebo in the upper arm on Day 1 and on Day 90. Randomization was stratified by region (Asia Pacific and Latin America) and age range (children aged 4-5 years, 6-11 years, and 12-16 years) to ensure each age range has the appropriate ratio of TDV to placebo in each region. After randomization dropouts were not replaced. Study Day 1 is defined to be the date of the first dose administration of TDV or placebo. The TDV was prepared as described in Example 1. Each subcutaneous dose of TDV was 0.5 mL and the concentration of the four dengue serotypes in the TDV vaccine in each dose was 3.6 $\log_{10}$ PFU/dose, 4.0 $\log_{10}$ PFU/dose, 4.6 $\log_{10}$ PFU/dose and 5.1 $\log_{10}$ PFU/dose of TDV-1, TDV-2, TDV-3 and TDV-4, respectively.

[00457] The „total concentration in pfu/0.5 ml" which serves as a base value for the calculation of the percentage concentration for each individual component of a tetravalent dengue vaccine is shown for one exemplary tetravalent vaccine composition comprising dengue serotype 1 in a concentration of 3.60 $\log_{10}$pfu/0.5 ml, a dengue serotype 2 concentration of 4.00 $\log_{10}$pfu/0.5 ml, a dengue serotype 3 concentration of 4.60 log10pfu/0.5 ml and a dengue serotype 4 concentration of 5.11 $\log_{10}$pfu/0.5 ml.

Primarily, the logarithmic values of the concentrations are converted into numerical values. The results of this conversion are $4x10^3$ pfu/0.5 ml for serotype 1, $1x10^4$ pfu/0.5ml for serotype 2, $4x10^4$ pfu/0.5 ml for serotype 3 and

$1.3x10^5$ pfu/0.5 ml for serotype 4. The total concentration in pfu/0.5 ml is the sum of the preceding numerical values resulting in $1.84 x10^5$ pfu/0.5 ml.

[00458] The "percentage concentration" for each of the serotypes 1, 2, 3 and 4 is obtained by dividing the numerical concentration value (expressed as pfu/0.5 ml) of an individual serotype by the total concentration (expressed in pfu/0.5 ml) and multiplying the result by 100 i.e.:
Percentage concentration of serotype 1 = ($4x10^3$ pfu/0.5 ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 2%
Percentage concentration of serotype 2 = ($1x10^4$ pfu/0.5ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 5%
Percentage concentration of serotype 3 = ($4x10^4$ pfu/0.5 ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 22%
Percentage concentration of serotype 4 = ($1.3x10^5$ pfu/0.5 ml ÷ $1.84 x10^5$ pfu/0.5 ml) x 100 = 71%.
The percentage concentrations are rounded to whole numbers.

[00459] Primary Outcome Measures included the vaccine efficacy (VE) of two doses of TDV in preventing virologically-confirmed dengue (VCD) fever induced by any dengue serotype [time frame: 30 days post-second vaccination (Day 120) until the end of Part 1]. VE is defined as 1 - (λv/λc), wherein λv and λc denote the hazard rates for the TDV and placebo groups, respectively. A virologically-confirmed dengue case is defined as febrile illness (defined as temperature ≥38°C on any 2 of 3 consecutive days) or illness clinically suspected to be dengue by the Investigator with a positive serotype-specific reverse transcriptase polymerase chain reaction (RT-PCR). A febrile illness will require an interval of at least 14 days from a previous febrile illness to avoid overlap of acute and convalescent visits from one episode with those from a second episode.

[00460] Secondary Outcome Measures include:

1) VE of two doses of TDV in preventing virologically-confirmed dengue fever induced by each dengue serotype [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].

2) VE of two doses of TDV in preventing virologically-confirmed dengue fever induced by any dengue serotype in participants dengue seronegative at baseline [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2 (up to 21 months)].

3) VE of two doses of TDV in preventing virologically-confirmed dengue fever induced by any dengue serotype in participants dengue seropositive at baseline [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].

4) VE of two doses of TDV in preventing hospitalization due to virologically-confirmed dengue fever induced by any dengue serotype [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].

5) VE of two doses of TDV in preventing virologically-confirmed severe dengue fever induced by any dengue serotype [time frame: from 30 days post-second vaccination (Day 120) until the end of Part 2].

6) Percentage of participants with solicited local injection site adverse events (AEs) in the safety subset [time frame: Days 1 through 7 after each vaccination] and severity of solicited local injection AEs. Solicited local AEs at injection site are defined as pain, erythema and swelling that occurred within 7 days after each vaccination.

7) Percentage of participants with solicited systemic adverse events (AEs) in the safety subset [time frame: Days 1 through 14 after each vaccination] and severity of solicited systemic AEs. Solicited systemic AEs in children (< 6 years) are defined as fever, irritability/fussiness, drowsiness and loss of appetite that occurred

within 14 days after each vaccination. Solicited systemic AEs in children (≥ 6 years) are defined as fever, headache, asthenia, malaise and myalgia that occurred within 14 days after each vaccination.

8) Percentage of participants with any unsolicited adverse events (AEs) in the safety subset [time frame: Days 1 through 28 after each vaccination]. Unsolicited AEs are any AEs that are not solicited local or systemic AEs, as defined above.

9) Percentage of participants with serious adverse events (SAEs) during Parts 1 and 2 [time frame: from Day 1 until the end of Parts 1 and 2]. A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability / incapacity, is a congenital anomaly / birth defect or is medically important due to other reasons than the above mentioned criteria.

10) Percentage of participants with fatal SAEs and SAEs related to study drug during the first and second half of Part 3 [time frame: for 3 years (18 month halves) beginning at the end of Part 2 (approximately 21 months after the first vaccination)].

11) Percentage of participants with a seropositive response for each of the four dengue serotypes in the immunogenicity subset [time frame: Day 1 and months 1, 3, 4, 9, 15 and then annually (up to 3 years)]. Seropositive response is defined as a reciprocal neutralizing titer ≥ 10. The four DENV serotypes are DEN-1, DEN-2, DEN-3 and DEN-4.

12) Percentage of participants with a seropositive response for multiple dengue serotypes in the immunogenicity subset [time frame: Day 1 and months 1, 3, 4, 9, 15 and then annually (up to 3 years)].

13) Geometric Mean Titers (GMTs) of neutralizing antibodies for each of the four dengue serotypes in the immunogenicity subset [time frame: Day 1 and months 1, 3, 4, 9, 15 and then annually (up to 3 years)]. GMTs of neutralizing antibodies will be measured via microneutralization test (MNT) as described in Example 2.

a) Study population

[00461] After screening, 20,099 participants were randomized, and 20,071 received at least one injection. In total, 97.4% of placebo participants (n/N: 6,521/6,698) and 97.3% of vaccinees (n/N: 13,038/13,401) completed Part 1 of the study (Figure 3). Reasons for study withdrawals included AEs, participants lost to follow-up, pregnancy, protocol violations, and withdrawal by participants (or parents/guardians). Baseline characteristics were similar across both treatment groups (Table 5). Mean age of study participants was 9.6 years, with baseline seronegativity of 27.7%, and enrollment was broadly balanced across regions (46.5% in Asia, 53.5% in Latin America). The highest seronegative rate was in Panama (62.2%), followed by Sri Lanka (38.5%), Thailand (34.4%), Brazil (28.8%), Nicaragua (22.3%), Colombia (15.4%), the Philippines (12.4%), and the Dominican Republic (2.8%).

Table 5. Baseline characteristics of study population (number, %)

	TDV	Placebo	Total
Per Protocol Set			
Number of Participants	12,704	6,317	19,021
Mean Age (Years, SD)	9.6 (3.35)	9.6 (3.34)	9.6 (3.35)
Baseline Seronegative[a]	3,533 (27.8)	1,726 (27.3)	5,259 (27.7)
Female	6,314 (49.7)	3,098 (49.0)	9,412 (49.5)

Male	6,390 (50.3)	3,219 (51.0)	9,609 (50.5)
Asia Pacific	5,896 (46.4)	2,942 (46.6)	8,838 (46.5)
Baseline Seronegative[a]	1,503 (25.5)	773 (26.3)	2,276 (25.8)
Latin America	6,808 (53.6)	3,375 (53.4)	10,183 (53.5)
Baseline Seronegative[a]	2,030 (29.8)	953 (28.2)	2,983 (29.3)
Safety Set[b]			
Number of Participants	13,380	6,687	20,071
Mean Age (Years, SD)	9.6 (3.36)	9.6 (3.34)	9.6 (3.35)
Baseline Seronegative[a]	3,714 (27.8)	1,832 (27.4)	5,547 (27.6)
Female	6,651 (49.7)	3,276 (49.0)	9,929 (49.5)
Male	6,729 (50.3)	3,411 (51.0)	10,142 (50.5)
Safety Set of Subset[b]			
Number of Participants	2,663	1,329	3,993
Baseline Seronegative[a]	740 (27.8)	369 (27.8)	1,109 (27.8)

[a]Seronegative for all serotypes; seropositive defined as reciprocal neutralizing antibody titer ≥10; SD, standard deviation.
[b]numbers of participants in TVD plus placebo groups are not equal to total numbers shown because misallocated participants (i.e. those who received both TVD and placebo due to an administrative error) are not included in the TDV and placebo group data.

b) Febrile illnesses and VCD

[00462] During Part 1, 5,754 and 4,663 episodes of febrile illness were reported in Asian and Latin American sites, respectively. Acute samples were obtained in 99.5% and 96.6% of these cases, with 98.3% and 85.1% of samples taken within five days, in Asia and Latin America, respectively. There were 278 VCD cases (76 hospitalized) in the safety set during the entire Part 1 period, of which 210 (58 hospitalized) were 30 days post-second vaccination in the PPS (Table 6; Table 8) and were included in primary endpoint analysis.

c) Distribution of VCD included in primary endpoint analysis

[00463] DENV-1 was reported in all countries with VCD and included all the 21 cases in Panama. In Sri Lanka, 54 of 60 VCD were DENV-2, and 87 of 109 VCD in the Philippines were DENV-3. All seven DENV-4 VCD were reported in the Philippines. No VCD were reported in Nicaragua or the Dominican Republic. Of the associated 58 hospitalized VCD, 43 were reported in Sri Lanka. A total of two severe dengue (both DENV-3) and five dengue hemorrhagic fever (DHF; three DENV-2; two DENV-3) cases were reported (Table 7). These seven were also the only such cases in the entire part 1 safety set.

d) Vaccine efficacy

[00464] VE against VCD of any serotype was 80.2% (95% CI: 73.3–85.3; P<0.001). A similar efficacy of 81% (95% CI: 64.1–90.0) between the doses and from first dose onwards in the safety set (Table 6) suggests that the vaccine was efficacious after the first dose. Exploratory analysis of the secondary efficacy endpoints showed a trend of differential efficacy by serotype, with the highest efficacy against DENV-2 (97.7%), followed by DENV-1 (73.7%), DENV-4 (63.2% with CI containing zero), and DENV-3 (62.6%; Table 7). Overall, efficacy was similar in baseline seronegatives (74.9%) and seropositives (82.2%; Figure 4 above); however, this varied by serotype. Efficacy against DENV-2 was not impacted by serostatus; efficacy against DENV-1 was slightly higher in baseline seropositives (79.8%; 95% CI: 51.3–91.6) than baseline seronegatives (67.2%; 95% CI: 23.2–86.0). No efficacy was observed against DENV-3 in baseline seronegatives (–38.7%; 95% CI: –335.7–55.8) compared to baseline seropositives (71.3%; 95% CI: 54.2–82.0). Efficacy by serostatus could not be calculated for DENV-4 because no cases were

observed in baseline seronegatives. In the primary endpoint timeframe of the PPS, only five VCD requiring hospitalization were reported in the vaccine group compared with 53 cases in the placebo group, with a VE of 95.4% (95% CI: 88.4–98.2; 97.2% for baseline seronegatives and 94.4% for baseline seropositives; Table 7; Figure 4 below), consistent with a VE of 93.3% (95% CI: 86.7–96.7) in the safety set from first dose onwards.

[00465] The primary vaccine efficacy (VE) of two doses of TDV in preventing virologically-confirmed dengue (VCD) fever induced by any dengue serotype is shown in Table 6.

Table 6. Vaccine efficacy of TDV in preventing virologically-confirmed dengue (VCD) fever against any serotype from 30 days post-second vaccination until end of part 1 Per Protocol Set (PPS), i.e. 12 months post-second vaccination. Safety set analysis from first dose to end of Part 1 study period, i.e. 12 months post-second vaccination

	Placebo n= 6317	**TDV (PPS) n= 12,704**
number of subject evaluated	6,316	12,700
number of subjects with febrile illness	1,712	3,195
number of febrile illness cases	2,591	4,692
virologically confirmed dengue fever (n [%])	149 [2.4]	61 [0.5]
Person-years at risk	5,670.1	11,578.7
incident density	2.6	0.5
relative risk	0.20	
95% CI of relative risk	(0.15, 0.27)	
vaccine efficacy (%)	80.2	
95% CI of vaccine efficacy	(73.3, 85.3)	
p-value for vaccine efficacy	<0.001	
	Placebo	**TDV (Safety Set)***
number of subject evaluated	6,687	13,380
virologically confirmed dengue fever (n [%])	199 [3.0]	78 [0.6]
Person-years at risk	8,072.0	16,351.5
incident density	2.5	0.5
vaccine efficacy (%)	80.9	
95% CI of vaccine efficacy	(75.2, 85.3)	

Note 1: Percentage of virologically confirmed dengue (VCD) fever are based on number of subjects evaluated.

Note 2: Person-years at risks is defined as cumulative time in years until start of VCD fever or until end of Part 1 study period or discontinuation date, whichever comes first. Incident density is defined as the number of cases per 100 person-years at risk. Percentages are based on total number (denominator) of analysis set participants evaluated and may not be equal to the total number of participants in the per protocol analysis set.

*One participant had two instances of VCD during Part 1, only the first VCD was included in efficacy calculation

Note 3: Vaccine efficacy (VE) and 2-sided 95% CIs are estimated from a Cox proportional hazard model with TDV as a factor, adjusted for age and stratified by region.

Note 4: Statistical significance will be concluded if the lower bound of the 95% CI for VE is above 25%. Since the hypotheses will be tested in a confirmatory manner at a 2-sided significance level of 5%, the calculated p-value should be compared with 0.025.

Note 5: Relative risk is calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.

[00466] For the efficacy evaluation shown in Table 6, a case of VCD was defined as febrile illness (defined as fever

≥38°C on any 2 of 3 consecutive days) with a positive serotype-specific RT-PCR (i.e., positive dengue detection RT-PCR) and occurring at any time starting from 30 days post-second vaccination (Day 120 [Month 4]) through the end of Part 1. The analysis was performed on the Per-Protocol Set (PPS) and Safety Set.

[00467] As used herein, the "Per-Protocol Set (PPS)" consist of all subjects in the Full Analysis Set (FAS) consisting of all randomized subjects who received at least one dose of TDV or placebo who had no major protocol violations. Major protocol violations are not receiving both doses of TDV or placebo administration, not receiving both doses in the correct interval, not having the correct administration of TDV or placebo, use of prohibited medications / vaccines by the subject, the subject meets any of the exclusion criteria of 2d, 3, 4 or 5 defined above or product preparation error.

[00468] The p-value is obtained by solving the critical value Z in the following equation:

Upper bound of 1-sided (1-p%) CI of HR=0.75, wherein HR is the hazard ratio and defined as HR= λV/λC.

$e^{[\hat{\beta}+Z*\hat{SE}]} = 0.75$, wherein $\hat{\beta}$ defines the treatment and $\hat{SE}$ the related standard error.

The 1-sided p-value is 1-(area to the left of the critical value Z from a standard normal distribution). Since the hypotheses will be tested in a confirmatory manner 2-sided at a significance level of 5%, the calculated 1-sided p-value should be compared with 0.025.

[00469] In summary in Part 1 of this study, a high vaccine efficacy of 80.2% against virologically-confirmed dengue of any serotype in children 4–16 years of age was found. It included an efficacy of 74.9% in baseline seronegatives and a robust 95.4% reduction in hospitalizations. Onset of protection could be seen after the first dose with 81% efficacy between doses. Overall, these results suggest a potential benefit for each vaccine recipient regardless of prior dengue exposure or age. This finding is significant because vaccine development against dengue has been challenging, especially for dengue naïve individuals, and dengue remains one of the WHO's top ten threats to global health in 2019.19 Furthermore, the onset of protection after the first dose has potential utility in the context of outbreak control or travel vaccination, offering a reduction in the risk of dengue after only one dose.

[00470] Severe forms of dengue were assessed as follows: Dengue Hemorrhagic Fever (DHF) as defined by the 1997 WHO definition. Severe Dengue through the Dengue Case Adjudication Committee. The Dengue Case Adjudication Committee (DCAC) consisted of four members: a voting chairperson, two voting members, and an independent non-voting statistician. The three DCAC voting members are all physicians and clinical dengue experts. DCAC members are not study investigators and do not have any conflict of interest that would bias their review of the trial data. All non-hospitalized cases were considered non-severe. The DCAC severe dengue case criteria applied in a blinded manner to virologically-confirmed hospitalized dengue cases are as follows: 1) bleeding abnormality, for a case to be considered severe there needs to be a significant intervention required in response to the bleeding episode such as blood transfusion, nasal packing, hormonal therapy, or, bleeding occurred into critical organs such as the brain; 2) plasma leakage, for a case to be considered severe there needs to be evidence of both plasma leakage and functional impairment (plasma leakage includes clinical evidence, radiological evidence, or hematocrit elevated >20% above normal levels or baseline; functional impairment defined as shock or respiratory distress); 3) liver, for a case to be considered severe there needs to be evidence of both hepatitis and functional impairment (hepatitis defined as an aspartate aminotransferase [AST] or alanine aminotransferase [ALT] >10 upper limit of normal range [ULN]; functional impairment defined as prothrombin [PT] >1.5 ULN or hypoalbuminemia); 4) renal, serum creatinine >2.5 times ULN or requiring dialysis; 5) cardiac, abnormalities intrinsic to the heart (i.e. not resulting from intravascular volume depletion) and with evidence of functional impairment (examples of intrinsic abnormality: myocarditis, pericarditis, and myopericarditis; example of functional impairment: new conduction abnormality resulting in irregular heart rhythm [i.e. not transient first-degree heart block]); 6) central nervous system, any

abnormality with the exception of a simple febrile convulsion or a brief delirium; 7) shock, all shock cases considered severe. At least 1 functional impairment (of criterion 3,4,5,6), needs to be present but the totality of data were considered by the members in their assessment.

[00471] Further results of part 1 and part 2 are presented in Tables 7a to c.

Table 7a: Distribution of cases contributing to primary endpoint by per protocol set subgroup (30 days after second vaccination until end of Part 1, i.e. 12 months after second vaccination)

	TDV Dengue Cases	TDV Incidence Density	Placebo Dengue Cases	Placebo Incidence Density	Vaccine Efficacy (95% CI)
VCD cases					
Baseline Seropositive[a]	41/9,165 (0.4%)	0.5	110/4,587 (2.4%)	2.7	82.2% (74.5%–87.6%)
Baseline Seronegative[a]	20/3,531 (0.6%)	0.6	39/1,726 (2.3%)	2.5	74.9% (57.0%–85.4%)
DENV-1	16/12,700 (0.1%)	0.1	30/6,316 (0.5%)	0.5	73.7% (51.7%–85.7%)
DENV-2	3/12,700 (<0.1%)	<0.1	64/6,316 (1.0%)	1.1	97.7% (92.7%–99.3%)
DENV-3	39/12,700 (0.3%)	0.3	51/6,316 (0.8%)	0.9	62.6% (43.3%–75.4%)
DENV-4[d]	3/12,700 (<0.1%)	<0.1	4/6,316 (<0.1%)	<0.1	63.2% (-64.6%–91.8%)
4–5 Years Old	13/1,619 (0.8%)	0.9	23/801 (2.9%)	3.2	72.8% (46.2%–86.2%)
6–11 Years Old	34/7,009 (0.5%)	0.5	85/3,491 (2.4%)	2.7	80.7% (71.3%–87.0%)
12–16 Years Old	14/4,072 (0.3%)	0.4	41/2,024 (2.0%)	2.2	83.3% (69.3%–90.9%)
Asia	54/5,894 (0.9%)	1.0	127/2,942 (4.3%)	4.9	79.5% (71.8%–85.1%)
Latin America	7/6,806 (0.1%)	0.1	22/3,374 (0.7%)	0.7	84.3% (63.1%–93.3%)
Hospitalized VCD cases					
Baseline Seropositive[a]	4/9,165 (<0.1%)	<0.1	35/4,587 (0.8%)	0.8	94.4% (84.3%–98.0%)
Baseline Seronegative[a]	1/3,531 (<0.1%)	<0.1	18/1,726 (1.0%)	1.2	97.2% (79.1%–99.6%)
Cases of DHF[b]					
All participants	1/12,700 (<0.1%)	<0.1	4/6,316 (<0.1%)	<0.1	87.3% (-13.5%–98.6%)
Severe VCD Cases[c]					
All participants	1/12,700 (<0.1%)	<0.1	1/6,316 (<0.1%)	<0.1	50.8% (-686.9%–96.9%)

VCD, virologically-confirmed dengue; DHF, dengue hemorrhagic fever

[a]Seronegative for all serotypes; baseline seropositive defined as reciprocal neutralizing antibody titer ≥10 to one or more serotypes.

[b]VCD cases meeting WHO 1997 DHF criteria; incidence density defined as the number of cases per 100 person-years at risk; percentages are based on total number (denominator) of per protocol set participants evaluated.

[c]two severe VCD were not classified as DHF.

[d] The number of cases identified was sufficient to provide reasonably precise estimates of vaccine efficacy against all individual serotypes, except DENV-4.

Table 7b: Distribution of cases contributing to secondary endpoint by per protocol set subgroup (30 days after second vaccination until end of Part 2, i.e. 18 months after second vaccination)

	TDV Dengue Cases	TDV Incidence Density	Placebo Dengue Cases	Placebo Incidence Density	Vaccine Efficacy (95% CI)
VCD cases					
Overall					73. 3% (66.5%-78.8%)
Baseline Seropositive[a]	75	0.6	150	2.4	76.1% (68.5%–81.9%)
Baseline Seronegative[a]	39	0.8	56	2.4	66.2% (49.1%–77.5%)
DENV-1	38	0.2	62	0.7	69.8% (54.8%–79.9%)
Baseline Seropositive[a]	21	0.2	37	0.6	72.0 (52.2%-83.6%)
Baseline Seronegative[a]	17	0.3	25	1	67.8 (40.3%-82.6%)
DENV-2	8	<0.1	80	0.9	95.1% (89.9%–97.6%)
Baseline Seropositive[a]	7	<0.1	54	0.9	93.7 (86.1%-97.1%)
Baseline Seronegative[a]	1	<0.1	26	1.1	98.1 (85.8%-99.7%)
Hospitalized VCD cases					
Overall	13	<0.1	66	0.8	90.4% (82.6%-94.7%)
Baseline Seropositive[a]	8	<0.1	45	0.7	91.4% (81.7%–95.9%)
Baseline Seronegative[a]	5	0.1	21	0.9	88.1% (68.5%–95.5%)

VCD, virologically-confirmed dengue;
[a]Seronegative for all serotypes; baseline seropositive defined as reciprocal neutralizing antibody titer ≥10 to one or more serotypes.

Table 7c: Distribution of cases contributing to secondary endpoint by safety set (first vaccination until end of Part 2, i.e. 21 months after first vaccination)

	TDV Dengue Cases	TDV Incidence Density	Placebo Dengue Cases	Placebo Incidence Density	Vaccine Efficacy (95% CI)
VCD cases					
Overall					75.3 % (69.5%-80.0%)
Overall in between[a]					81.0% (64.1%-90.0%)
Baseline Seropositive[b]	89	0.5	187	2.3	77.2% (70.6%–82.3%)
Baseline Seronegative[b]	42	0.7	70	2.3	70.6% (56.9%–79.9%)
DENV-1	41	0.2	78	0.7	73.9% (61.9%–82.1%)
DENV-2	14	<0.1	109	1.0	93.7% (89.0%–96.4%)
Hospitalized VCD cases					
Overall	17	<0.1	81	0.7	89.7% (82.6%-93.9%)

VCD, virologically-confirmed dengue;
[a]In between: VCD after first vaccination and before second vaccination.
[b]Seronegative for all serotypes; baseline seropositive defined as reciprocal neutralizing antibody titer ≥10 to one or more serotypes.

Table 7d: Dengvaxia® VCD (first vaccination until 25 months after first vaccination (i.e.13 month after third vaccination), ITT from CYD15, 9 to 16 years of age)[a]

	Vaccine Efficacy (95% CI)
Overall VCD	64.7 % (58.7%-69.8%)
Baseline Seropositive[b]	83.7% (62.2%-93.7%)
Baseline Seronegative[b]	43.2% (-61.5%–80.0%)
DENV-1	58.8% (40.2%–65.9%)
DENV-2	50.2%(31.8%-63.6%)
Overall Hospitalized VCD	80.3% (64.7%-89.5%)

[a]Luis Villar et al. Efficacy of a tetravalent dengue vaccine in Children in Latin America: N Engl J of Med 2015 Vol. 372 No2, 113-123

[00472] Clinical signs and symptoms of virologically-confirmed dengue cases during Part 1 study period in safety set data are shown in **Table 8**.

Table 8: Clinical signs and symptoms of virologically-confirmed dengue cases during Part 1 study period (safety set data)

	TDV (N=13,380)	Placebo (N=6,687)	Relative Risk
Number of VCD Cases	78	200	–
Median Duration of Febrile Illness (days; 95% CI)[a]	6.0 (5.7–7.4)	6.0 (5.9–6.8)	–
Median Duration of Fever (days; 95% CI)	4.0 (3.9–4.6)	5.0 (4.5–5.0)	–
Number of Hospitalized VCD Cases	9	67	–
Median Duration of Hospitalization (days; 95% CI)	5.0 (2.8–5.4)	5.0 (4.6–5.4)	–
Evidence of Bleeding (%, n/N)	3.8% (3/78)	3.5% (7/200)	1.10
Plasma Leakage (%, n/N)	2.6% (2/78)	6.5% (13/200)	0.39
Plasma Leakage - Pleural Effusion (%, n/N)	1.3% (1/78)	1.5% (3/200)	–
Plasma Leakage - Ascites (%, n/N)	1.3% (1/78)	3.0% (6/200)	–
Plasma Leakage - Radiological Signs (%, n/N)	40.0% (2/5)	19.6% (10/51)	–
Plasma Leakage - Hematocrit Increase ≥20% (%, n/N)[b]	3.8% (2/53)	9.5% (13/137)	–
Platelet Count $\leq 100x10^9$ (%, n/N)[c]	6.4% (5/78)	22.0% (44/200)	0.29
Platelet Count $\leq 50x10^9$ (%, n/N)[c]	3.8% (3/78)	11.0% (22/200)	0.35
ALT or AST ≥1000 U/L (%, n/N)[c]	0% (0/78)	0% (0/200)	–

VCD, virologically-confirmed dengue; ALT, alanine aminotransferase; AST, aspartate aminotransferase

[a]Duration of febrile illness defined as start date of earliest symptom to end date of latest symptom plus one day (symptoms considered include fever and any general symptoms).

[b]Hematocrit increase defined as maximum hematocrit between Day 3 and Day 7 inclusive, from onset of fever ≥20% increase over minimum hematocrit before Day 3 or after Day 7 from onset of fever.

[c]For platelet, ALT, and AST data, assessments within 14 days of onset of febrile illness have been considered.

N refers to number of VCD cases with available data for the specific parameter

e) Immunogenicity

[00473] The highest geometric mean titers (GMTs) were observed against DENV-2 regardless of baseline serostatus (Table 10). A very high tetravalent seropositivity rate (99.5%) in baseline seronegatives one month after the second dose (Tables 9 and 10) was observed.

[00474] Seropositivity rate (% of seropositive subjects) for each of the four dengue serotypes is determined at prevaccination on Day 1 (Month 0), post-first vaccination on Day 30 (Month 1), prevaccination on Day 90 (Month 3), post-second vaccination on Day 120 (Month 4), Day 270 (Month 9), Day 450 (Month 15), and then annually. Seropositivity rates (% participants, 95% CI) by dengue serotype per protocol set for immunogenicity data for Day 0, Day 30, Day 90, Day 120, and Day 270 are shown in Table 9.

[00475] Seropositivity rates (% participants, 95% CI) by dengue serotype against three or more serotypes (trivalent) and against all four serotypes (tetravalent) per protocol set for immunogenicity data for Day 0, Day 30, Day 90, Day 120, and Day 270 are shown in Table 9. The tetravalent seropositivity rates were high (>91%) in baseline seronegatives six months after second dose.

Table 9: Seropositivity rates (% participants, 95% CI) by dengue serotype (per protocol set for immunogenicity data)

BASELINE SEROPOSITIVE		BASELINE SERONEGATIVE	
TDV N=1,816	Placebo N=902	TDV N=702	Placebo N=345
DENV-1			
89.1 (87.6–90.5)	90.6 (88.5–92.4)	0 (0–0.5)	0 (0–1.1)
99.5 (99.1–99.8)	88.6 (86.3–90.7)	94.1 (92.0–95.8)	4.9 (2.8–7.8)
99.3 (98.8–99.6)	90.2 (88.1–92.1)	91.6 (89.3–93.5)	6.1 (3.8–9.2)
>99.9 (99.7–100)	90.3 (88.1–92.3)	99.5 (98.6–99.9)	8.3 (5.5–11.9)
99.6 (99.1–99.8)	89.8 (87.5–91.8)	95.1 (93.0–96.6)	9.0 (6.0–12.8)
DENV-2			
96.5 (95.6–97.3)	97.2 (95.9–98.2)	0 (0–0.5)	0 (0–1.1)
99.9 (99.6–100)	93.3 (91.4–94.9)	98.6 (97.4–99.4)	10.7 (7.5–14.5)
>99.9 (99.7–100)	94.0 (92.2–95.5)	99.0 (98.0–99.6)	12.2 (8.9–16.1)
99.9 (99.6–100)	93.6 (91.7–95.2)	100 (99.4–100)	14.7 (11.0–19.1)
100 (99.8–100)	94.6 (92.8–96.1)	100 (99.4–100)	18.3 (14.1–23.2)
DENV-3			
88.1 (86.5–89.6)	88.0 (85.7–90.1)	0 (0–0.5)	0 (0–1.1)
99.8 (99.4–99.9)	87.6 (85.1–89.7)	96.1 (94.3–97.4)	4.0 (2.1–6.7)
99.5 (99.1–99.8)	87.3 (84.9–89.4)	94.4 (92.5–96.0)	2.0 (0.8–4.1)
99.8 (99.5–100)	87.9 (85.5–90.1)	100 (99.4–100)	5.1 (2.9–8.2)
99.7 (99.4–99.9)	87.1 (84.6–89.4)	96.4 (94.6–97.7)	7.7 (4.9–11.3)
DENV-4			
88.1 (86.5–89.6)	87.4 (85.0–89.5)	0 (0–0.5)	0 (0–1.1)
99.6 (99.2–99.9)	86.6 (84.1–88.8)	90.5 (88.0–92.6)	1.8 (0.7–3.9)
99.3 (98.8–99.7)	86.9 (84.5–89.0)	92.0 (89.8–93.9)	2.9 (1.4–5.3)
>99.9 (99.7–100)	88.3 (85.9–90.4)	99.8 (99.1–100)	4.8 (2.7–7.8)
99.7 (99.3–99.9)	87.6 (85.1–89.9)	97.0 (95.4–98.2)	6.3 (3.9–9.7)
Three or More Serotypes			
87.5 (85.9–89.0)	87.3 (84.9–89.4)	0 (0–0.5)	0 (0–1.1)
99.8 (99.5–100)	87.2 (84.7–89.4)	96.5 (94.8–97.8)	1.2 (0.3–3.1)
99.7 (99.3–99.9)	87.7 (85.3–89.7)	94.9 (93.0–96.4)	1.7 (0.6–3.7)
99.9 (99.6–100)	88.4 (86.0–90.5)	99.8 (99.1–100)	4.2 (2.2–7.0)
99.7 (99.4–99.9)	87.3 (84.7–89.5)	97.5 (96.0–98.6)	5.7 (3.3–8.9)
All Four Serotypes			
83.5 (81.7–85.2)	83.5 (80.9–85.8)	0 (0–0.5)	0 (0–1.1)
99.1 (98.5–99.5)	82.9 (80.2–85.4)	85.3 (82.4–87.9)	0.9 (0.2–2.6)
98.6 (97.9–99.1)	83.6 (81.0–86.0)	84.3 (81.4–86.9)	1.4 (0.5–3.3)
99.8 (99.5–100)	85.2 (82.6–87.6)	99.5 (98.6–99.9)	3.5 (1.8–6.2)
99.2 (98.7–99.6)	84.6 (81.9–87.0)	91.3 (88.7–93.4)	5.3 (3.1–8.5)

Seropositivity rates (% participants, 95% CI) by dengue serotype (per protocol set for immunogenicity data; seropositive defined as a reciprocal neutralizing antibody titer ≥10; baseline seronegative defined as seronegative to all serotype; baseline seropositive defined as seropositive to one or more serotypes; N refers to number of participants in the analysis set; number of participants evaluated at each time point may vary)

[00476] Geometric mean titers (GMTs) of neutralizing antibodies (microneutralization test [MNT]) for each dengue serotype are determined at pre-vaccination on Day 1 (Month 0), post-first vaccination on Day 30 (Month 1), pre-vaccination on Day 90 (Month 3), post-second vaccination on Day 120 (Month 4), Day 270 (Month 9), Day 450 (Month 15), and then annually. Geometric mean titers (95% CI) by dengue serotype per protocol set for immunogenicity data for

Day 0, Day 30, Day 90, Day 120, and Day 270 are shown in Table 10.

Table 10: Geometric mean titers (95% CI) by dengue serotype (per protocol set for immunogenicity data)

	BASELINE SEROPOSITIVE		BASELINE SERONEGATIVE	
	TDV N=1,816	Placebo N-902	TDV N=702	Placebo N=345
DENV-1				
Day 1	410 (365–461)	445 (377–524)	5.0 (5.0–5.0)	5.0 (5.0–5.0)
Day 30	2,404 (2,204–2,622)	430 (361–512)	118 (106–131)	5.8 (5.3–6.3)
Day 90	1,945 (1,791–2,112)	410 (349–481)	91 (82–102)	5.9 (5.4–6.3)
Day 120	2,115 (1,957–2,286)	451 (381–534)	184 (169–201)	6.3 (5.7–7.0)
Day 270	1,447 (1,329–1,574)	415 (350–492)	87 (79–97)	6.3 (5.7–6.9)
DENV-2				
Day 1	745 (674–825)	802 (697–924)	5.0 (5.0–5.0)	5.0 (5.0–5.0)
Day 30	6,697 (6,301–7,117)	744 (635–870)	6,277 (5,648–6,977)	6.6 (6.0–7.3)
Day 90	4,826 (4,571–5,096)	729 (629–845)	1,682 (1,544–1,834)	7.0 (6.3–7.9)
Day 120	4,897 (4,646–5,163)	766 (655–896)	1,730 (1,614–1,855)	7.7 (6.7–8.8)
Day 270	3,692 (3,496–3,898)	776 (665–906)	929 (856–1,010)	8.7 (7.4–10.2)
DENV-3				
Day 1	357 (321–398)	356 (305–415)	5.0 (5.0–5.0)	5.0 (5.0–5.0)
Day 30	2,255 (2,094–2,428)	349 (298–409)	194 (173–218)	5.5 (5.2–5.9)
Day 90	1,563 (1,453–1,682)	321 (277–374)	94 (85–104)	5.5 (5.1–5.9)
Day 120	1,761 (1,646–1,885)	353 (301–414)	228 (212–246)	6.0 (5.4–6.6)
Day 270	1,089 (1,009–1,175)	307 (261–360)	72 (66–78)	6.3 (5.7–7.0)
DENV-4				
Day 1	218 (198–241)	234 (203–270)	5.0 (5.0–5.0)	5.0 (5.0–5.0)
Day 30	1,303 (1,221–1,391)	222 (191–258)	111 (98–125)	5.4 (5.0–5.7)
Day 90	1,002 (940–1,069)	215 (187–248)	63 (57–70)	5.5 (5.1–5.9)
Day 120	1,129 (1,066–1,196)	241 (208–280)	144 (134–155)	5.8 (5.3–6.4)
Day 270	778 (730–830)	229 (197–266)	64 (59–70)	6.2 (5.6–6.9)

[00477] Vaccine viremia is assessed by three PCRs: dengue detection RT-PCR, vaccine screening PCR and TDV sequencing in subjects with febrile illness within 30 days after each vaccination.

f) Safety

[00478] Rates of serious adverse events (SAEs) were similar in the vaccine and placebo groups (3.1% and 3.8% of participants, respectively; Table 11). One vaccinee and four placebo recipients experienced SAEs considered to be related to receiving blinded investigational product by the investigator (two experienced hypersensitivity, two were diagnosed with dengue, and one with DHF). There were five deaths during Part 1, and all were considered unrelated to the investigational product or study procedures. Total rates of unsolicited AEs were similar between the vaccine and placebo groups. The most commonly (≥1% of vaccine-recipients) reported unsolicited AEs within four weeks of any dose by preferred term were pyrexia (vaccine group 1.5%; placebo 1.4%), nasopharyngitis (vaccine 2.7%; placebo 3.0%), upper respiratory tract infection (vaccine 2.6%; placebo 2.9%), and viral infection (vaccine 1.1%; placebo 0.9%). Solicited local reactions were reported more frequently in the vaccine group.

Table 11a: Overview of safety data. Subjects with at least one adverse event after any vaccine dose. Data presented as number of events (percentage of subjects; number [n] of subjects/total [N] subjects) unless otherwise stated (safety set data)

	TDV	Placebo
Safety Set	N=13,380	N=6,687
SAEs	3.1% (409/13,380)	3.8% (255/6,687)
Non-IP-Related[a] SAEs	3.0% (408/13,380)	3.8% (251/6,687)
IP-Related[a] SAEs	<0.1% (1/13,380)	<0.1% (4/6,687)
SAEs Leading to IP Withdrawal and / or Trial Discontinuation	0.1% (18/13,380)	0.1% (8/6,687)
Deaths	<0.1% (4/13,380)	<0.1% (1/6,687)
IP-Related Deaths	0% (0/13,380)	0% (0/6,687)
Safety Subset	N=2,663	N=1,329
Unsolicited AEs Occurring Within 4 Weeks of Any Dose	18.4% (490/2,663)	18.8% (250/1,329)
IP-Related[a] Unsolicited AEs Occurring Within 4 Weeks of Any Dose	1.0% (27/2,663)	1.6% (21/1,329)
Solicited Systemic AEs Occurring Within 2 Weeks of Any Dose[b]	42.0% (1,107/2,635)	38.0% (501/1,317)
IP-Related[a] Solicited Systemic AEs Occurring Within 2 Weeks of Any Dose	31.2% (821/2,635)	28.2% (371/1,317)
Solicited Local Reactions Occurring Within 1 Week of Any Dose[c]	36.7% (967/2,633)	25.7% (338/1,317)

AE, adverse event; SAE, serious adverse event; IP, investigational product/TDV
[a]IP-related, defined as related to the investigational product as assessed by investigator
[b]only participants with diary data available were evaluated
[c]all injection site (solicited local) reactions considered to be IP-related

Table 11b: Number of participants (%) with serious adverse events after any vaccination during Part 1 by MedDRA (Medical Dictionary for Regulatory Activities)System Organ Class in the order of decreasing frequency (safety set data presented by TDV and placebo group for events that occurred in > 3 participants due to risk of unblinding).

MedDRA System Organ Class	TDV N=13,380	Placebo N=6,687	Total* N=20,071
Any Serious Adverse Events	409 (3.1)	255 (3.8)	664 (3.3)
Infections and infestations	235 (1.8)	179 (2.7)	414 (2.1)
Injury, poisoning and procedural complications	87 (0.7)	37 (0.6)	124 (0.6)
Gastrointestinal disorders	23 (0.2)	9 (0.1)	32 (0.2)
Nervous system disorders	14 (0.1)	6 (<0.1)	20 (<0.1)
Respiratory, thoracic and mediastinal disorders	14 (0.1)	6 (<0.1)	20 (<0.1)
Renal and urinary disorders	15 (0.1)	3 (<0.1)	18 (<0.1)
Blood and lymphatic system disorders	8 (<0.1)	2 (<0.1)	10 (<0.1)
Pregnancy, puerperium and perinatal conditions	8 (<0.1)	2 (<0.1)	10 (<0.1)
Skin and subcutaneous tissue disorders	7 (<0.1)	3 (<0.1)	10 (<0.1)
Psychiatric disorders	7 (<0.1)	2 (<0.1)	9 (<0.1)
General disorders and administration site conditions	5 (<0.1)	3 (<0.1)	8 (<0.1)
Immune system disorders	3 (<0.1)	4 (<0.1)	7 (<0.1)
Metabolism and nutrition disorders	6 (<0.1)	1 (<0.1)	7 (<0.1)
Musculoskeletal and connective tissue	1 (<0.1)	5 (<0.1)	6 (<0.1)
Social circumstances	2 (<0.1)	4 (<0.1)	6 (<0.1)
Congenital, familial and genetic disorders	3 (<0.1)	2 (<0.1)	5 (<0.1)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	3 (<0.1)	1 (<0.1)	4 (<0.1)
Endocrine disorders	–	–	3 (<0.1)
Hepatobiliary disorders	–	–	3 (<0.1)
Reproductive system and breast disorders	–	–	3 (<0.1)
Vascular disorders	–	–	3 (<0.1)
Cardiac disorders	–	–	2 (<0.1)
Eye disorders	–	–	2 (<0.1)
Investigations	–	–	1 (<0.1)
Product issues	–	–	1 (<0.1)
Surgical and medical procedures	–	–	1 (<0.1)

*Total column includes participants who received both TAK-003 and placebo due to administration error and are excluded from the TAK-003 and placebo groups. N in column header refers to number of participants in the safety set

Table 11c: Number of participants (%) with unsolicited adverse events of any severity up to 28-days after any vaccination by MedDRA System Organ Class in the order of decreasing frequency (Subset of safety set data presented by TDV and placebo group for events that occurred in > 6 participants due to risk of unblinding).

MedDRA System Organ Class	TDV N=2,663	Placebo N=1,329	Total N=3,993
Any Unsolicited Adverse Events	487 (18.3)	249 (18.7)	736 (18.4)
Infections and infestations	368 (13.8)	190 (14.3)	558 (14.0)
Injury, poisoning and procedural complications	21 (0.8)	22 (1.7)	43 (1.1)
Gastrointestinal disorders	33 (1.2)	9 (0.7)	42 (1.1)
General disorders and administration site conditions	30 (1.1)	11 (0.8)	41 (1.0)
Skin and subcutaneous tissue disorders	27 (1.0)	7 (0.5)	34 (0.9)
Nervous system disorders	18 (0.7)	13 (1.0)	31 (0.8)
Respiratory, thoracic and mediastinal disorders	18 (0.7)	10 (0.8)	28 (0.7)
Blood and lymphatic system disorders	6 (0.2)	5 (0.4)	11 (0.3)
Musculoskeletal and connective tissue disorders	6 (0.2)	5 (0.4)	11 (0.3)
Immune system disorders	–	–	6 (0.2)
Psychiatric disorders	–	–	3 (<0.1)
Reproductive system and breast disorders	–	–	3 (<0.1)
Ear and labyrinth disorders	–	–	2 (<0.1)
Cardiac disorders	–	–	1 (<0.1)
Congenital, familial and genetic disorders	–	–	1 (<0.1)
Eye disorders	–	–	1 (<0.1)
Renal and urinary disorders	–	–	1 (<0.1)
Social circumstances	–	–	1 (<0.1)

*Total column includes participants who received both TAK-003 and placebo due to administration error and are excluded from the TAK-003 and placebo groups. N in column header refers to number of participants in the subset of safety set.

Table 11d: Summary of diary reported injection site reactions up to 7 days and systemic adverse events up to 14 days after any vaccination (Subset of safety set data). Data presented as number of participants with events / number of evaluated participants in the analysis set (% of evaluated participants with events).

Solicited Events	TDV	Placebo
Injection site reactions (Age < 6 years)		
Any	106/331 (32.0)	43/169 (25.4)
Pain	104/331 (31.4)	43/169 (25.4)
Erythema	5/331 (1.5)	1/169 (0.6)
Swelling	11/331 (3.3)	2/169 (1.2)
Injection site reactions (Age ≥ 6 years)		
Any	861/2302 (37.4)	295/1148 (25.7)
Pain	853/2302 (37.1)	293/1148 (25.5)
Erythema	33/2301 (1.4)	1/1147 (<0.1)
Swelling	33/2300 (1.4)	6/1147 (0.5)
Systemic adverse events (Age < 6 years)		
Any	88/331 (26.6)	35/169 (20.7)
Irritability/Fussiness	41/331 (12.4)	16/169 (9.5)
Drowsiness	45/331 (13.6)	21/169 (12.4)
Loss of Appetite	57/331 (17.2)	22/169 (13.0)
Fever (Body temperature >= 38°C or 100.4°F)	45/327 (13.8)	23/169 (13.6)
Systemic adverse events (Age ≥ 6 years)		
Any	941/2302 (40.9)	422/1147 (36.8)
Headache	715/2302 (31.1)	326/1147 (28.4)
Asthenia	404/2302 (17.5)	187/1147 (16.3)
Malaise	510/2301 (22.2)	226/1147 (19.7)
Myalgia	554/2302 (24.1)	216/1147 (18.8)
Fever (Body temperature >= 38°C or 100.4°F)	221/2279 (9.7)	124/1134 (10.9)

[00479] Additionally, a study to assess the efficacy of a booster dose as a follow-on study of the above-described phase III study, such that booster will be given at 4 to 4.5 years post the second dose in a large enough subset of the above-described phase III study, wherein said subset e.g. includes at least 20 subjects or at least 200 subjects, is possible.

Example 4: Concomitant administration of a hepatitis A vaccine and a dengue vaccine

4.1 Introduction, Purpose and Objectives of the Study

[00480] A randomized, observer blind, phase 3 trial was conducted in 900 healthy adult subjects aged 18 to 60 years (distributed across the entire age range) in non-endemic countries for dengue disease and hepatitis A virus (HAV) to investigate the immunogenicity and safety of two doses of tetravalent dengue vaccine TDV (subcutaneous (SC) injection), and of the simultaneous on the same day administration of a single dose of HAV vaccine (containing an inactivated HAV; intramuscular (IM) injection) and TDV (SC injection).

[00481] A purpose of the study was to assess whether HAV vaccine can be safely administered simultaneously on the same day with TDV as travel vaccines before an international travel of a subject to HAV and dengue (DENV)-endemic countries.

[00482] The primary objective of this study was demonstrate non-inferiority (NI) of the immune response to one dose of HAV vaccine simultaneously administered on the same day with one dose TDV on the same day, compared to one dose HAV vaccine simultaneously on the same day administered with placebo on the same day, in DENV/HAV-naïve subjects one month after vaccination.

[00483] The secondary objectives of this study were to describe TDV-induced immunogenicity after a single dose of TDV in DENV/HAV-naïve subjects; to describe TDV-induced immunogenicity after two doses of TDV administered 90 days apart in DENV/HAV-naïve subjects; to describe HAV vaccine-induced immunogenicity in DENV/HAV-naïve subjects; and to assess the safety profile after each vaccine injection in all trial groups.

4.2 Eligibility Criteria

[00484] Criteria for inclusion include:

1. The participant is aged 18 to 60 years, inclusive.
2. Participants who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs) and the clinical judgment of the Investigator.
3. The participant signs and dates a written informed consent form and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.
4. Participants who can comply with trial procedures and are available for the duration of follow-up.

[00485] Exclusion criteria include:

1. Participants with an elevated oral temperature (≥38°C or 100.4°F) within 3 days of the intended date of vaccination.
2. Known hypersensitivity or allergy to any of the vaccine components (including excipients of the investigational vaccines or placebo).
3. Participants with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the Investigator, may interfere with the participant's ability to participate in the trial.
4. Participants with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (e.g., Guillain-Barré syndrome).

5. Participants with history or any illness that, in the opinion of the Investigator, might interfere with the results of the trial or pose additional risk to the participant due to participation in the trial.
6. Known or suspected impairment/alteration of immune function, including:
    1. Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
    2. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).
    3. Administration of immunoglobulins and/or any blood products within the 3 months prior to Day 1 (M0) or planned administration during the trial.
    4. Receipt of immunostimulants within 60 days prior to Day 1(M0).
    5. Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).
    6. Human immunodeficiency virus (HIV) infection or HIV-related disease.
    7. Hepatitis A virus (HAV) infection.
    8. Hepatitis C virus infection.
    9. Genetic immunodeficiency.
7. Abnormalities of splenic or thymic function.
8. Participants with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
9. Participants with any serious chronic or progressive disease according to judgment of the Investigator (e.g., neoplasm, insulin dependent diabetes, cardiac, renal or hepatic disease).
10. Participants with body mass index (BMI) greater than or equal to 35 kg/m^2 (=weight in kg/[height in meters^2]).
11. Participants participating in any clinical trial with another investigational product 30 days prior to Day 1 (M0) or intent to participate in another clinical trial at any time during the conduct of this trial.
12. Participants who received any other vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this trial or who are planning to receive any vaccine within 28 days of trial vaccine administration.
13. Previous HAV vaccination (in a clinical trial or with an approved product).
14. Participants involved in the trial conduct or their first degree relatives.
15. Participants with history of substance or alcohol abuse within the past 2 years.
16. Female participants who are pregnant or breastfeeding.
17. Females of childbearing potential who are sexually active, and who have not used any of the acceptable contraceptive methods for at least 2 months prior to Day 1 (M0).
    1. Of childbearing potential is defined as status post onset of menarche and not meeting any of the following conditions: bilateral tubal ligation (at least 1 year previously), bilateral oophorectomy (at least 1 year previously) or hysterectomy
    2. Acceptable birth control methods are defined as one or more of the following:

i. Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring).

ii. Barrier method (condom with spermicide or diaphragm with spermicide) each and every time during intercourse.

iii. Intrauterine device (IUD). iv. Monogamous relationship with vasectomized partner (partner must have been vasectomized for at least 6 months prior to Day 1 [M0]).

Other contraceptive methods may be considered in agreement with the Sponsor and implemented only after approval of a substantial amendment by the regulatory authorities and by the appropriate ethics committee.

18. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (Day 90 [M3]). In addition, they must be advised not to donate ova during this period.
19. Any positive or indeterminate pregnancy test.
20. Previous and planned vaccination (during the trial conduct) against any flaviviruses including dengue, yellow fever (YF), Japanese Encephalitis (JE) viruses or tick-borne encephalitis.
21. Previous participation in any clinical trial of a dengue or other flavivirus (e.g., West Nile [WN] virus) candidate vaccine, except for participants who received placebo in those trials.
22. Participants with a current or previous infection with a flavivirus such as dengue, Zika, YF, JE, WN fever, tick-borne encephalitis or Murray Valley encephalitis and participants with a history of prolonged (≥1 year) habitation in a dengue endemic area.
23. Participants with contraindications, warnings and/or precautions to vaccination with the HAV vaccine as specified within the product information.

4.3 Study Design & Vaccinations

[00486] Eligible subjects were randomized equally (1:1:1 ratio) to one of the following 3 trial groups (300 subjects per group):

- Group 1: HAV vaccine (IM) and placebo (SC), simultaneously on the same day administered on day 1 (month 0); placebo (SC) administered at day 90 (month 3).
- Group 2: TDV (SC) and placebo (IM), simultaneously on the same day administered on day 1 (month 0); TDV (SC) administered at day 90 (month 3).
- Group 3: TDV (SC) and HAV vaccine (IM), simultaneously on the same day administered on day 1 (month 0); TDV (SC) administered at day 90 (month 3).

A more detailed scheme of the study design is shown in Figure 6. Up to 28 days prior to the first vaccination, enrolment was carried out and blood samples were taken for screening anti-HAV antibodies. On day 1, pre-vaccination blood samples were taken. On day 30 (after the first vaccination on day 1) post-vaccination blood samples were taken. On day 120 (after the first vaccination on day 1) another blood sample was taken. Safety follow-up took place on day 270 (after the first vaccination on day 1).

[00487] The TDV was prepared as described in Example 1. Each subcutaneous dose of the TDV had a volume of 0.5 ml and the concentration of the four dengue serotypes in the TDV in each dose was 5.1 $\log_{10}$pfu/0.5 ml, 4.5 $\log_{10}$pfu/0.5 ml, 5.4 $\log_{10}$pfu/0.5 ml and 5.9 $\log_{10}$pfu/0.5 ml of TDV-1, TDV-2, TDV-3 and TDV-4, respectively. Each subcutaneous dose comprises the TDV dispersed in 0.5 ml of an aqueous solution containing Pluronic F127 (10.6 mg/ml), trehalose dihydrate (170 mg/ml) and human serum albumin (1.08 mg/ml).

[00488] The HAV vaccine includes an inactivated hepatitis A virus, derived from a hepatitis A virus strain HM-175 (see definitions above), and is commercially available under the tradename HAVRIX® as described above. The intramuscular dose of the HAV vaccine administered to groups 1) and 3) was 1 ml and each 1 ml dose has a viral antigen activity of about 1440 EL.U., wherein the viral antigen is adsorbed on 0.5 mg of aluminum in the form of aluminum hydroxide. The hepatitis A vaccine contains excipients in the form of an amino acid supplement (about 0.3% w/v) and in the form of polysorbate (about 0.05 mg/ml) dissolved in a phosphate-buffered saline solution.

[00489] Simultaneously on the same day administered trial vaccines were injected to opposite arms. Normal saline solution for injection (0.9% NaCl) was used as placebo. A blood sample for an anti-HAV antibody test were collected at screening from all subjects to exclude subjects who are positive for anti-HAV antibodies up to 28 days prior to vaccination (see Figure 6). All subjects were followed-up for 6 months after the second vaccination at day 90 (month 3), so the trial duration was 270 days or 9 months for each subject (not including the screening period). Outside the context of this trial, subjects in Groups 1 and 3 will be offered a HAV vaccine booster dose after the completion of trial procedures at day 270 (month 9).

[00490] Dengue neutralizing antibodies (microneutralization test (MNT50)) were measured using blood samples collected at pre-first trial vaccination (day 1 (month 0)), 1 month post first trial vaccination (day 30 (month 1)), and 1 month post second trial vaccination (day 120 (month 4)). Blood samples for the measurement of anti-HAV antibodies (enzyme-linked immunosorbent assay (ELISA)) were collected at pre-first trial vaccination (day 1 (month 0)) and 1 month post first trial vaccination (day 30 (month 1)).

4.4 Primary Endpoint

[00491] The primary endpoint included the proportion of HAV/DENV-naive subjects at baseline who are seroprotected against HAV at day 30 (month 1) as measured by enzyme-linked immunosorbent assay (ELISA)). In other words, the primary endpoint includes the seroprotection rates (SPRs). Seroprotection is defined as serum anti-HAV antibody levels ≥10 mIU/mL. Immunological naivety to HAV/DENV is defined as anti-HAV antibody levels <10 mIU/mL and reciprocal neutralizing titers for all 4 dengue serotypes <10.

4.5 Secondary Endpoints

[00492] a) Secondary Immunogenicity Endpoints

[00493] The secondary endpoints included the geometric mean titers of neutralizing antibodies (GMTs) (microneutralization test (MNT50)) for each of the 4 dengue serotypes at day 30 (month 1) and day 120 (month 4) which were determined in HAV/DENV-naive subjects at baseline; the proportion of HAV/DENV-naive subjects at baseline who are seropositive for each of the 4 dengue serotypes at day 30 (month 1) and day 120 (month 4) (seroprotection rate); and geometric mean concentrations (GMC) of anti-HAV antibodies at day 30 (month 1) in subjects HAV/DENV-naive at baseline.

[00494] Seropositivity for dengue virus is defined as a reciprocal neutralizing titer ≥10 for any of the four dengue serotypes within the secondary immunogenicity endpoints.

[00495] b) Secondary Safety Endpoints

[00496] Secondary safety endpoints included the frequency and severity of solicited local adverse events (AE) for 7

days after each trial vaccination; the frequency and severity of solicited systemic AEs for 14 days after each trial vaccination; the percentage of subjects with any unsolicited AEs for 28 days after each trial vaccination; the percentage of subjects with serious adverse events (SAE) throughout the trial; and the percentage of subjects with medically attended adverse events (MAAE) throughout the trial.

4.6 Analysis Sets of the Study

[00497] Table 12 below displays each analysis set of the present study. In total, 1199 subjects belonging to the group "all screened" included all subjects who signed the informed consent, regardless of whether the subjects were screen failures. After initial screening, 900 subjects were included into the "randomized set" which includes all randomized subjects, regardless of whether any dose of the IPs was received. The safety set, consisting of 897 subjects, includes all randomized subjects who received ≥1 dose of the IPs. The immunogenicity subjects included a total of 359 subjects and is subdivided into the following four subsets. The HAV-full analysis set (HAV-FAS) includes all randomized subjects in the immunogenicity subset who received ≥ 1 dose of the trial vaccine with available day 1 and day 30 anti-HAV antibody measurements. The HAV-per-protocol set (HAV-PPS) includes all HAV- and DENV-naïve subjects from the HAV-FAS who have no major protocol violations. The TDV-full analysis set (TDV-FAS) includes all randomized subjects in the immunogenicity subset who received ≥ 1 dose of trial vaccine and with available day 1 and ≥ 1 post-dose measurements. The TDV-per-protocol set (TDV-PPS), consisting of 197 subjects, includes all HAV- and DENV-naïve subjects from the TDV-FAS who have no major protocol violations.

Table 12. Analysis sets of the study.

	HAV/Pbo	TDV/Pbo	HAV/TDV	Total
All Screened[1]	NA	NA	NA	1199
Randomized Set[2]	300	300	300	900
Safety Set (SS)[3]	299	300	298	897
Immunogenicity Subset	119	120	120	359
• **HAV Full Analysis Set (HAV-FAS)[4]**	115	117	114	346
• **HAV Per-Protocol Set (HAV-PPS)[5]**	75	71	81	227
• **TDV-FAS[6]**	116	117	115	348
• **TDV-PPS[7]**	67	63	67	197
Subjects analyzed for primary non-inferiority objective[8]	**69/115**	**NA**	**79/114**	**148/227**

[1]All Screened: All subjects who signed the informed consent, regardless of whether subjects were screen failures

[2]Randomized Set: All randomized subjects, regardless of whether any dose of the trial vaccines was received.

[3]Safety Set: All randomized subjects who received ≥ 1 dose of trial vaccines.

[4]HAV-FAS: All randomized subjects in the immunogenicity subset who received ≥ 1 dose of trial vaccine, with available Day 1 and Day 30 HAV measurements.

[5]HAV-PPS: All HAV & DENV-naïve subjects from the HAV-FAS who have no major protocol violations.

[6]TDV-FAS: All randomized subjects in the immunogenicity subset who received ≥ 1 dose of trial vaccine and available Day 1 and ≥ 1 post-dose measurement.

[7]TDV-PPS: All HAV & DENV-naïve subjects from the TDV-FAS who have no major protocol violations.

[8] Subject excluded from TDV-PPS but included into analysis for primary non-inferiority objective had their Day 30 measurement outside the protocol defined visit window.

[00498] From a total number of 359 subjects in the immunogenicity subset (including all subjects which received ≥ 1 vaccination), 13 subjects have been excluded from HAV-FAS because of not providing a valid baseline and post-dosing measurement (on day 30) for HAV. Furthermore, HAV-PPS includes all HAV- and DENV-naïve subjects of HAV-FAS who had no major protocol violations. The subjects analyzed for primary non-inferiority objective are based on the HAV-PPS subjects of the HAV/Pbo and HAV/TDV group, wherein based on HAV-PPS of these two groups (HAV/Pbo and HAV//TDV), some subjects were not included in the 30 days analysis (6 subjects of the HAV/Pbo group and 2 Subjects of the HAV/TDV group), since these subjects had their day 30 measurement outside the visit window defined in the protocol. Therefore, a total of 69 subjects was included into the TDV/Pbo group and a total of 79 subjects was included in the HAV/TDV group for analyzing the primary non-inferiority objective.

[00499] From a total number of 359 subjects in the immunogenicity subset (including all subjects which received ≥ 1 vaccination), a total of 11 subjects have been excluded from TDV-FAS, because they did not provide a valid baseline and at least one post-dosing measurement (day 30 and/or day 120) for TDV. Furthermore, a total number of 151 subjects had been excluded from the TDV-PPS for not being HAV & DENV-naïve at baseline or for not receiving both vaccinations 1 and 2 or if vaccination 2 (usually on day 90) is outside the window -15/+25 days or if major protocol violations occur.

4.7 HAV Baseline Serostatus and Demographic & Baseline Characteristics

[00500] The safety set evaluated for baseline HAV antibody levels included a total of 362 subjects of which 27.3% were HAV seropositive at baseline (see Table 13).

[00501] HAS-FAS included a total of 346 subjects evaluated for baseline HAV antibody levels (see Table 13). HAV-naivety was defined as anti-HAV antibody (ab) level of < 10 mIU/ml. However, the ELISA used for serological analysis could not be validated below levels of 12.5 mIU/ml. The qualitative screening test had a specification that effectively amounted to a lower limit of quantification of 70 mIU/ml. In view of these criteria, 72.5% of the subjects of said HAS-FAS evaluated for baseline HAV antibody levels were HAV naive at baseline (see Table 13).

Table 13. HAV baseline serostatus in the safety set and in the HAV-FAS

	HAV/Pbo	TDV/Pbo	HAV/TDV	Total
Safety set evaluated for baseline HAV antibody levels	**119**	**121**	**122**	**362**
• HAV seropositive at baseline	31 (26.1%)	38 (31.4%)	30 (24.6%)	99 (27.3%)
HAV-FAS Evaluated For Baseline HAV antibody levels	**115 (100%)**	**117 (100%)**	**114 (100%)**	**346 (100%)**
• HAV seronegative at baseline <12.5 mIU/ml (a)	86 (74.8%)	79 (67.5%)	86 (74.4%)	251 (72.5%)
• HAV seropositive at baseline (b)	29 (25.2%)	38 (32.5%)	28 (24.6%)	95 (27.5%)
• baseline 12.5-70 mIU/ml	18	23	13	54
• baseline 70-1000 mIU/ml	11	14	14	39
• baseline >1000	0	1	1	2

(a) HAV-naivety was defined as anti-HAV ab level of< 10 mIU/ml; The ELISA used for serological analysis could not be validated below levels of 12.5 mIU/ml.

(b) The qualitative screening test had a specification that effectively amounted to a lower limit of quantification of 70 mIU/ml

[00502] The HAV-PPS includes a total number of 227 subjects (see Table 14). The mean age of the total number of subjects of the HAV-PPS, which are DENV- and HAV-naïve was 34.8. 31.3% of the total number of subjects of the HAV-PPS were female (see Table 14). In total, 97.8% of the total subjects of the HAV-PPS were of an ethnicity which is NOT Hispanic or Latino, and, in particular, 89.9% of the HAV-PPS participants were of race "white European", especially in order to reflect the situation of travelers of HAV- and dengue non-endemic countries (see Table 14).

[00503] The safety set includes a total number of 897 subjects (see Table 14). The mean age of the total number of subjects of the safety set was 35.4 years of which 31.3% were female. In total, 97.7% of the total subjects of the HAV-PPS were of an ethnicity which is NOT Hispanic or Latino, and, in particular, 87.1% of the HAV-PPS participants were of race "white European", especially in order to reflect the situation of travelers of HAV- and dengue non-endemic countries (see Table 14).

Table 14. Demographic and baseline characteristics (HAV-PPS and Safety Set)

		HAV/Pbo	**TDV/Pbo**	**HAV/TDV**	**Total**
HAV-PPS (DENV/HAV-naïve)	**N**	**75**	**71**	**81**	**227**
Age Years	Mean (SD)	34.3 (11.68)	35.5 (11.24)	34.8 (11.70)	34.8 (11.51)
Gender	Female n (%)	24 (32.0%)	30 (42.3%)	17 (21.0%)	71 (31.3%)
Ethnicity NOT Hispanic or Latino	n (%)	72 (96.0%)	71 (100.0%)	79 (97.5%)	222 (97.8%)
Race White European	n (%)	64 (85.3%)	64 (90.1%)	76 (93.8%)	204 (89.9%)
		HAV/Pbo	**TDV/Pbo**	**HAV/TDV**	**Total**
Safety set	**N**	**299**	**300**	**298**	**897**
Age Years	Mean (SD)	34.7 (12.04)	36.0 (11.88)	35.5 (11.96%)	35.4 (11.96)
Gender	Female n (%)	107 (35.8%)	120 (40.0%)	90 (30.2%)	317 (35.3%)
Ethnicity NOT Hispanic or Latino	n (%)	289 (96.7%)	293 (97.7%)	294 (98.7%)	876 (97.7%)
Race White European	n (%)	255 (85.3%)	265 (88.3%)	261 (87.6%)	781 (87.1%)

4.8 Study Results

[00504] a) Primary endpoint and sensitivity analyses

[00505] The present study was successful in meeting the primary objective of non-inferiority for the simultaneous on the same day administration of HAV and TDV. Table 15 displays the seroprotection rates (SPRs) of groups 1 (received HAV/Pbo) and 3 (received HAV/TDV) on day 30 after the first vaccination (on day 1), the SPR differences between the HAV/Pbo group and the HAV/TDV group on day 30, and the confidence intervals (CIs) of these SPR differences for HAV and DENV-baseline naïve subjects. These values (SPRs, SPR differences; CIs) were used for the primary endpoint evaluation of the study. Table 15 further shows these values for the results of three sensitivity analyses (also used for non-inferiority assessments), wherein the subjects had different, i.e. mixed, HAV/TDV serostatuses at baseline. Non-inferiority between the hepatitis A vaccine and the tetravalent dengue vaccine, when simultaneously on the same day administered, in the present study is concluded, if the seroprotection rate (SPR) difference between group 1 (received HAV and placebo on the same day 1) and group 3 (received HAV and TDV on the same day 1) has an upper bound of a two-sided 95% confidence interval, calculated using the Newcombe score method, which is lower than the 10% non-inferiority margin. This criterion is fulfilled for each of the groups in Table 15. In the primary endpoint group, the **upper bound of the 95% CI** of the SPR difference is **4.31%** which is less than the **non-inferiority margin of 10%** (see second line from above in Table 15).

[00506] As mentioned above, sensitivity analyses 1 to 3 were used to evaluate populations that included subjects who were seropositive for dengue and/or for hepatitis A at baseline, in particular reflecting "real life" travel clinic settings in non-endemic countries in which subjects, i.e. travelers who plan to go to dengue and HAV endemic countries, are not always aware of their HAV and/or dengue serostatus before requesting pre-travel vaccinations.

[00507] The object of **non-inferiority** of the simultaneous on the same day administration was also met in sensitivity analyses **1 to 3 (upper bounds of the 95% CI of the SPR differences: 3.21%; 2.93%; and 2.55% which are each less than the non-inferiority margin of 10%)**. Furthermore, the SPRs of the HAV/TDV groups (98.8%; 99.0%; 99.1%, respectively, see Table 15) were respectively higher than the SPRs of the HAV/Pbo group (96.2%; 96.9%; 97.2%, respectively, see Table 15).

[00508] Therefore, due to the non-inferiority of the simultaneous on the same day administration of the HAV vaccine and TDV to subjects with mixed baseline serostatus (and baseline naivety) with respect to HAV and all dengue serotypes, there is no need for determining or knowing the subject's baseline serostatus with respect to each of the two diseases, prior to simultaneously on the same day administering the HAV vaccine and TDV.

Table 15. Primary endpoint: Non-inferiority (NI) assessments & sensitivity analysis

Analysis	Analysis Set	HAV/Pbo (Group 1) SPR% (n/n)	HAV/TDV (Group3) SPR% (n/n)	SPR difference	95% CI[1] (of SPR difference)
Primary endpoint	HAV PPS – includes baseline HAV- and DENV- subjects	**97.1% (67/69)**	**98.7% (78/79)**	-1.63	**(-8.78, 4.31)**
Sensitivity Analysis 1	HAV PPS – includes baseline HAV-, DENV-, and DENV+ subjects	96.2% (76/79)	98.8% (83/84)	-2.61	(-9.46, 3.21)
Sensitivity Analysis 2	HAV PPS – includes baseline HAV-; HAV+ (12.5-70mIU/ml); DENV-; DENV+	96.9% (93/96)	99.0% (96/97)	-2.09	(-7.82, 2.93)
Sensitivity Analysis 3	HAV PPS – includes baseline HAV-; HAV+; DENV-; DENV+	97.2% (103/106)	99.1% (109/110)	-1.92	(-7.14, 2.55)

[1]CI = Confidence Interval

Non-inferiority Assessment: Seroprotection Rates (SPRs) for HAV Group 1 (HAV + placebo simultaneous on the same day administration) vs Group 3 (HAV + TDV simultaneous on the same day administration)

Rates difference for primary comparison (Group 1 – Group 3) are presented together with 95% CI calculated using Newcombe score method

NI of simultaneous on the same day administration of HAV and TDV to HAV alone will be concluded if the upper bound of the 95% CI is less than NI margin of 10%.

[00509] b) Secondary immunogenicity endpoints

[00510] Table 16 shows GMTs (with respect to each of the four dengue virus serotypes DENV-1 to DENV-4) when measured on day 1 pre-first vaccination, on day 30 after the first vaccination (on day 1), and on day 120 after the first vaccination of the subjects (on day 1) of the DENV-PPS including the groups receiving HAV/TDV, TDV and placebo, as well as HAV and placebo (Pbo), respectively. Table 16 shows positive trends in favor of the simultaneous on the same day administration group (received HAV/TDV) with respect to all dengue GMTs.

[00511] Said positive trend in favor of the simultaneous on the same day administration and the day 30 synergism of the simultaneously on the same day administered vaccines is also confirmed in Table 17.

Table 16. Immunogenicity of DENV-PPS: GMTs of DENV MNT50. In particular, DENV GMTs against each serotype with respect to mean titers of neutralizing antibodies measured by MNT50 for each dengue serotype by trial visit are shown.

		DENV-1			DENV-2			DENV-3			DENV-4		
		HAV/p	**TDV/p**	**HAV/TDV**	**HAV/p**	**TDV/p**	**HAV/TDV**	**HAV/p**	**TDV/p**	**HAV/TDV**	**HAV/p**	**TDV/p**	**HAV/TDV**
Day 1	n	67	63	67	67	63	67	67	63	67	67	63	67
	GMT (SD[1])	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)	5.0 (1.00)
Day 30	n	62	60	65	62	60	65	62	60	65	62	60	65
	GMT (SD)	5.0 (1.00)	108.2 (5.64)	**152.5** (4.62)	6.0 (1.87)	2897.9 (13.86)	**3960.0** (8.79)	5.3 (1.49)	95.4 (6.24)	**140.5** (4.50)	5.0 (1.00)	74.3 (5.03)	**142.1** (6.19)
Day 120	n	50	55	62	50	55	62	50	55	62	50	55	62
	GMT (SD)	5.0 (1.00)	171.3 (6.23)	173.7 (4.28)	5.7 (1.72)	2064.1 (3.60)	1764.3 (4.03)	5.0 (1.00)	83.8 (3.66)	92.6 (2.80)	5.0 (1.00)	56.1 (3.19)	81.4 (3.49)

[1] SD = standard deviation.

Table 17. Secondary endpoint: HAV Geometric Mean Concentrations (GMCs)

	HAV/Pbo	TDV/Pbo	HAV/TDV	Total
Baseline				
• n	75	71	81	227
• Geometric Mean (SD[1])	6.3 (1.00)	6.3 (1.00)	6.3 (1.00)	6.3 (1.00)
• 95% CI[2]	(NE, NE)	(NE, NE)	(NE, NE)	(NE, NE)
• Median	6.3	6.3	6.3	6.3
• Min-max	6, 6	6, 6	6, 6	6, 6
Day 30				
• n	69	66	79	214
• Geometric Mean (SD)	80.5 (3.01)	6.7 (1.27)	**93.0 (2.44)**	39.5 (4.27)
• 95% CI	(61.8, 105.0)	(6.4, 7.2)	(76.1, 113.6)	(32.5, 48.0)
• Median	81.5	6.3	94.5	52.4
• Min-max	6, 1044	6, 16	6, 1859	6, 1859

[1] SD = standard deviation.
[2] CI = confidence interval.

[00512] c) Secondary safety endpoints

[00513] The safety set was investigated for solicited adverse events, solicited systemic adverse events, unsolicited adverse events and serious adverse events throughout the study. Tables 18a to 21c show the study results of the safety set with respect to each secondary safety endpoint.

[00514] Table 18a. Frequency of Solicited Local AEs and Solicited Systemic AEs after first vaccination – Safety Set

	HAV/Pbo (N=270)	TDV/Pbo (N=271)	HAV/TDV (N=257)
Solicited Local (within 7 days)	141/289 (48.8)	152/292 (52.1)	196/285 (68.8)
Solicited Systemic (within 14 days)	139/289 (48.1)	132/292 (45.2)	141/285 (49.5)
Related to IP	98/289 (33.9)	106/292 (36.3)	117/285 (41.1)
Not related to IP	41/289 (14.2)	26/292 (8.9)	24/285 (8.4)

Note: For solicited AEs, excluding prolonged solicited AEs, percentages are calculated based on number of subjects with non-missing data (n) evaluated in each trial group. Subjects with 1 or more AEs for a particular category of AEs are counted only once using the most related event.

Table 18b. Frequency of Solicited Local AEs and Solicited Systemic AEs after second vaccination – Safety Set

	Pbo (N=270)	TDV (N=271)	TDV (N=257)
Solicited Local (within 7 days)	28/255 (11.0)	100/264 (37.9)	103/251 (41.0)
Solicited Systemic (within 14 days)	73/254 (28.7)	82/263 (31.2)	85/251 (33.9)
Related to IP	49/254 (19.3)	60/263 (22.8)	61/251 (24.3)
Not related to IP	24/254 (9.4)	22/263 (8.4)	24/251 (9.6)

Note: For solicited AEs, excluding prolonged solicited AEs, percentages are calculated based on number of subjects with non-missing data (n) evaluated in each trial group. Subjects with 1 or more AEs for a particular category of AEs are counted only once using the most related event.

Table 18c. Frequency of Solicited Local AEs and Solicited Systemic AEs after any vaccination – Safety Set

	HAV/Pbo (n=299)	TDV/Pbo (n=300)	HAV/TDV (n=298)
Solicited Local (within 7 days)	151/289 (52.2)	175/292 (59.9)	216/285 (75.8)
Solicited Systemic (within 14 days)	161/289 (55.7)	167/292 (57.2)	167/285 (58.6)
Related to IP	121/289 (41.9)	133/292 (45.5)	141/285 (49.5)
Not related to IP	40/289 (13.8)	34/292 (11.6)	26/285 (9.1)

Note: For solicited AEs, excluding prolonged solicited AEs, percentages are calculated based on number of subjects with non-missing data (n) evaluated in each trial group. Subjects with 1 or more AEs for a particular category of AEs are counted only once using the most related event.

Table 19a. Overview of Unsolicited AE up to 28 Days Post-vaccination (after first vaccination) – Safety Set

	HAV/Pbo N=299	TDV/Pbo N=300	HAV/TDV N=298
[Any AE], n (%)	43 (14.4%)	51 (17.0%)	56 (18.8%)
Mild	32 (10.7%)	32 (10.7%)	43 (14.4%)
Moderate	11 (3.7%)	18 (6.0%)	12 (4.0%)
Severe	0	1 (0.3%)	1 (0.3%)

Notes:

- This summary includes all unsolicited AEs with a date of onset within 28 days after each trial vaccination.
- N is the number of subjects who received the specific vaccination. Percentages are calculated based on N for corresponding column.
- Table shows the number of subjects that reported unsolicited AE
- Subjects with 1 or more AEs for a particular category of adverse event are counted only once using the most related/most severe/most serious event.

Table 19b. Overview of Unsolicited AE up to 28 Days Post-vaccination (after second vaccination) – Safety Set

	Pbo N=270	TDV N =271	TDV N=257
Any AE, n (%)	39 (14.4%)	27 (10.0%)	30 (11.7%)
Mild	17 (6.3%)	14 (5.2%)	15 (5.8%)
Moderate	22 (8.1%)	13 (4.8%)	13 (5.1%)
Severe	0	0	2 (0.8%)

Notes:

- This summary includes all unsolicited AEs with a date of onset within 28 days after each trial vaccination.
- N is the number of subjects who received the specific vaccination. Percentages are calculated based on N for corresponding column.
- Table shows the totals of AE that were experienced by the number of subjects that reported unsolicited AE
  Subjects with 1 or more AEs for a particular category of adverse event are counted only once using the most related/most severe/most serious event.

Table 20. Most Common Unsolicited AEs Up to 28 Days Post-Vaccination (after Any Vaccination)* – Safety Set

AE, n (%)	HAV/Pbo N=299	TDV/Pbo N=300	HAV/TDV N=298
Nasopharyngitis	9 (3.0%)	8 (2.7%)	11 (3.7%)

* Reported by ≥ 2.0 % of subjects.

Table 21a. Safety: Overview of Serious Adverse Events (SAEs) After any dose – Safety Set

Number of events, number (%) subjects with SAE	HAV/Pbo (n=299)		TDV/Pbo (n=300)		HAV/TDV (n=298)		Total (N=597)	
	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects
SAEs – any	**3**	**2 (0.7%)**	**10**	**8 (2.7%)**	**10**	**7 (2.3%)**	**23**	**17 (2.8%)**
• After 1st dose up to 2nd dose	0	0	3	3 (1.0%)	3	2 (0.7%)	6	5 (0.8%)
• After 2nd dose up to trial end	3	2 (0.7%)	7	5 (1.8%)	7	5 (1.9%)	17	12 (2.0%)
SAEs – related	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
SAE – premature vaccine and/or trial discontinuation	**0**	**0**	**1**	**1 (0.3%)**	**0**	**0**	**1**	**1 (0.2%)**
Deaths	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

p = Placebo

Table 21b. Safety: Serious Adverse Events by System Organ Class and Preferred Term after first and after second dose - Safety Set

Number (%) subjects with SAE SOC		HAV/Pbo (N=299)	TDV/Pbo (N=300)	HAV/TDV (N=298)
After 1st dose up to 2nd dose – any SAE		***0***	***3 (1.0)***	***2 (0.7)***
Gastrointestinal disorders	Crohn's disease*	0	1 (0.3)	0
Injury, poisoning and procedural complications	Intentional overdose	0	0	1 (0.3)
Neoplasms benign, malignant and unspecified	Bladder cancer stage II	0	1 (0.3)	0
Nervous system disorders	Loss of consciousness**	0	1 (0.3)	0
Psychiatric disorders	Intentional self-injury	0	0	1 (0.3)
		Pbo (N=270)	**TDV (N=271)**	**TDV (N=257)**
After 2nd dose up to end of trial – any SAE		***2 (0.7)***	***5 (1.8)***	***5 (1.9)***
Cardiac disorders	Supraventricular tachycardia	0	0	1 (0.4)
Gastrointestinal disorders	Abdominal pain	0	1 (0.4)	0
	Abdominal strangulated hernia	1 (0.4)	0	0
	Intestinal ischaemia	0	0	1 (0.4)
	Mesenteric vein thrombosis	0	0	1 (0.4)
	Oesophagitis	0	1 (0.4)	0

* Subject had a history of irritable bowel syndrome
**Occurred >2 months after vaccination

Table 21c. Safety: Serious Adverse Events by System Organ Class and Preferred TermAfter first and after second dose - Safety Set – *Continued*

Number (%) subjects with SAE **SOC** / **PT**		**Pbo (N=270)**	**TDV (N=271)**	**TDV (N=257)**
Infections and infestations	Appendicitis	0	1 (0.4)	0
	Wound infection	1 (0.4)	0	0
Injury, poisoning and procedural complications	Abdominal injury	0	1 (0.4)	0
	Cervical vertebral fracture	0	0	1 (0.4)
	Fractured coccyx	1 (0.4)	0	0
	Joint dislocation	0	1 (0.4)	0
	Lower limb fracture	0	0	1 (0.4)
	Thermal burn	0	1 (0.4)	0
Neoplasms benign, malignant and unspecified	Invasive ductal breast carcinoma	0	1 (0.4)	0
	Prostate cancer	0	0	1 (0.4)
Respiratory, thoracic and mediastinal disorders	Acute respiratory distress syndrome	0	0	1 (0.4)

FIRST ITEM LIST OF THE INVENTION

1. A method of preventing hepatitis A and dengue disease in a subject or subject population, the method comprising simultaneously on the same day administering a hepatitis A vaccine and a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

2. The method according to item 1, wherein the hepatitis A vaccine is an inactivated virus vaccine.

3. The method according to item 1 or 2, wherein the dengue vaccine composition 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 chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log10 pfu/0.5 mL.

4. The method according to any one of the preceding items, wherein the subject population or subject is seronegative to all dengue serotypes.

5. The method according to any one of the preceding items, wherein said unit dose of the dengue vaccine composition is administered by subcutaneous injection and said hepatitis A vaccine is administered by intramuscular injection, and wherein said injections are preferably administered to the arm, more preferably to the deltoid region of the arm.

6. The method according to item 5, wherein said unit dose of the dengue vaccine composition and said hepatitis A vaccine are administered to different anatomical sites, such as to opposite arms.

7. The method according to any one of the preceding items, wherein two of said unit doses of the dengue vaccine composition are administered within 12 months or more, or within six months, or within three months.

8. The method according to item 7 comprising the administration of two of said unit doses of the dengue vaccine composition and one dose of said hepatitis A vaccine, in particular according to the following schedule

- a first simultaneous administration of the first unit dose of the dengue vaccine composition and said hepatitis A vaccine on day 0, and
- a second administration of the second unit dose of the dengue vaccine composition after said first simultaneous administration, such as about 3 months later.

9. The method according to any one of the preceding items, wherein the subject population or subject is of 2 to 60 years of age.

10. The method according to any one of the preceding items, wherein the subject population or subject is from a dengue endemic region.

11. The method according to any one of items 1 to 9, wherein the subject population or subject is from a dengue non-endemic region, preferably from a dengue non-endemic and hepatitis-A non-endemic region.

12. The method according to any one of the preceding items, wherein the hepatitis A vaccine comprises a hepatitis A virus derived from a hepatitis A virus strain HM-175.

13. The method according to any one of the preceding items, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and the inactivated hepatitis A virus is derived from a hepatitis A virus strain HM-175.

14. The method according to any one of the preceding items, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and wherein the inactivated hepatitis A virus is adsorbed on aluminum.

15. The method according to item 14, wherein the aluminum is aluminum hydroxide or aluminum hydroxyphosphate sulfate.

16. The method according to any one of the preceding items, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and wherein the hepatitis A vaccine comprises a phosphate-buffered saline solution and excipients dissolved therein in the form of an amino acid and in and in the form of polysorbate.

17. The method according to any one of the preceding items, wherein the hepatitis A vaccine includes a hepatitis A virus expressing a viral antigen in a concentration ranging from 500 ELISA Units (EL.U.) to 2000 ELISA Units (EL.U.).

18. The method according to any one of the preceding items, wherein the method does not include a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition or
wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition.

19. The method according to item 18, wherein the method does not include a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject at any time before, during and after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition or
wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown at any time before, during or after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition.

20. The method according to any of the preceding items, wherein 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 and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection without determination whether there was a previous dengue infection and/or a previous hepatitis A infection, and

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

(C) administering at least one further unit dose of the tetravalent dengue virus composition to the subject within 3 to 12 months of administration of the first unit dose, and optionally

(D) administering at least one further dose of the hepatitis A vaccine to the subject within 6 to 18 months of administration of the first unit dose.

21. The method according to any one of the preceding items, wherein 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 and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection, and

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

(C) administering two further unit doses of the tetravalent dengue virus composition to the subject at about 6 and about 12 months of administration of the first unit dose and administering a hepatitis A vaccine to the subject at either about 6 or about 12 months of administration of the first unit dose.

22. The method according to item 21, wherein step (A) is carried out without determination whether there was a previous hepatitis A infection.

23. The method according to any one of items 3 to 22, wherein upon reconstitution of the dengue vaccine composition 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 (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 6%, at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

24. The method according to any one of the preceding items, wherein the method provides compatibility between the dengue vaccine composition and the hepatitis A vaccine.

25. The method according to any one of the preceding items, wherein the method provides synergy between the dengue vaccine composition and the hepatitis A vaccine.

26. The method according to any one of the preceding items, wherein the method provides non-inferiority in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects divided into one subject population and into one control subject population, wherein the subject population receives simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and the control subject population receives simultaneously on the same day a hepatitis A vaccine and a placebo administration.

27. The method according to any one of the preceding items, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

28. The method according to any one of the preceding items, wherein the method provides a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline and seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

a) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, and

b) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

29. The method according to any one of items 1 to 26, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% or of at least 99% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline.

30. The method according to any one of items 1 to 26, wherein the method provides a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

a) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, wherein the subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline, and

b) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo, wherein the control subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

31. The method according to any one of the preceding items, wherein the subject or subject population is exposed to a hepatitis A virus outbreak and/or a dengue virus outbreak.

32. The method according to any of the preceding items, wherein the method provides an anti-hepatitis A virus antibody Geometric Mean Concentration (GMC) of at least 70 mIU/ml or at least 80 mIU/ml or at least 90 mIU/ml on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

33. The method according to any one of the preceding items, wherein the simultaneous on the same day administration of the hepatitis A vaccine and the unit dose of the dengue vaccine composition to the subject or the subject population is safe.

34. The method according to any one of the preceding items, wherein there are no serious adverse events related to the simultaneous on the same day administration.

35. The method according to any one of the preceding items, wherein the method provides the Geometric Mean Titer (GMT) of neutralizing antibodies measured by MNT50 of

- at least 110 or at least 140 or at least 150 for dengue serotype 1,
- at least 3000 or at least 3500 or at least 3900 for dengue serotype 2,
- at least 100 or at least 120 or at least 140 for dengue serotype 3, and/or
- at least 80 or at least 110 or at least 140 for dengue serotype 4,

on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

36. The method according to any one of the preceding items, wherein the subject or subject population is 18 to 60 years of age.

37. A kit against hepatitis A and dengue disease comprising

a box containing at least

(a) a first container holding a hepatitis A vaccine, and

(b) a second container holding a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

SECOND ITEM LIST OF THE INVENTION

1. Use of a hepatitis A vaccine for the manufacture of a medicament for preventing hepatitis A in a subject or subject population and a use of a unit dose of a dengue vaccine composition for the manufacture of medicament for preventing dengue disease in the subject or subject population, the prevention of hepatitis A and dengue disease comprising simultaneously on the same day administering the hepatitis A vaccine and the unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

2. Use according to item 1, wherein the hepatitis A vaccine is an inactivated virus vaccine.

3. Use according to item 1 or 2, wherein the dengue vaccine composition 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 chimeric dengue serotype 2/4 strain in a concentration of at least 4.5 log10 pfu/0.5 mL.

4. Use according to any one of the preceding items, wherein the subject population or subject is seronegative to all dengue serotypes.

5. Use according to any one of the preceding items, wherein said unit dose of the dengue vaccine composition is administered by subcutaneous injection and said hepatitis A vaccine is administered by intramuscular injection, and wherein said injections are preferably administered to the arm, more preferably to the deltoid region of the arm.

6. Use according to item 5, wherein said unit dose of the dengue vaccine composition and said hepatitis A vaccine are administered to different anatomical sites, such as to opposite arms.

7. Use according to any one of the preceding items, wherein two of said unit doses of the dengue vaccine composition are administered within 12 months or more, or within six months, or within three months.

8. Use according to item 7 comprising the administration of two of said unit doses of the dengue vaccine composition and one dose of said hepatitis A vaccine, in particular according to the following schedule

- a first simultaneous administration of the first unit dose of the dengue vaccine composition and said hepatitis A vaccine on day 0, and
- a second administration of the second unit dose of the dengue vaccine composition after said first simultaneous administration, such as about 3 months later.

9. Use according to any one of the preceding items, wherein the subject population or subject is of 2 to 60 years of age.

10. Use according to any one of the preceding items, wherein the subject population or subject is from a dengue endemic region.

11. Use according to any one of items 1 to 9, wherein the subject population or subject is from a dengue non-endemic region, preferably from a dengue non-endemic and hepatitis-A non-endemic region.

12. Use according to any one of the preceding items, wherein the hepatitis A vaccine comprises a hepatitis A virus derived from a hepatitis A virus strain HM-175.

13. Use according to any one of the preceding items, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and the inactivated hepatitis A virus is derived from a hepatitis A virus strain HM-175.

14. Use according to any one of the preceding items, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and wherein the inactivated hepatitis A virus is adsorbed on aluminum.

15. Use according to item 14, wherein the aluminum is aluminum hydroxide or aluminum hydroxyphosphate sulfate.

16. Use according to any one of the preceding items, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and wherein the hepatitis A vaccine comprises a phosphate-buffered saline solution and excipients dissolved therein in the form of an amino acid and in and in the form of polysorbate.

17. Use according to any one of the preceding items, wherein the hepatitis A vaccine includes a hepatitis A virus expressing a viral antigen in a concentration ranging from 500 ELISA Units (EL.U.) to 2000 ELISA Units (EL.U.).

18. Use according to any one of the preceding items, wherein the method does not include a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition or
wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition.

19. Use according to item 18, wherein the method does not include a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject at any time before, during and after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition or
wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown at any time before, during or after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition.

20. Use according to any of the preceding items, wherein 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 and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection without determination whether there was a previous dengue infection and/or a previous hepatitis A infection, and
(B) administering a first unit dose of the tetravalent dengue virus composition and a hepatitis A vaccine to the subject, and optionally

(C) administering at least one further unit dose of the tetravalent dengue virus composition to the subject within 3 to 12 months of administration of the first unit dose, and optionally

(D) administering at least one further dose of the hepatitis A vaccine to the subject within 6 to 18 months of administration of the first unit dose.

21. Use according to any one of the preceding items, wherein 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 and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection, and

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

(C) administering two further unit doses of the tetravalent dengue virus composition to the subject at about 6 and about 12 months of administration of the first unit dose and administering a hepatitis A vaccine to the subject at either about 6 or about 12 months of administration of the first unit dose.

22. Use according to item 21, wherein step (A) is carried out without determination whether there was a previous hepatitis A infection.

23. Use according to any one of items 3 to 22, wherein upon reconstitution of the dengue vaccine composition 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 (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 6%, at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

24. Use according to any one of the preceding items, wherein the method provides compatibility between the dengue vaccine composition and the hepatitis A vaccine.

25. Use according to any one of the preceding items, wherein the method provides synergy between the dengue vaccine composition and the hepatitis A vaccine.

26. Use according to any one of the preceding items, wherein the method provides non-inferiority in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects divided into one subject population and into one control subject population, wherein the subject population receives simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and the control subject population receives simultaneously on the same day a hepatitis A vaccine and a placebo administration.

27. Use according to any one of the preceding items, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

28. Use according to any one of the preceding items, wherein the method provides a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline and seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

c) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, and

d) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

29. Use according to any one of items 1 to 26, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% or of at least 99% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline.

30. Use according to any one of items 1 to 26, wherein the method provides a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

c) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, wherein the subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline, and

d) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo, wherein the control subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

31. Use according to any one of the preceding items, wherein the subject or subject population is exposed to a hepatitis A virus outbreak and/or a dengue virus outbreak.

32. Use according to any of the preceding items, wherein the method provides an anti-hepatitis A virus antibody Geometric Mean Concentration (GMC) of at least 70 mIU/ml or at least 80 mIU/ml or at least 90 mIU/ml on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

33. Use according to any one of the preceding items, wherein the simultaneous on the same day administration of the hepatitis A vaccine and the unit dose of the dengue vaccine composition to the subject or the subject population is safe.

34. Use according to any one of the preceding items, wherein there are no serious adverse events related to the simultaneous on the same day administration.

35. Use according to any one of the preceding items, wherein the method provides the Geometric Mean Titer (GMT) of neutralizing antibodies measured by MNT50 of

- at least 110 or at least 140 or at least 150 for dengue serotype 1,
- at least 3000 or at least 3500 or at least 3900 for dengue serotype 2,
- at least 100 or at least 120 or at least 140 for dengue serotype 3, and/or
- at least 80 or at least 110 or at least 140 for dengue serotype 4,

on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

36. Use according to any one of the preceding items, wherein the subject or subject population is 18 to 60 years of age.

37. A kit against hepatitis A and dengue disease comprising

a box containing at least

(a) a first container holding a hepatitis A vaccine, and

(b) a second container holding a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

2020331884 23 Dec 2024

CLAIMS

1. A method of effective vaccination against hepatitis A and dengue disease in a subject or in a subject population, the method comprising simultaneously on the same day administering the hepatitis A vaccine and a unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

2. Use of a hepatitis A vaccine in the manufacture of a medicament for preventing hepatitis A in a subject or subject population and of a unit dose of a dengue vaccine composition in the manufacture of a medicament for preventing dengue disease in the subject or subject population, wherein the prevention of hepatitis A and dengue disease comprises simultaneously on the same day administering the hepatitis A vaccine and the unit dose of a dengue vaccine composition, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

3. Use of a unit dose of a dengue vaccine composition in the manufacture of a medicament for preventing dengue disease and hepatitis A in a subject or subject population, wherein the prevention of dengue disease and hepatitis A comprises simultaneously on the same day administering the unit dose of a dengue vaccine composition and a hepatitis A vaccine, wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

4. A hepatitis A vaccine and a unit dose of a dengue vaccine composition which are used to prevent hepatitis A and dengue disease in a subject or in a subject population, wherein preventing dengue disease and hepatitis A comprises that the hepatitis A vaccine and the unit dose of the dengue vaccine composition are to be administered to the subject or subject population simultaneously on the same day and wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

5. A unit dose of a dengue vaccine composition which is used to prevent dengue disease and hepatitis A in a subject or in a subject population, wherein preventing dengue disease and hepatitis A comprises that the unit dose of the dengue vaccine composition and a hepatitis A vaccine are to be administered to the subject or subject population simultaneously on the same day and wherein said unit dose comprises a tetravalent dengue virus composition including four live, attenuated dengue virus strains.

6. The method, use, or composition according to any one of claims 1-5, wherein the hepatitis A vaccine is an inactivated virus vaccine.

7. The method, use, or composition according to any one of claims 1-6, wherein the dengue vaccine composition 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

2020331884 23 Dec 2024

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

8. The method, use, or composition according to any one of claims 1-7, wherein the subject population or subject is seronegative to all dengue serotypes.

9. The method, use, or composition according to any one of claims 1-8, wherein said unit dose of the dengue vaccine composition is administered by subcutaneous injection and said hepatitis A vaccine is administered by intramuscular injection.

10. The method, use, or composition according to claim 9, wherein said unit dose of the dengue vaccine composition and said hepatitis A vaccine are administered to different anatomical sites.

11. The method, use, or composition according to any one of claims 1-10, wherein two of said unit doses of the dengue vaccine composition are administered within 12 months or more, or within six months, or within three months.

12. The method, use, or composition according to claim 11, comprising the administration of said first and second unit doses of the dengue vaccine composition and one dose of said hepatitis A vaccine, according to the following schedule:

- simultaneous administration of the first unit dose of the dengue vaccine composition and said hepatitis A vaccine on day 0, and
- administration of the second unit dose of the dengue vaccine composition after said first simultaneous administration, about 3 months later.

13. The method, use, or composition according to any one of claims 1-12, wherein the subject population or subject is of 2 to 60 years of age.

14. The method, use, or composition according to any one of claims 1-12, wherein the subject population or subject is more than 60 years of age.

15. The method, use, or composition according to any one of claims 1-14, wherein the subject population or subject is from a dengue endemic region.

16. The method, use, or composition according to any one of claims 1-15, wherein the subject population or subject is from a dengue non-endemic region.

17. The method, use, or composition according to any one of claims 1-16, wherein the hepatitis A vaccine comprises a hepatitis A virus derived from a hepatitis A virus strain HM-175.

18. The method, use, or composition according to any one of claims 1-17, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus.

2020331884 23 Dec 2024

19. The method, use, or composition according to any one of claims 1-18, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and wherein the inactivated hepatitis A virus is adsorbed on aluminum.

20. The method, use, or composition according to claim 19, wherein the aluminum is aluminum hydroxide or aluminum hydroxyphosphate sulfate.

21. The method, use, or composition according to any one of claims 1-20, wherein the hepatitis A vaccine comprises an inactivated hepatitis A virus and wherein the hepatitis A vaccine comprises a phosphate-buffered saline solution and excipients dissolved therein in the form of an amino acid and in and in the form of polysorbate.

22. The method, use, or composition according to any one of claims 1-21, wherein the hepatitis A vaccine includes a hepatitis A virus expressing a viral antigen in a concentration ranging from 500 ELISA Units (EL.U.) to 2000 ELISA Units (EL.U.).

23. The method, use, or composition according to any one of claims 1-22, not including a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition, wherein the determination of the previous dengue infection in the subject is characterized by a laboratory confirmed history of dengue or a validated serological test, or
wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown before the administration of the hepatitis A vaccine and before the administration of the unit dose of the dengue vaccine composition.

24. The method, use, or composition according to claim 23, not including a step of determination whether there was a previous dengue infection and/or a previous hepatitis A infection in the subject population or in the subject at any time before, during and after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition, wherein the determination of the previous dengue infection in the subject is characterized by a laboratory confirmed history of dengue or a validated serological test, or
wherein the hepatitis A serostatus and/or the dengue serostatus of the subject population or of the subject is unknown at any time before, during or after the steps of administration of the hepatitis A vaccine and of the unit dose of the dengue vaccine composition.

25. The method, use, or composition according to any of claims 1-24, comprising a primary vaccination consisting of the steps of:
(A) selecting a subject for administration of the unit doses of the tetravalent dengue virus composition and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection without determination whether there was a previous dengue infection and/or a previous hepatitis A infection, and

2020331884 23 Dec 2024

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

(C) administering at least one further unit dose of the tetravalent dengue virus composition to the subject within 3 to 12 months of administration of the first unit dose, and optionally

(D) administering at least one further dose of the hepatitis A vaccine to the subject within 6 to 18 months of administration of the first unit dose.

26. The method, use, or composition according to any one of claims 1-25, comprising a primary vaccination consisting of the steps of:

(A) selecting a subject for administration of the unit doses of the tetravalent dengue virus composition and the hepatitis A vaccine in need for protection against dengue infection and hepatitis A infection, and

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

(C) administering two further unit doses of the tetravalent dengue virus composition to the subject at about 6 and about 12 months of administration of the first unit dose and administering a hepatitis A vaccine to the subject at either about 6 or about 12 months of administration of the first unit dose.

27. The method, use, or composition according to claim 26, wherein step (A) is carried out without determination whether there was a previous hepatitis A infection.

28. The method, use, or composition according to any one of claims 7 to 27, wherein upon reconstitution of the dengue vaccine composition 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 (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 6%, at least 8%, or at least 10%, or at least 12%, or at least 14%, or at least 16%, or at least 18%.

29. The method, use, or composition according to any one of claims 1-28, providing compatibility between the dengue vaccine composition and the hepatitis A vaccine.

30. The method, use, or composition according to any one of claims 1-29, providing synergy between the dengue vaccine composition and the hepatitis A vaccine.

31. The method, use, or composition according to any one of claims 1-30, providing non-inferiority in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects divided into one subject population and into one control subject population, wherein the subject population receives simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and the control subject population receives simultaneously on the same day a hepatitis A vaccine and a placebo administration.

2020331884 23 Dec 2024

32. The method, use, or composition according to any one of claims 1-31, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

33. The method, use, or composition according to any one of claims 1-32, providing a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline and seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

a) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, and

b) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

34. The method, use, or composition according to any one of claims 1 to 31, wherein the hepatitis A vaccine provides a hepatitis A seroprotection rate of at least 95% or of at least 98% or of at least 99% on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline.

35. The method, use, or composition according to any one of claims 1 to 31, providing a hepatitis A seroprotection rate difference with respect to a hepatitis A mono-administration, the difference being determined in a non-inferiority clinical study including at least 60 or at least 120 healthy subjects being seronegative with respect to hepatitis A at baseline, wherein the healthy subjects include healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

the healthy subjects being divided into

a) a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of the hepatitis A vaccine and the unit dose of the dengue vaccine composition, wherein the subject population includes healthy subject(s) which

2020331884 23 Dec 2024

are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline, and

b) a control subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day an administration (on day 0/1) of a hepatitis A vaccine and a placebo, wherein the control subject population includes healthy subject(s) which are seropositive with respect to at least one dengue virus serotype at baseline and healthy subject(s) which are seronegative with respect to all dengue virus serotypes at baseline,

wherein the difference is determined between the hepatitis A seroprotection rate of the control subject population on day 30 after the administration (on day 0/1) and the hepatitis A seroprotection rate of the subject population on day 30 after the administration (on day 0/1), and

wherein the difference has an upper bound within a two-sided 95% confidence interval which is lower than 10%.

36. The method, use, or composition according to any one of claims 1-35, wherein the subject or subject population is exposed to a hepatitis A virus outbreak and/or a dengue virus outbreak, optionally wherein the dengue virus outbreak is due to a dengue serotype 2 and/or due to a dengue serotype 1.

37. The method, use, or composition according to any one of claims 1-36, providing an anti-hepatitis A virus antibody Geometric Mean Concentration (GMC) of at least 70 mIU/ml or at least 80 mIU/ml or at least 90 mIU/ml on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

38. The method, use, or composition according to any one of claims 1-37, wherein the simultaneous on the same day administration of the hepatitis A vaccine and the unit dose of the dengue vaccine composition to the subject or the subject population is safe.

39. The method, use, or composition according to claim 38, wherein there are no serious adverse events related to the simultaneous on the same day administration of the hepatitis A vaccine and the unit dose of the dengue vaccine composition.

40. The method, use, or composition according to any one of claims 1-39, providing the Geometric Mean Titer (GMT) of neutralizing antibodies measured by MNT50 of

- at least 110 or at least 140 or at least 150 for dengue serotype 1,
- at least 3000 or at least 3500 or at least 3900 for dengue serotype 2,
- at least 100 or at least 120 or at least 140 for dengue serotype 3, and/or
- at least 80 or at least 110 or at least 140 for dengue serotype 4,

on day 30 after an administration (on day 0/1) to a subject population of at least 30 or at least 50 healthy subjects receiving simultaneously on the same day the hepatitis A vaccine and the unit dose of the dengue vaccine composition and being seronegative with respect to hepatitis A at baseline and being seronegative with respect to all dengue virus serotypes at baseline.

2020331884 23 Dec 2024

41. The method, use, or composition according to any one of claims 1-40, wherein the subject or subject population is 18 to 60 years of age.

42. The method, use, or composition according to claim 16, wherein the subject population or subject is from a dengue non-endemic region and a hepatitis A non-endemic region.

43. The method, use, or composition according to claim 18, wherein the inactivated hepatitis A virus is derived from a hepatitis A virus strain HM-175.

44. The method, use, or composition according to claim 9, wherein said unit dose of the dengue vaccine composition and said hepatitis A vaccine are both administered to the arm.

45. The method, use, or composition according to claim 44, wherein said unit dose of the dengue vaccine composition and said hepatitis A vaccine are both administered to the deltoid region of the arm.

46. The method, use, or composition of any one of claims 1-45, wherein the dengue vaccine provides (i) a reduced risk of dengue disease with hospitalization, or (ii) a reduced risk of severe dengue, or (iii) a reduced risk of dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS) as compared to an unvaccinated subject in a subject population.

47. The method, use, or composition of any one of claims 1-46, to reduce the risk of dengue disease in a subject or subject population after administering one dose or one amount of the dengue vaccine composition, optionally wherein the administration of the one dose or one amount results in a reduction of risk of dengue disease within three months, within two months, within one month, or within two weeks after said one dose or one amount.

48. The method, use, or composition of any one of claims 1-47, wherein the dengue disease is due to dengue serotype 1 and/or dengue serotype 2.

49. The method, use, or composition of any one of claims 1-48, wherein the subject or subject population has been subject to prior vaccination against Yellow Fever or Japanese Encephalitis, wherein said prior vaccination against Yellow Fever or Japanese Encephalitis refers to a vaccination prior to said simultaneous administration.

50. The method, use, or composition of any one of claims 1-49, including one or more dengue booster vaccinations after the primary vaccination.

51. The method, use, or composition of any one of claims 1-50, 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, optionally

wherein the dengue vaccine composition includes at least one chimeric dengue virus and at least one non-chimeric dengue virus.

2020331884 23 Dec 2024

52. The method, use, or composition of any one of claims 1-51, wherein each of the four live, attenuated dengue virus strains comprises dengue structural proteins including capsid protein (C), pre-membrane protein (prM), and envelope protein (E), and dengue non-structural proteins including NS1, NS2A, NS2B, NS3, NS4A, and NS5.

53. The method, use, or composition of any one of claims 1-52, wherein the dengue vaccine composition is a tetravalent dengue virus composition including four live, attenuated dengue virus strains, representing serotype 1, serotype 2, serotype 3 and serotype 4, optionally
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 a) to c) from the wild type as follows:

a) 5'-noncoding region (NCR)-57
b) NS1-53 Gly-to-Asp and
c) NS3-250 Glu-to-Val.

54. The method, use, or composition of claim 53, wherein the dengue serotype 1 is a chimeric dengue serotype 2/1 strain, the dengue serotype 2 is a non-chimeric serotype 2 strain, the dengue serotype 3 is as a chimeric dengue serotype 2/3 strain, and the dengue serotype 4 is a chimeric dengue serotype 2/4 strain.

55. The method, use, or composition of claim 54, 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,
wherein in the DENV-2/1 chimeric strain the structural proteins prM and E from serotype 2 strain are replaced with the corresponding structural proteins derived from DENV-1 16007, and
wherein in the DENV-2/3 chimeric strain the structural proteins prM and E from serotype 2 strain are replaced with the corresponding structural proteins derived from a DENV-3 16562, and
wherein in the DENV-2/4 chimeric strain the structural proteins prM and E from serotype 2 strain are replaced with the corresponding structural proteins derived from a DENV-4 1036.

56. The method, use, or composition of any one of claims 53 to 55, wherein the
dengue serotype 1 is characterized by amino acid sequence of SEQ ID NO. 2, and
dengue serotype 2 is characterized by amino acid sequence of SEQ ID NO. 4, and
dengue serotype 3 is characterized by amino acid sequence of SEQ ID NO. 6, and
dengue serotype 4 is characterized by amino acid sequence of SEQ ID NO. 8.

57. The method, use, or composition of any one of claims 1 to 56, wherein the dengue vaccine composition 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.

2020331884 23 Dec 2024

58. The method, use, or composition of any one of claims 1 to 57, wherein the unit dose of a dengue vaccine composition is administered without the need to determine the occurrence of a previous dengue infection in a subject.

59. The method, use, or composition of any one of claims 1 to 58, wherein the dengue vaccine composition is a tetravalent dengue virus composition, wherein the tetravalent dengue virus composition is TAK-003.

60. The method, use, or composition of any one of claims 1 to 59, wherein the hepatitis A vaccine and the unit dose of a tetravalent dengue virus composition are in separate formulations and/or in separate containers, such as in separate vials.

FIGURES

Figure 1

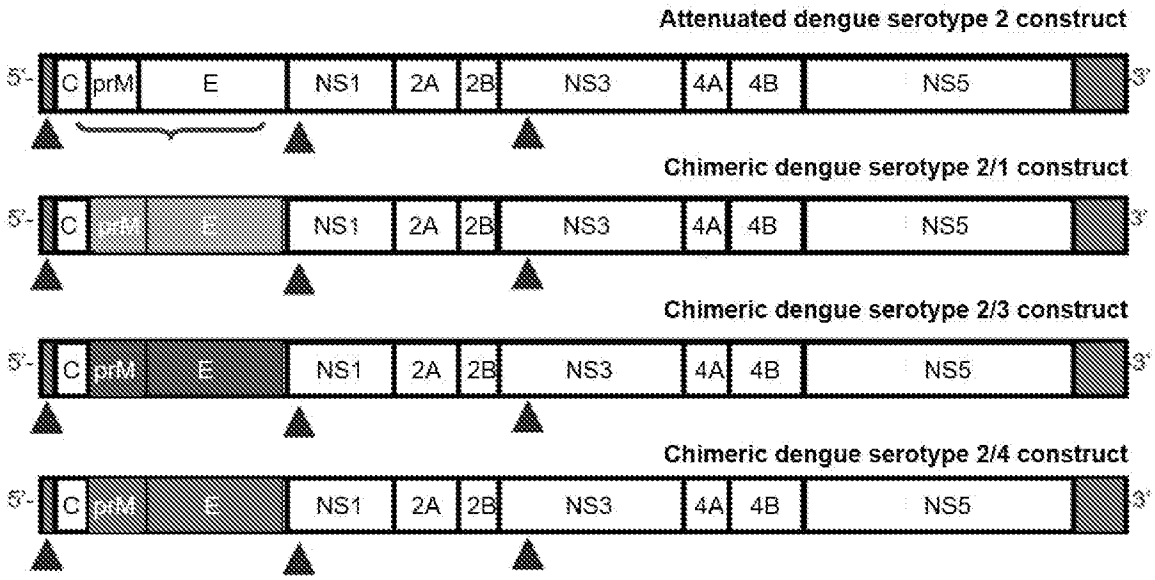


Figure 2

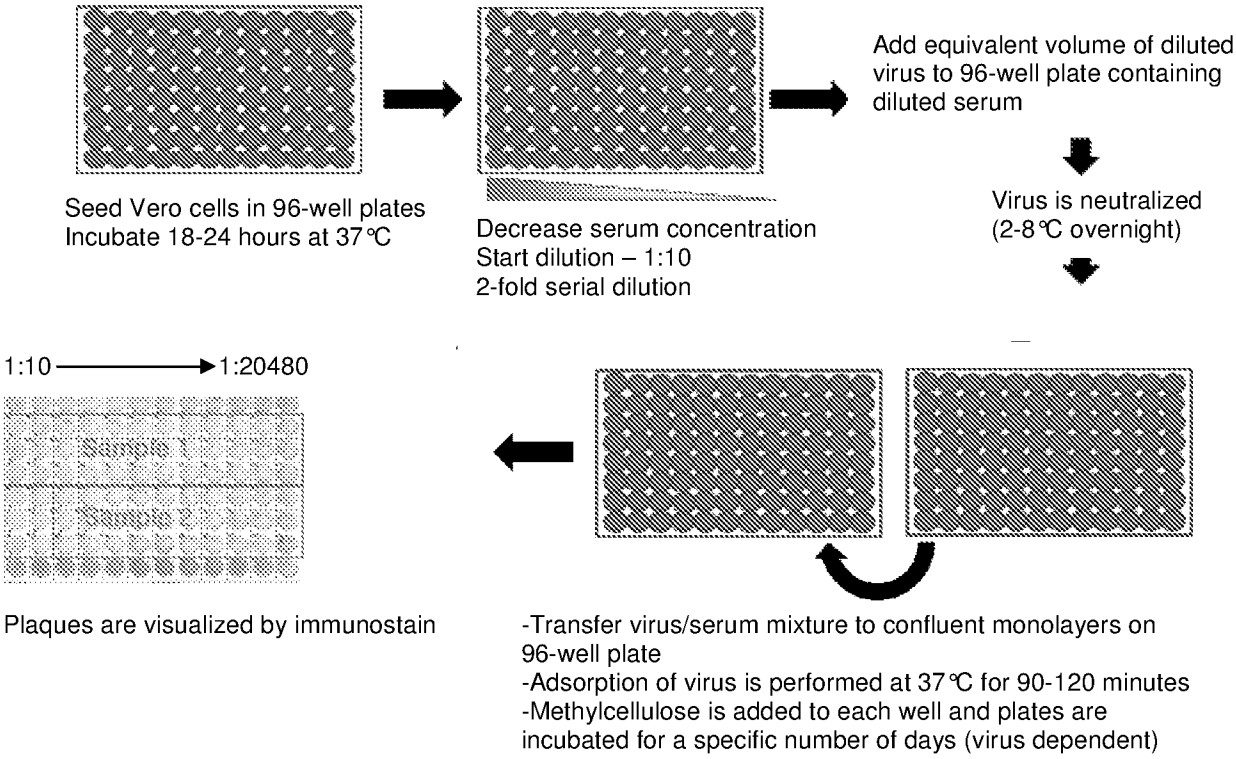

Add equivalent volume of diluted virus to 96-well plate containing diluted serum
Virus is neutralized (2-8℃ overnight)
Seed Vero cells in 96-well plates
Incubate 18-24 hours at 37℃
Decrease serum concentration
Start dilution – 1:10
2-fold serial dilution
1:10
1:20480
Sample 1
Sample 2
Plaques are visualized by immunostain
-Transfer virus/serum mixture to confluent monolayers on 96-well plate
-Adsorption of virus is performed at 37℃ for 90-120 minutes
-Methylcellulose is added to each well and plates are incubated for a specific number of days (virus dependent)

Figure 3

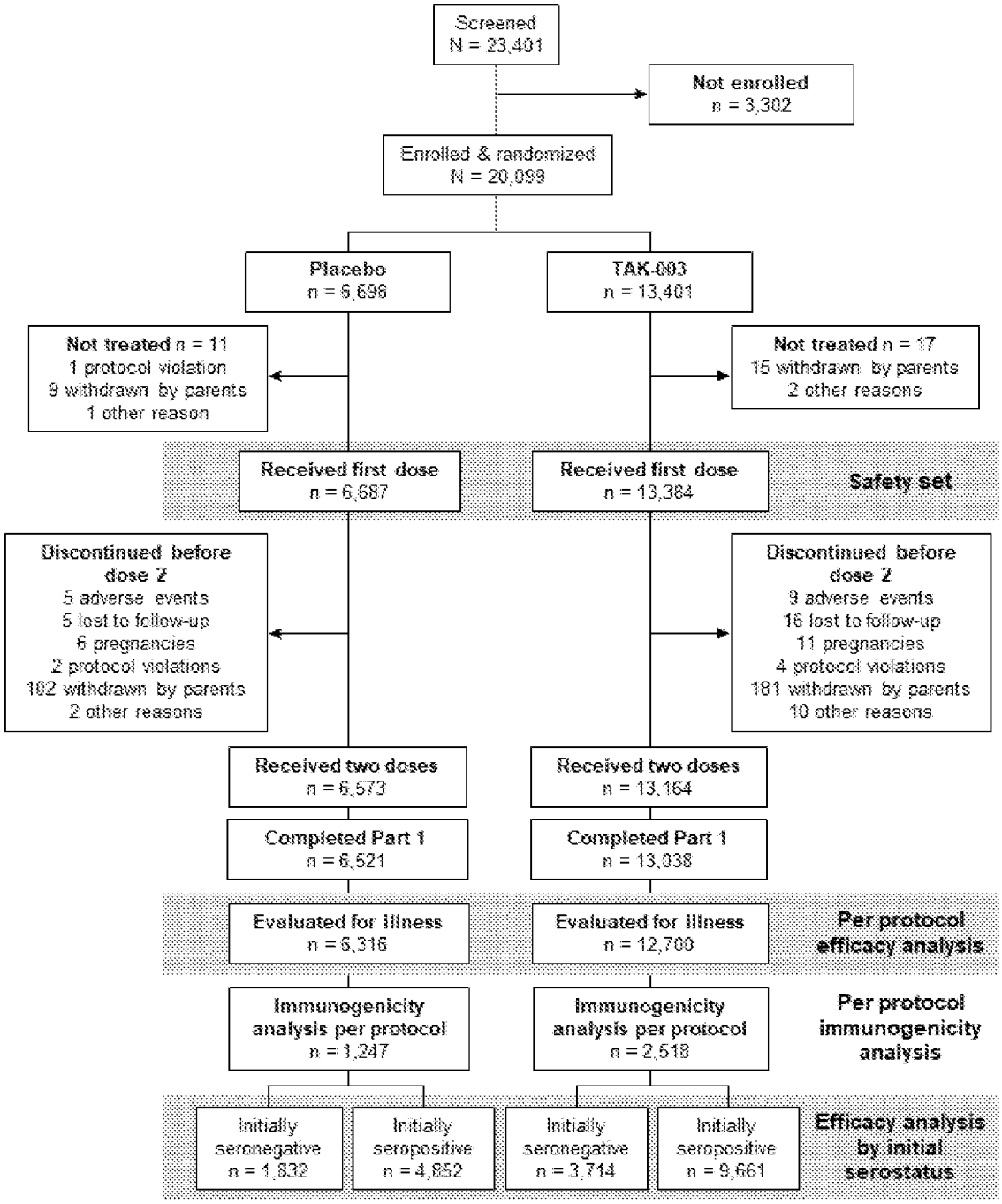
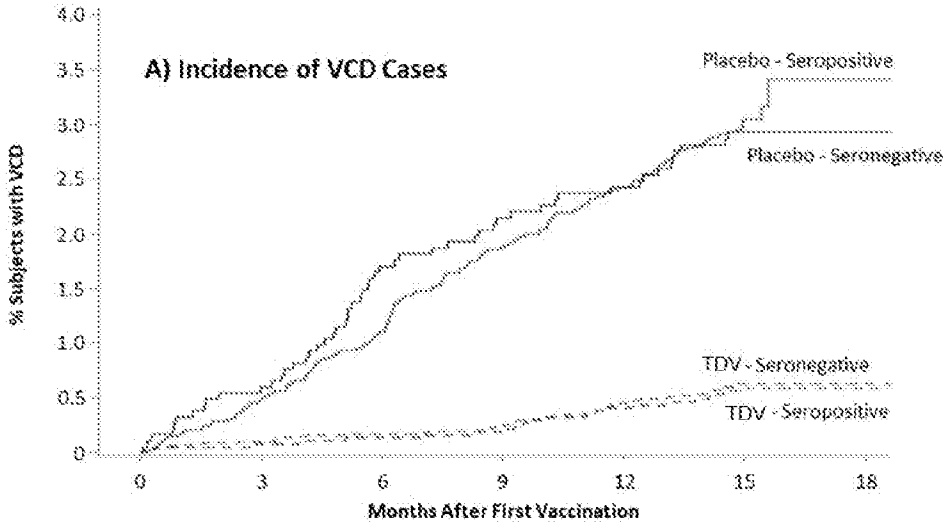
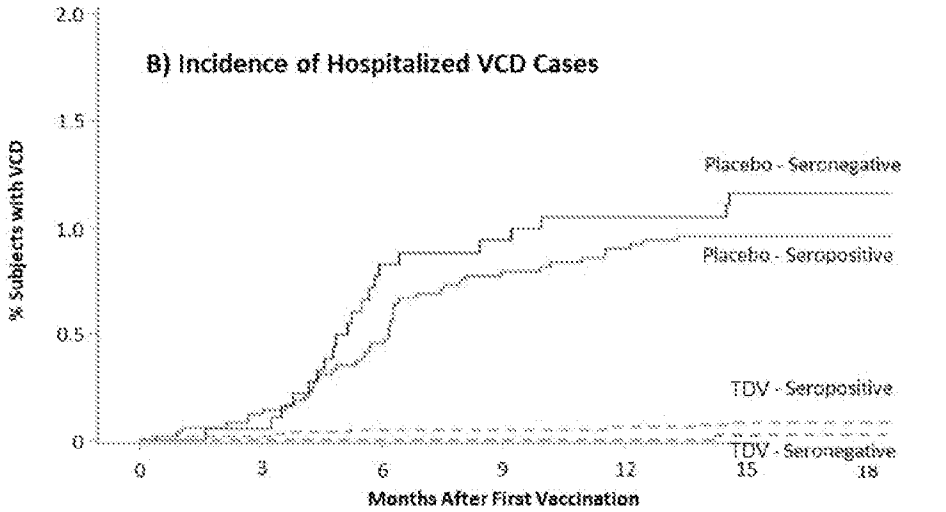

Screened
N = 23,401
Not enrolled
n = 3,302
Enrolled & randomized
N = 20,099
Placebo
n = 6,698
TAK-003
n = 13,401
Not treated n = 11
1 protocol violation
9 withdrawn by parents
1 other reason
Not treated n = 17
15 withdrawn by parents
2 other reasons
Received first dose
n = 6,687
Received first dose
n = 13,384
Safety set
Discontinued before dose 2
5 adverse events
5 lost to follow-up
6 pregnancies
2 protocol violations
182 withdrawn by parents
2 other reasons
Discontinued before dose 2
9 adverse events
16 lost to follow-up
11 pregnancies
4 protocol violations
181 withdrawn by parents
10 other reasons
Received two doses
n = 6,573
Received two doses
n = 13,164
Completed Part 1
n = 6,521
Completed Part 1
n = 13,038
Evaluated for illness
n = 6,316
Evaluated for illness
n = 12,700
Per protocol efficacy analysis
Immunogenicity analysis per protocol
n = 1,247
Immunogenicity analysis per protocol
n = 2,518
Per protocol immunogenicity analysis
Initially seronegative
n = 1,832
Initially seropositive
n = 4,852
Initially seronegative
n = 3,714
Initially seropositive
n = 9,661
Efficacy analysis by initial serostatus

Figure 4



	Month 0	Month 3.0	Month 14.5	Month 15.9	Month 18.0
Placebo - Seronegative	n=1,832	n=1,817	n=1,744	n=94	n=7
Placebo - Seropositive	n=4,852	n=4,805	n=4,607	n=218	n=6
TDV - Seronegative	n=3,714	n=3,690	n=3,615	n=157	n=10
TDV - Seropositive	n=9,661	n=9,591	n=9,391	n=417	n=10



	Month 0	Month 3.0	Month 14.5	Month 15.9	Month 18.0
Placebo - Seronegative	n=1,832	n=1,827	n=1,776	n=94	n=7
Placebo - Seropositive	n=4,852	n=4,823	n=4,700	n=220	n=6
TDV - Seronegative	n=3,714	n=3,693	n=3,635	n=157	n=10
TDV - Seropositive	n=9,661	n=9,599	n=9,435	n=417	n=10

Figure 5

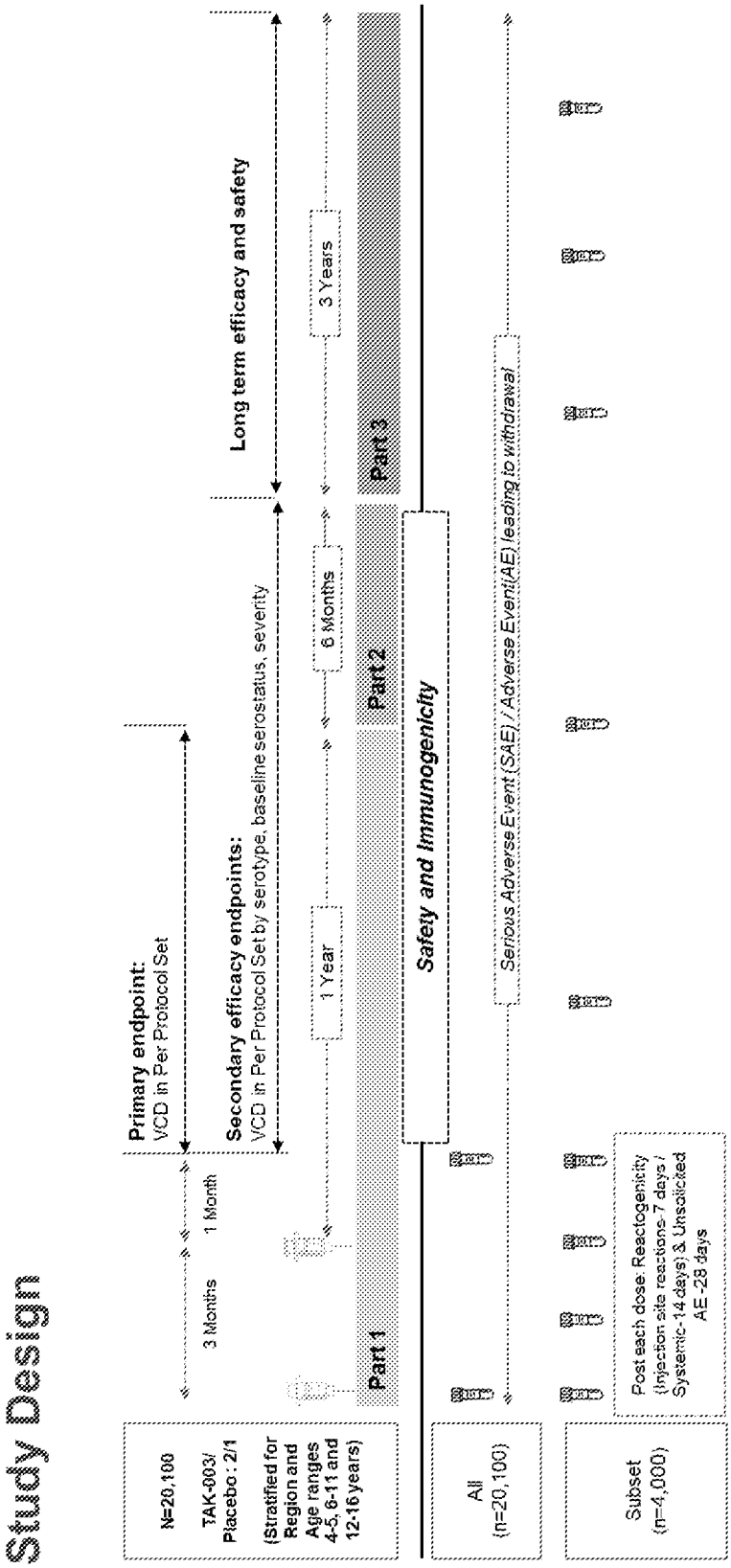
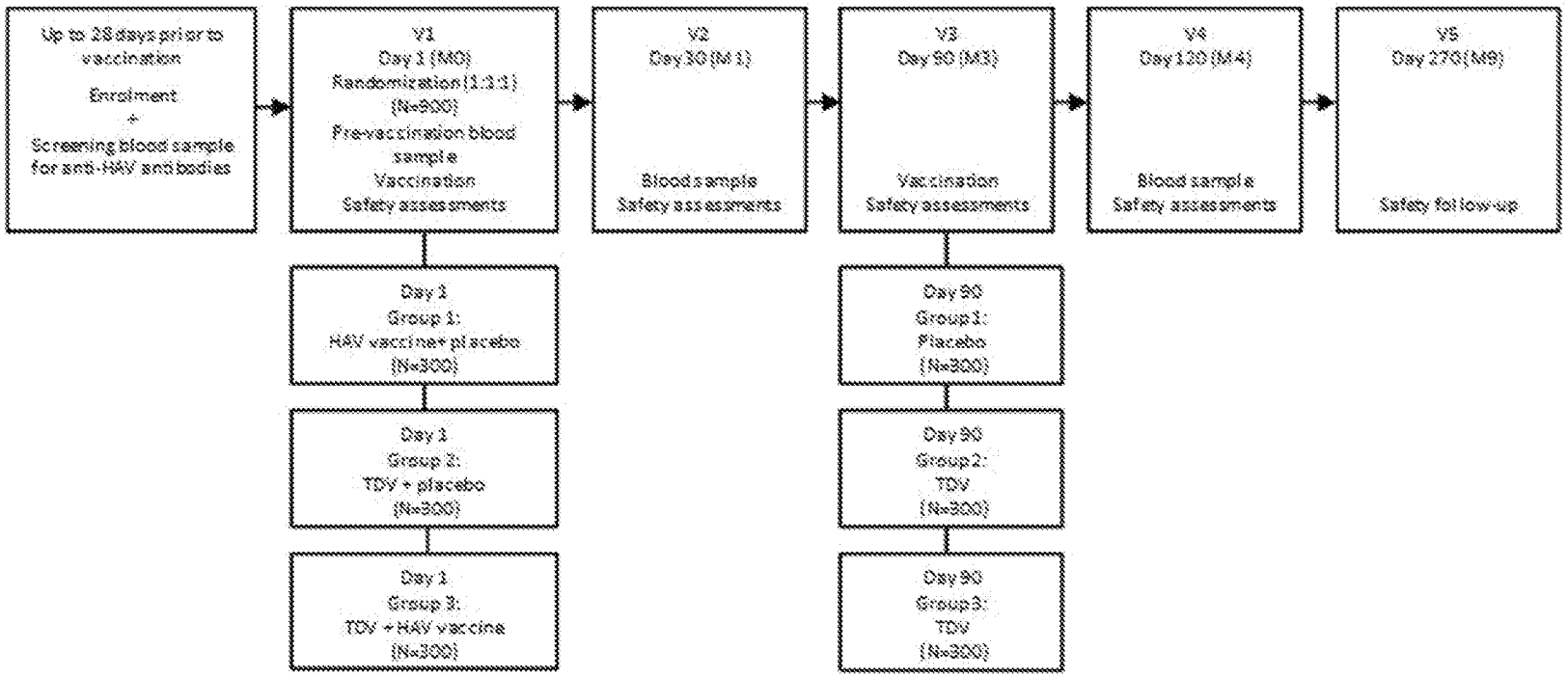

Study Design
N=20,100
TAK-003/ Placebo : 2/1
(Stratified for Region and Age ranges 4-5, 6-11 and 12-16 years)
Primary endpoint:
VCD in Per Protocol Set
3 Months
1 Month
Secondary efficacy endpoints:
VCD in Per Protocol Set by serotype, baseline serostatus, severity
Long term efficacy and safety
1 Year
6 Months
3 Years
Part 1
Part 2
Part 3
Safety and Immunogenicity
Serious Adverse Event (SAE) / Adverse Event(AE) leading to withdrawal
All (n=20,100)
Subset (n=4,000)
Post each dose: Reactogenicity (Injection site reactions-7 days / Systemic-14 days) & Unsolicited AE -28 days

Figure 6

Scheme of Trial Design (HAV + TDV Co-administration)



M=Month, V=Visit

Notes:

(i) A blood sample for measurement of Baseline anti-HAV antibodies will be collected at Screening from all subjects.

(ii) Blood samples for immunogenicity assessments will only be collected from subjects included in the immunogenicity subset (120 subjects in each group).

(iii) Safety will be assessed in all subjects.

SEQUENCE LISTING

<110> Takeda Vaccines, Inc.

<120> METHODS FOR PREVENTING DENGUE AND HEPATITIS A

<130> T08269WO3/ES

<150> EP19 195 692.9
<151> 2019-09-05

<150> US16/561,953
<151> 2019-09-05

<150> PCT/US2019/049749
<151> 2019-09-05

<150> PID201907241
<151> 2019-08-16

<160> 16

<170> PatentIn version 3.5

<210> 1
<211> 10723
<212> DNA
<213> chimeric dengue seroytpe 2/1 (MVS)

<400> 1
agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgggg gagagccgca tatgatagtt 480

agcaagcagg aaagaggaaa gtcacttttg ttcaagacct ctgcaggtgt caacatgtgc 540

accctcattg cgatggattt gggagagttg tgtgaggaca cgatgaccta caaatgcccc 600

cggatcactg aggcggaacc agatgacgtt gactgttggt gcaatgccac ggacacatgg 660

gtgacctatg gaacgtgctc tcaaactggc gaacaccgac gagacaaacg ttccgtcgca 720

ttggccccac acgtggggct tggcctagaa acaagagccg aaacgtggat gtcctctgaa 780

ggtgcttgga aacagataca aaaagtagag acttgggctc tgagacatcc aggattcacg 840

gtgatagccc tttttctagc acatgccata ggaacatcca tcacccagaa agggatcatt 900

ttcattttgc tgatgctggt aacaccatct atggccatgc gatgcgtggg aataggcaac 960

agagacttcg tggaaggact gtcaggagca acatgggtgg atgtggtact ggagcatgga 1020

agttgcgtca ccaccatggc aaaaaacaaa ccaacactgg acattgaact cttgaagacg 1080

gaggtcacaa accctgcagt tctgcgtaaa ttgtgcattg aagctaaaat atcaaacacc 1140

accaccgatt cgagatgtcc aacacaagga gaagccacac tggtggaaga acaagacgcg 1200

aactttgtgt gccgacgaac gttcgtggac agaggctggg gcaatggctg tgggctattc 1260

ggaaaaggta gtctaataac gtgtgccaag tttaagtgtg tgacaaaact agaaggaaag 1320

atagttcaat atgaaaacct aaaatattca gtgatagtca ccgtccacac tggagatcag 1380

caccaggtgg gaaatgagac tacagaacat ggaacaactg caaccataac acctcaagct 1440

cctacgtcgg aaatacagct gaccgactac ggaaccctta cattagattg ttcacctagg 1500

acagggctag attttaacga gatggtgttg ctgacaatga aagaaagatc atggcttgtc 1560

cacaaacaat ggttcctaga cttaccactg ccttggacct ctggggcttc aacatcccaa 1620

gagacttgga acagacaaga tttactggtc acatttaaga cagctcatgc aaagaagcag 1680

gaagtagtcg tactaggatc acaagaagga gcaatgcaca ctgcgctgac tggagcgaca 1740

gaaatccaaa cgtcaggaac gacaacaatt ttcgcaggac acctaaaatg cagactaaaa 1800

atggacaaac taactttaaa agggatgtca tatgtgatgt gcacaggctc attcaagtta 1860

gagaaagaag tggctgagac ccagcatgga actgttctgg tgcaggttaa atatgaagga 1920

acagacgcac catgcaagat tccctttcg acccaagatg agaaaggagc aacccagaat 1980

gggagattaa taacagccaa ccccatagtc actgacaaag aaaaaccagt caatattgag 2040

gcagaaccac cctttggtga gagctacatc gtggtaggag caggtgaaaa agctttgaaa 2100

ctaagctggt tcaagaaagg aagcagcata gggaaaatgt ttgaagcaac tgcccgagga 2160

gcacgaagga tggccattct gggagacacc gcatgggact tcggttctat aggaggagtg 2220

ttcacgtcta tgggaaaact ggtacaccag gtttttggaa ctgcatatgg agttttgttt 2280

agcggagttt cttggaccat gaaaatagga atagggattc tgctgacatg gctaggatta 2340

aattcaagga acacgtccct ttcgatgatg tgcatcgcag ccggcattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccctaccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagatcaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggatttt 5460

atgacagcca ctccccgggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc gaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> 2
<211> 3391
<212> PRT
<213> chimeric dengue serotype 2/1 (MVS)

<400> 2

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val

65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Gly Gly Glu Pro His Met Ile Val
115 120 125

Ser Lys Gln Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ser Ala Gly
130 135 140

Val Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Leu Cys Glu
145 150 155 160

Asp Thr Met Thr Tyr Lys Cys Pro Arg Ile Thr Glu Ala Glu Pro Asp
165 170 175

Asp Val Asp Cys Trp Cys Asn Ala Thr Asp Thr Trp Val Thr Tyr Gly
180 185 190

Thr Cys Ser Gln Thr Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
195 200 205

Leu Ala Pro His Val Gly Leu Gly Leu Glu Thr Arg Ala Glu Thr Trp
210 215 220

Met Ser Ser Glu Gly Ala Trp Lys Gln Ile Gln Lys Val Glu Thr Trp
225 230 235 240

Ala Leu Arg His Pro Gly Phe Thr Val Ile Ala Leu Phe Leu Ala His
245 250 255

Ala Ile Gly Thr Ser Ile Thr Gln Lys Gly Ile Ile Phe Ile Leu Leu
260 265 270

Met Leu Val Thr Pro Ser Met Ala Met Arg Cys Val Gly Ile Gly Asn
275 280 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
290 295 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305 310 315 320

Leu Asp Ile Glu Leu Leu Lys Thr Glu Val Thr Asn Pro Ala Val Leu
325 330 335

Arg Lys Leu Cys Ile Glu Ala Lys Ile Ser Asn Thr Thr Thr Asp Ser
340 345 350

```
Arg Cys Pro Thr Gln Gly Glu Ala Thr Leu Val Glu Glu Gln Asp Ala
        355                 360                 365

Asn Phe Val Cys Arg Arg Thr Phe Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Ile Thr Cys Ala Lys Phe Lys
385                 390                 395                 400

Cys Val Thr Lys Leu Glu Gly Lys Ile Val Gln Tyr Glu Asn Leu Lys
                405                 410                 415

Tyr Ser Val Ile Val Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Thr Glu His Gly Thr Thr Ala Thr Ile Thr Pro Gln Ala
        435                 440                 445

Pro Thr Ser Glu Ile Gln Leu Thr Asp Tyr Gly Thr Leu Thr Leu Asp
    450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Thr
465                 470                 475                 480

Met Lys Glu Arg Ser Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Thr Ser Gly Ala Ser Thr Ser Gln Glu Thr Trp Asn
            500                 505                 510

Arg Gln Asp Leu Leu Val Thr Phe Lys Thr Ala His Ala Lys Lys Gln
        515                 520                 525

Glu Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Thr Ser Gly Thr Thr Thr Ile Phe Ala
545                 550                 555                 560

Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Thr Leu Lys Gly
                565                 570                 575

Met Ser Tyr Val Met Cys Thr Gly Ser Phe Lys Leu Glu Lys Glu Val
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val Lys Tyr Glu Gly
        595                 600                 605

Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly
    610                 615                 620
```

```
Ala Thr Gln Asn Gly Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp
625                 630                 635                 640

Lys Glu Lys Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser
                645                 650                 655

Tyr Ile Val Val Gly Ala Gly Glu Lys Ala Leu Lys Leu Ser Trp Phe
            660                 665                 670

Lys Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly
        675                 680                 685

Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Ile Gly Gly Val Phe Thr Ser Met Gly Lys Leu Val His Gln Val Phe
705                 710                 715                 720

Gly Thr Ala Tyr Gly Val Leu Phe Ser Gly Val Ser Trp Thr Met Lys
                725                 730                 735

Ile Gly Ile Gly Ile Leu Leu Thr Trp Leu Gly Leu Asn Ser Arg Asn
            740                 745                 750

Thr Ser Leu Ser Met Met Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
        755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895
```

```
Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910


Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925


Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940


Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960


Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975


Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990


Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005


Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020


Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035


Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050


Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065


Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080


Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095


Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110


Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125


His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140


Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155


Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
```

1160 1165 1170

Asn Met Ser Phe Arg Asp Leu Gly Arg Val Met Val Met Val Gly
1175 1180 1185

Ala Thr Met Thr Asp Asp Ile Gly Met Gly Val Thr Tyr Leu Ala
1190 1195 1200

Leu Leu Ala Ala Phe Lys Val Arg Pro Thr Phe Ala Ala Gly Leu
1205 1210 1215

Leu Leu Arg Lys Leu Thr Ser Lys Glu Leu Met Met Thr Thr Ile
1220 1225 1230

Gly Ile Val Leu Leu Ser Gln Ser Thr Leu Pro Glu Thr Ile Leu
1235 1240 1245

Glu Leu Thr Asp Ala Leu Ala Leu Gly Met Met Val Leu Lys Met
1250 1255 1260

Val Arg Asn Met Glu Lys Tyr Gln Leu Ala Val Thr Ile Met Ala
1265 1270 1275

Ile Leu Cys Val Pro Asn Ala Val Ile Leu Gln Asn Ala Trp Lys
1280 1285 1290

Val Ser Cys Thr Ile Leu Ala Val Val Ser Val Ser Pro Leu Phe
1295 1300 1305

Leu Thr Ser Ser Gln Gln Lys Thr Asp Trp Ile Pro Leu Ala Leu
1310 1315 1320

Thr Ile Lys Gly Leu Asn Pro Thr Ala Ile Phe Leu Thr Thr Leu
1325 1330 1335

Ser Arg Thr Ser Lys Lys Arg Ser Trp Pro Leu Asn Glu Ala Ile
1340 1345 1350

Met Ala Val Gly Met Val Ser Ile Leu Ala Ser Ser Leu Leu Lys
1355 1360 1365

Asn Asp Ile Pro Met Thr Gly Pro Leu Val Ala Gly Gly Leu Leu
1370 1375 1380

Thr Val Cys Tyr Val Leu Thr Gly Arg Ser Ala Asp Leu Glu Leu
1385 1390 1395

Glu Arg Ala Ala Asp Val Lys Trp Glu Asp Gln Ala Glu Ile Ser
1400 1405 1410

Gly Ser Ser Pro Ile Leu Ser Ile Thr Ile Ser Glu Asp Gly Ser
1415 1420 1425

```
Met Ser  Ile Lys Asn Glu Glu  Glu Asp Gln Thr Leu  Thr Ile Leu
    1430                1435                1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                1450                1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                1465                1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                1480                1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                1495                1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                1510                1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                1525                1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                1540                1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                1555                1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                1570                1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                1585                1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                1615                1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680
```

```
Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                 1690                 1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                 1705                 1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                 1720                 1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                 1735                 1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                 1750                 1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                 1765                 1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                 1780                 1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                 1795                 1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                 1810                 1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                 1825                 1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                 1840                 1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                 1855                 1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                 1870                 1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                 1885                 1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                 1900                 1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                 1915                 1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                 1930                 1935
```

```
Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                2110                2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                2125                2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                2140                2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                2155                2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                2170                2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                2185                2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
```

2195 2200 2205

Ile Val Leu Leu Ile Pro Glu Pro Glu Lys Gln Arg Thr Pro Gln
2210 2215 2220

Asp Asn Gln Leu Thr Tyr Val Val Ile Ala Ile Leu Thr Val Val
2225 2230 2235

Ala Ala Thr Met Ala Asn Glu Met Gly Phe Leu Glu Lys Thr Lys
2240 2245 2250

Lys Asp Leu Gly Leu Gly Ser Ile Ala Thr Gln Gln Pro Glu Ser
2255 2260 2265

Asn Ile Leu Asp Ile Asp Leu Arg Pro Ala Ser Ala Trp Thr Leu
2270 2275 2280

Tyr Ala Val Ala Thr Thr Phe Val Thr Pro Met Leu Arg His Ser
2285 2290 2295

Ile Glu Asn Ser Ser Val Asn Val Ser Leu Thr Ala Ile Ala Asn
2300 2305 2310

Gln Ala Thr Val Leu Met Gly Leu Gly Lys Gly Trp Pro Leu Ser
2315 2320 2325

Lys Met Asp Ile Gly Val Pro Leu Leu Ala Ile Gly Cys Tyr Ser
2330 2335 2340

Gln Val Asn Pro Ile Thr Leu Thr Ala Ala Leu Phe Leu Leu Val
2345 2350 2355

Ala His Tyr Ala Ile Ile Gly Pro Gly Leu Gln Ala Lys Ala Thr
2360 2365 2370

Arg Glu Ala Gln Lys Arg Ala Ala Ala Gly Ile Met Lys Asn Pro
2375 2380 2385

Thr Val Asp Gly Ile Thr Val Ile Asp Leu Asp Pro Ile Pro Tyr
2390 2395 2400

Asp Pro Lys Phe Glu Lys Gln Leu Gly Gln Val Met Leu Leu Val
2405 2410 2415

Leu Cys Val Thr Gln Val Leu Met Met Arg Thr Thr Trp Ala Leu
2420 2425 2430

Cys Glu Ala Leu Thr Leu Ala Thr Gly Pro Ile Ser Thr Leu Trp
2435 2440 2445

Glu Gly Asn Pro Gly Arg Phe Trp Asn Thr Thr Ile Ala Val Ser
2450 2455 2460

```
Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                 2470                 2475

Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                 2485                 2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                 2500                 2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                 2515                 2520

Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                 2530                 2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                 2545                 2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                 2560                 2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                 2575                 2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                 2590                 2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                 2605                 2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                 2620                 2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                 2635                 2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                 2650                 2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                 2665                 2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                 2680                 2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                 2695                 2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                 2710                 2715
```

```
Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                2725                2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                2740                2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                2755                2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                2770                2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                2785                2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                2800                2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                2815                2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                2830                2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                2845                2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                2860                2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                2875                2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                2890                2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                2905                2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                2920                2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                2935                2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                2950                2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                2965                2970
```

```
Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                 2980                 2985


Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                 2995                 3000


His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                 3010                 3015


Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                 3025                 3030


Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                 3040                 3045


Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                 3055                 3060


Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                 3070                 3075


Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                 3085                 3090


Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                 3100                 3105


Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120


Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135


Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                 3145                 3150


Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                 3160                 3165


Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                 3175                 3180


Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                 3190                 3195


Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                 3205                 3210


Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                 3220                 3225


Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
```

```
     3230                3235                3240

 Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
     3245                3250                3255

 His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
     3260                3265                3270

 Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
     3275                3280                3285

 His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
     3290                3295                3300

 Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
     3305                3310                3315

 Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
     3320                3325                3330

 Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
     3335                3340                3345

 Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
     3350                3355                3360

 Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
     3365                3370                3375

 Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
     3380                3385                3390


 <210>  3
 <211>  10723
 <212>  DNA
 <213>  dengue serotype 2 (MVS)

 <400>  3
 agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta       60

 gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg      120

 aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag      180

 ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg      240

 gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga      300

 tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt      360

 ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg      420

 attccaacag tgatggcgtt ccatttaacc acacgtaacg gagaaccaca catgatcgtc      480

 agcagacaag agaaagggaa aagtcttctg tttaaaacag aggttggcgt gaacatgtgt      540

 accctcatgg ccatggacct tggtgaattg tgtgaagaca caatcacgta cgagtgtccc      600
```

cttctcaggc agaatgagcc agaagacata gactgttggt gcaactctac gtccacgtgg 660

gtaacttatg ggacgtgtac caccatggga gaacatagaa gagaaaaaag atcagtggca 720

ctcgttccac atgtgggaat gggactggag acacgaactg aaacatggat gtcatcagaa 780

ggggcctgga aacatgtcca gagaattgaa acttggatct tgagacatcc aggcttcacc 840

atgatggcag caatcctggc atacaccata ggaacgacac atttccaaag agccctgatc 900

ttcatcttac tgacagctgt cactccttca atgacaatgc gttgcatagg aatgtcaaat 960

agagactttg tggaaggggt ttcaggagga agctgggttg acatagtctt agaacatgga 1020

agctgtgtga cgacgatggc aaaaaacaaa ccaacattgg attttgaact gataaaaaca 1080

gaagccaaac agcctgccac cctaaggaag tactgtatag aggcaaagct aaccaacaca 1140

acaacagaat ctcgctgccc aacacaaggg gaacccagcc taaatgaaga gcaggacaaa 1200

aggttcgtct gcaaacactc catggtagac agaggatggg gaaatggatg tggactattt 1260

ggaaagggag gcattgtgac ctgtgctatg ttcagatgca aaaagaacat ggaaggaaaa 1320

gttgtgcaac cagaaaactt ggaatacacc attgtgataa cacctcactc aggggaagag 1380

catgcagtcg gaaatgacac aggaaaacat ggcaaggaaa tcaaaataac accacagagt 1440

tccatcacag aagcagaatt gacaggttat ggcactgtca caatggagtg ctctccaaga 1500

acgggcctcg acttcaatga gatggtgttg ctgcagatgg aaaataaagc ttggctggtg 1560

cacaggcaat ggttcctaga cctgccgtta ccatggttgc ccggagcgga cacacaaggg 1620

tcaaattgga tacagaaaga gacattggtc actttcaaaa atccccatgc gaagaaacag 1680

gatgttgttg ttttaggatc ccaagaaggg gccatgcaca cagcacttac aggggccaca 1740

gaaatccaaa tgtcatcagg aaacttactc ttcacaggac atctcaagtg caggctgaga 1800

atggacaagc tacagctcaa aggaatgtca tactctatgt gcacaggaaa gtttaaagtt 1860

gtgaaggaaa tagcagaaac acaacatgga acaatagtta tcagagtgca atatgaaggg 1920

gacggctctc catgcaagat ccctttgag ataatggatt tggaaaaaag acatgtctta 1980

ggtcgcctga ttacagtcaa cccaattgtg acagaaaaag atagcccagt caacatagaa 2040

gcagaacctc catttggaga cagctacatc atcataggag tagagccggg acaactgaag 2100

ctcaactggt ttaagaaagg aagttctatc ggccaaatgt ttgagacaac aatgaggggg 2160

gcgaagagaa tggccatttt aggtgacaca gcctgggatt ttggatcctt gggaggagtg 2220

tttacatcta taggaaaggc tctccaccaa gtctttggag caatctatgg agctgccttc 2280

agtggggttt catggactat gaaaatcctc ataggagtca ttatcacatg gataggaatg 2340

aattcacgca gcacctcact gtctgtgaca ctagtattgg tgggaattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctcccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccgtattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> 4
<211> 3391
<212> PRT
<213> dengue seroytpe 2 (MVS)

<400> 4

```
Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1               5                   10                  15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
            20                  25                  30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
        35                  40                  45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65                  70                  75                  80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
                85                  90                  95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
            100                 105                 110

Met Ala Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val
        115                 120                 125

Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Val Gly
    130                 135                 140

Val Asn Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu
145                 150                 155                 160

Asp Thr Ile Thr Tyr Glu Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu
                165                 170                 175

Asp Ile Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly
            180                 185                 190

Thr Cys Thr Thr Met Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala
        195                 200                 205

Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp
225                 230                 235                 240

Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile Leu Ala Tyr
                245                 250                 255

Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu
            260                 265                 270
```

```
Thr Ala Val Thr Pro Ser Met Thr Met Arg Cys Ile Gly Met Ser Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val
    290                 295                 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu
                325                 330                 335

Arg Lys Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys
        355                 360                 365

Arg Phe Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Arg
385                 390                 395                 400

Cys Lys Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu
                405                 410                 415

Tyr Thr Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly
            420                 425                 430

Asn Asp Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser
        435                 440                 445

Ser Ile Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu
    450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln
465                 470                 475                 480

Met Glu Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile
            500                 505                 510

Gln Lys Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln
        515                 520                 525

Asp Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr
```

545 550 555 560

Gly His Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly
565 570 575

Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile
580 585 590

Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly
595 600 605

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys
610 615 620

Arg His Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu
625 630 635 640

Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser
645 650 655

Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe
660 665 670

Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly
675 680 685

Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
690 695 700

Leu Gly Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe
705 710 715 720

Gly Ala Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys
725 730 735

Ile Leu Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser
740 745 750

Thr Ser Leu Ser Val Thr Leu Val Leu Val Gly Ile Val Thr Leu Tyr
755 760 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
770 775 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785 790 795 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
805 810 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
820 825 830

```
Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095
```

```
Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Asp Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Lys Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                1240                1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                1255                1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                1270                1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                1285                1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                1300                1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                1315                1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                1330                1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                1345                1350
```

```
Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                1360                1365


Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                1375                1380


Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                1390                1395


Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                1405                1410


Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                1420                1425


Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
    1430                1435                1440


Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                1450                1455


Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                1465                1470


Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                1480                1485


Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                1495                1500


Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                1510                1515


Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                1525                1530


Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                1540                1545


Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                1555                1560


Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                1570                1575


Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                1585                1590


Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605


Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
```

1610 1615 1620

Leu Tyr Gly Asn Gly Val Val Thr Arg Ser Gly Ala Tyr Val Ser
1625 1630 1635

Ala Ile Ala Gln Thr Glu Lys Ser Ile Glu Asp Asn Pro Glu Ile
1640 1645 1650

Glu Asp Asp Ile Phe Arg Lys Arg Arg Leu Thr Ile Met Asp Leu
1655 1660 1665

His Pro Gly Ala Gly Lys Thr Lys Arg Tyr Leu Pro Ala Ile Val
1670 1675 1680

Arg Glu Ala Ile Lys Arg Gly Leu Arg Thr Leu Ile Leu Ala Pro
1685 1690 1695

Thr Arg Val Val Ala Ala Glu Met Glu Glu Ala Leu Arg Gly Leu
1700 1705 1710

Pro Ile Arg Tyr Gln Thr Pro Ala Ile Arg Ala Val His Thr Gly
1715 1720 1725

Arg Glu Ile Val Asp Leu Met Cys His Ala Thr Phe Thr Met Arg
1730 1735 1740

Leu Leu Ser Pro Val Arg Val Pro Asn Tyr Asn Leu Ile Ile Met
1745 1750 1755

Asp Glu Ala His Phe Thr Asp Pro Ala Ser Ile Ala Ala Arg Gly
1760 1765 1770

Tyr Ile Ser Thr Arg Val Glu Met Gly Glu Ala Ala Gly Ile Phe
1775 1780 1785

Met Thr Ala Thr Pro Pro Gly Ser Arg Asp Pro Phe Pro Gln Ser
1790 1795 1800

Asn Ala Pro Ile Ile Asp Glu Glu Arg Glu Ile Pro Glu Arg Ser
1805 1810 1815

Trp Asn Ser Gly His Glu Trp Val Thr Asp Phe Lys Gly Lys Thr
1820 1825 1830

Val Trp Phe Val Pro Ser Ile Lys Ala Gly Asn Asp Ile Ala Ala
1835 1840 1845

Cys Leu Arg Lys Asn Gly Lys Lys Val Ile Gln Leu Ser Arg Lys
1850 1855 1860

Thr Phe Asp Ser Glu Tyr Val Lys Thr Arg Thr Asn Asp Trp Asp
1865 1870 1875

```
Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                2110                2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                2125                2130
```

```
Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                 2140                 2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                 2155                 2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                 2170                 2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                 2185                 2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                 2200                 2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                 2215                 2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                 2230                 2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                 2245                 2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                 2260                 2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                 2275                 2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                 2290                 2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                 2305                 2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                 2320                 2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                 2335                 2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Leu Phe  Leu Leu Val
    2345                 2350                 2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                 2365                 2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                 2380                 2385
```

```
Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                 2395                 2400


Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                 2410                 2415


Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                 2425                 2430


Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                 2440                 2445


Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                 2455                 2460


Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                 2470                 2475


Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                 2485                 2490


Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                 2500                 2505


Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                 2515                 2520


Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                 2530                 2535


Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                 2545                 2550


Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                 2560                 2565


Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                 2575                 2580


Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                 2590                 2595


Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                 2605                 2610


Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                 2620                 2625


Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                 2635                 2640


Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
```

```
     2645                2650                2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
     2660                2665                2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
     2675                2680                2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
     2690                2695                2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
     2705                2710                2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
     2720                2725                2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
     2735                2740                2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
     2750                2755                2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
     2765                2770                2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
     2780                2785                2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
     2795                2800                2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
     2810                2815                2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
     2825                2830                2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
     2840                2845                2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
     2855                2860                2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
     2870                2875                2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
     2885                2890                2895

Ala Leu  Gly Ala Val Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
     2900                2905                2910
```

```
Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                 2920                 2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                 2935                 2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                 2950                 2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                 2965                 2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                 2980                 2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                 2995                 3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                 3010                 3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                 3025                 3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                 3040                 3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                 3055                 3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                 3070                 3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                 3085                 3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                 3100                 3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                 3145                 3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                 3160                 3165
```

```
Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                3175                3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                3190                3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                3205                3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                3220                3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                3235                3240

Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                3250                3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                3265                3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                3280                3285

His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                3295                3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                3310                3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                3325                3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                3340                3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                3355                3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                3370                3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                3385                3390
```

<210> 5
<211> 10717
<212> DNA
<213> chimeric dengue serotype 2/3 (MVS)

<400> 5
agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgatg gagagccgcg catgattgtg 480

gggaagaatg aaagaggaaa atccctactt ttcaagacag cctctggaat caacatgtgc 540

acactcatag ctatggatct gggagagatg tgtgatgaca cggtcactta caaatgcccc 600

cacattaccg aagtggagcc tgaagacatt gactgctggt gcaaccttac atcgacatgg 660

gtgacttatg gaacatgcaa tcaagctgga gagcatagac gcgataagag atcagtggcg 720

ttagctcccc atgttggcat gggactggac acacgcactc aaacctggat gtcggctgaa 780

ggagcttgga gacaagtcga gaaggtagag acatgggccc ttaggcaccc agggtttacc 840

atactagccc tatttcttgc ccattacata ggcacttcct tgacccagaa agtggttatt 900

tttatactat taatgctggt taccccatcc atgacaatga gatgtgtagg agtaggaaac 960

agagattttg tggaaggcct atcgggagct acgtgggttg acgtggtgct cgagcacggt 1020

gggtgtgtga ctaccatggc taagaacaag cccacgctgg acatagagct tcagaagacc 1080

gaggccaccc aactggcgac cctaaggaag ctatgcattg agggaaaaat taccaacata 1140

acaaccgact caagatgtcc cacccaaggg gaagcgattt tacctgagga gcaggaccag 1200

aactacgtgt gtaagcatac atacgtggac agaggctggg gaaacggttg tggtttgttt 1260

ggcaagggaa gcttggtgac atgcgcgaaa tttcaatgtt tagaatcaat agagggaaaa 1320

gtggtgcaac atgagaacct caaatacacc gtcatcatca cagtgcacac aggagaccaa 1380

caccaggtgg gaaatgaaac gcagggagtc acggctgaga taacacccca ggcatcaacc 1440

gctgaagcca ttttacctga atatggaacc ctcgggctag aatgctcacc acggacaggt 1500

ttggatttca atgaaatgat ctcattgaca atgaagaaca aagcatggat ggtacataga 1560

caatggttct ttgacttacc cctaccatgg acatcaggag cttcagcaga aacaccaact 1620

tggaacagga aagagcttct tgtgacattt aaaaatgcac atgcaaaaaa gcaagaagta 1680

gttgttcttg gatcacaaga gggagcaatg catacagcac tgacaggagc tacagagatc 1740

caaacctcag gaggcacaag tatctttgcg gggcacttaa aatgtagact caagatggac 1800

aaattggaac tcaaggggat gagctatgca atgtgcttga gtagctttgt gttgaagaaa 1860

gaagtctccg aaacgcagca tgggacaata ctcattaagg ttgagtacaa aggggaagat 1920

gcaccctgca agattccttt ctccacggag gatggacaag gaaaagctct caatggcaga 1980

ctgatcacag ccaatccagt ggtgaccaag aaggaggagc ctgtcaacat tgaggctgaa 2040

cctccttttg gagaaagtaa catagtaatt ggaattggag acaaagccct gaaaatcaac 2100

tggtacaaga agggaagctc gattgggaag atgttcgagg ccactgccag aggtgcaagg 2160

cgcatggcca tcttgggaga cacagcctgg gactttggat cagtgggtgg tgttttgaat 2220

tcattaggga aaatggtcca ccaaatattt gggagtgctt acacagccct atttggtgga 2280

gtctcctgga tgatgaaaat tggaataggt gtcctcttaa cctggatagg gttgaactca 2340

aaaaatactt ctatgtcatt ttcatgcatc gcggccggca ttgtgacact gtatttggga 2400

gtcatggtgc aggccgatag tggttgcgtt gtgagctgga aaaacaaaga actgaaatgt 2460

ggcagtggga ttttcatcac agacaacgtg cacacatgga cagaacaata caagttccaa 2520

ccagaatccc cttcaaaact agcttcagct atccagaaag cccatgaaga ggacatttgt 2580

ggaatccgct cagtaacaag actggagaat ctgatgtgga aacaaataac accagaattg 2640

aatcacattc tatcagaaaa tgaggtgaag ttaactatta tgacaggaga catcaaagga 2700

atcatgcagg caggaaaacg atctctgcgg cctcagccca ctgagctgaa gtattcatgg 2760

aaaacatggg gcaaagcaaa aatgctctct acagagtctc ataaccagac ctttctcatt 2820

gatggccccg aaacagcaga atgccccaac acaaatagag cttggaattc gttggaagtt 2880

gaagactatg gctttggagt attcaccacc aatatatggc taaaattgaa agaaaaacag 2940

gatgtattct gcgactcaaa actcatgtca gcggccataa aagacaacag agccgtccat 3000

gccgatatgg gttattggat agaaagtgca ctcaatgaca catggaagat agagaaagcc 3060

tctttcattg aagttaaaaa ctgccactgg ccaaaatcac acaccctctg gagcaatgga 3120

gtgctagaaa gtgagatgat aattccaaag aatctcgctg gaccagtgtc tcaacacaac 3180

tatagaccag gctaccatac acaaataaca ggaccatggc atctaggtaa gcttgagatg 3240

gactttgatt tctgtgatgg aacaacagtg gtagtgactg aggactgcgg aaatagagga 3300

ccctctttga gaacaaccac tgcctctgga aaactcataa cagaatggtg ctgccgatct 3360

tgcacattac caccgctaag atacagaggt gaggatgggt gctggtacgg gatggaaatc 3420

agaccattga aggagaaaga agagaatttg gtcaactcct tggtcacagc tggacatggg 3480

caggtcgaca acttttcact aggagtcttg ggaatggcat tgttcctgga ggaaatgctt 3540

aggacccgag taggaacgaa acatgcaata ctactagttg cagtttcttt tgtgacattg 3600

atcacaggga acatgtcctt tagagacctg ggaagagtga tggttatggt aggcgccact 3660

atgacggatg acataggtat gggcgtgact tatcttgccc tactagcagc cttcaaagtc 3720

agaccaactt ttgcagctgg actactcttg agaaagctga cctccaagga attgatgatg 3780

actactatag gaattgtact cctctcccag agcaccatac cagagaccat tcttgagttg 3840

actgatgcgt tagccttagg catgatggtc ctcaaaatgg tgagaaatat ggaaaagtat 3900

caattggcag tgactatcat ggctatcttg tgcgtcccaa acgcagtgat attacaaaac 3960

gcatggaaag tgagttgcac aatattggca gtggtgtccg tttccccact gttcttaaca 4020

tcctcacagc aaaaaacaga ttggatacca ttagcattga cgatcaaagg tctcaatcca 4080

acagctattt ttctaacaac cctctcaaga accagcaaga aaaggagctg gccattaaat 4140

gaggctatca tggcagtcgg gatggtgagc attttagcca gttctctcct aaaaaatgat 4200

attcccatga caggaccatt agtggctgga gggctcctca ctgtgtgcta cgtgctcact 4260

ggacgatcgg ccgatttgga actggagaga gcagccgatg tcaaatggga agaccaggca 4320

gagatatcag gaagcagtcc aatcctgtca ataacaatat cagaagatgg tagcatgtcg 4380

ataaaaaatg aagaggaaga acaaacactg accatactca ttagaacagg attgctggtg 4440

atctcaggac tttttcctgt atcaatacca atcacggcag cagcatggta cctgtgggaa 4500

gtgaagaaac aacgggccgg agtattgtgg gatgttcctt cacccccacc catgggaaag 4560

gctgaactgg aagatggagc ctatagaatt aagcaaaaag ggattcttgg atattcccag 4620

atcggagccg gagtttacaa agaaggaaca ttccatacaa tgtggcatgt cacacgtggc 4680

gctgttctaa tgcataaagg aaagaggatt gaaccatcat gggcggacgt caagaaagac 4740

ctaatatcat atggaggagg ctggaagtta gaaggagaat ggaaggaagg agaagaagtc 4800

caggtattgg cactggagcc tggaaaaaat ccaagagccg tccaaacgaa acctggtctt 4860

ttcaaaacca acgccggaac aataggtgct gtatctctgg acttttctcc tggaacgtca 4920

ggatctccaa ttatcgacaa aaaaggaaaa gttgtgggtc tttatggtaa tggtgttgtt 4980

acaaggagtg gagcatatgt gagtgctata gcccagactg aaaaaagcat tgaagacaac 5040

ccagagatcg aagatgacat tttccgaaag agaagactga ccatcatgga cctccaccca 5100

ggagcgggaa agacgaagag ataccttccg gccatagtca gagaagctat aaaacggggt 5160

ttgagaacat taatcttggc ccccactaga gttgtggcag ctgaaatgga ggaagccctt 5220

agaggacttc caataagata ccagacccca gccatcagag ctgtgcacac cgggcgggag 5280

attgtggacc taatgtgtca tgccacattt accatgaggc tgctatcacc agttagagtg 5340

ccaaactaca acctgattat catggacgaa gcccatttca cagacccagc aagtatagca 5400

gctagaggat acatctcaac tcgagtggag atgggtgagg cagctgggat ttttatgaca 5460

gccactcccc cgggaagcag agacccattt cctcagagca atgcaccaat catagatgaa 5520

gaaagagaaa tccctgaacg ctcgtggaat tccggacatg aatgggtcac ggattttaaa 5580

gggaagactg tttggttcgt tccaagtata aaagcaggaa atgatatagc agcttgcctg 5640

aggaaaaatg gaaagaaagt gatacaactc agtaggaaga cctttgattc tgagtatgtc 5700

aagactagaa ccaatgattg ggacttcgtg gttacaactg acatttcaga aatgggtgcc 5760

aatttcaagg ctgagagggt tatagacccc agacgctgca tgaaaccagt catactaaca 5820

gatggtgaag agcgggtgat tctggcagga cctatgccag tgacccactc tagtgcagca 5880

caaagaagag ggagaatagg aagaaatcca aaaaatgaga atgaccagta catatacatg 5940

ggggaacctc tggaaaatga tgaagactgt gcacactgga aagaagctaa aatgctccta 6000

gataacatca acacgccaga aggaatcatt cctagcatgt tcgaaccaga gcgtgaaaag 6060

gtggatgcca ttgatggcga ataccgcttg agaggagaag caaggaaaac ctttgtagac 6120

ttaatgagaa gaggagacct accagtctgg ttggcctaca gagtggcagc tgaaggcatc 6180

aactacgcag acagaaggtg gtgttttgat ggagtcaaga acaaccaaat cctagaagaa 6240

aacgtggaag ttgaaatctg gacaaaagaa ggggaaagga agaaattgaa acccagatgg 6300

ttggatgcta ggatctattc tgacccactg gcgctaaaag aatttaagga atttgcagcc 6360

ggaagaaagt ctctgaccct gaacctaatc acagaaatgg gtaggctccc aaccttcatg 6420

actcagaagg caagagacgc actggacaac ttagcagtgc tgcacacggc tgaggcaggt 6480

ggaagggcgt acaaccatgc tctcagtgaa ctgccggaga ccctggagac attgctttta 6540

ctgacacttc tggctacagt cacgggaggg atctttttat tcttgatgag cgcaaggggc 6600

atagggaaga tgaccctggg aatgtgctgc ataatcacgg ctagcatcct cctatggtac 6660

gcacaaatac agccacactg gatagcagct tcaataatac tggagttttt tctcatagtt 6720

ttgcttattc cagaacctga aaaacagaga acaccccaag acaaccaact gacctacgtt 6780

gtcatagcca tcctcacagt ggtggccgca accatggcaa acgagatggg tttcctagaa 6840

aaaacgaaga aagatctcgg attgggaagc attgcaaccc agcaacccga gagcaacatc 6900

ctggacatag atctacgtcc tgcatcagca tggacgctgt atgccgtggc cacaacattt 6960

gttacaccaa tgttgagaca tagcattgaa aattcctcag tgaatgtgtc cctaacagct 7020

atagccaacc aagccacagt gttaatgggt ctcgggaaag gatggccatt gtcaaagatg 7080

gacatcggag ttccccttct cgccattgga tgctactcac aagtcaaccc cataactctc 7140

acagcagctc ttttcttatt ggtagcacat tatgccatca tagggccagg actccaagca 7200

aaagcaacca gagaagctca gaaaagagca gcggcgggca tcatgaaaaa cccaactgtc 7260

gatggaataa cagtgattga cctagatcca ataccttatg atccaaagtt tgaaaagcag 7320

ttgggacaag taatgctcct agtcctctgc gtgactcaag tattgatgat gaggactaca 7380

tgggctctgt gtgaggcttt aaccttagct accgggccca tctccacatt gtgggaagga 7440

aatccaggga ggttttggaa cactaccatt gcggtgtcaa tggctaacat ttttagaggg 7500

agttacttgg ccggagctgg acttctcttt tctattatga agaacacaac caacacaaga 7560

aggggaactg gcaacatagg agagacgctt ggagagaaat ggaaaagccg attgaacgcg 7620

ttgggaaaaa gtgaattcca gatctacaag aaaagtggaa tccaggaagt ggatagaacc 7680

ttagcaaaag aaggcattaa aagaggagaa acggaccatc acgctgtgtc gcgaggctca 7740

gcaaaactga gatggttcgt tgagagaaac atggtcacac cagaagggaa agtagtggac 7800

ctcggttgtg gcagaggagg ctggtcatac tattgtggag gactaaagaa tgtaagagaa 7860

gtcaaaggcc taacaaaagg aggaccagga cacgaagaac ccatccccat gtcaacatat 7920

gggtggaatc tagtgcgtct tcaaagtgga gttgacgttt tcttcatccc gccagaaaag 7980

tgtgacacat tattgtgtga cataggggag tcatcaccaa atcccacagt ggaagcagga 8040

cgaacactca gagtccttaa cttagtagaa aattggttga acaacaacac tcaattttgc 8100

ataaaggttc tcaacccata tatgccctca gtcatagaaa aaatggaagc actacaaagg 8160

aaatatggag gagccttagt gaggaatcca ctctcacgaa actccacaca tgagatgtac 8220

tgggtatcca atgcttccgg gaacatagtg tcatcagtga acatgatttc aaggatgttg 8280

atcaacagat ttacaatgag atacaagaaa gccacttacg agccggatgt tgacctcgga 8340

agcggaaccc gtaacatcgg gattgaaagt gagataccaa acctagatat aattgggaaa 8400

agaatagaaa aaataaagca agagcatgaa acatcatggc actatgacca agaccaccca 8460

tacaaaacgt gggcatacca tggtagctat gaaacaaaac agactggatc agcatcatcc 8520

atggtcaacg gagtggtcag gctgctgaca aaaccttggg acgtcgtccc catggtgaca 8580

cagatggcaa tgacagacac gactccattt ggacaacagc gcgtttttaa agagaaagtg 8640

gacacgagaa cccaagaacc gaaagaaggc acgaagaaac taatgaaaat aacagcagag 8700

tggctttgga aagaattagg gaagaaaaag acacccagga tgtgcaccag agaagaattc 8760

acaagaaagg tgagaagcaa tgcagccttg ggggccatat tcactgatga gaacaagtgg 8820

aagtcggcac gtgaggctgt tgaagatagt aggttttggg agctggttga caaggaaagg 8880

aatctccatc ttgaaggaaa gtgtgaaaca tgtgtgtaca acatgatggg aaaaagagag 8940

aagaagctag gggaattcgg caaggcaaaa ggcagcagag ccatatggta catgtggctt 9000

ggagcacgct tcttagagtt tgaagcccta ggattcttaa atgaagatca ctggttctcc 9060

agagagaact ccctgagtgg agtggaagga gaagggctgc acaagctagg ttacattcta 9120

agagacgtga gcaagaaaga gggaggagca atgtatgccg atgacaccgc aggatgggat 9180

acaagaatca cactagaaga cctaaaaaat gaagaaatgg taacaaacca catggaagga 9240

gaacacaaga aactagccga ggccattttc aaactaacgt accaaaacaa ggtggtgcgt 9300

gtgcaaagac caacaccaag aggcacagta atggacatca tatcgagaag agaccaaaga 9360

ggtagtggac aagttggcac ctatggactc aatactttca ccaatatgga agcccaacta 9420

atcagacaga tggagggaga aggagtcttt aaaagcattc agcacctaac aatcacagaa 9480

gaaatcgctg tgcaaaactg gttagcaaga gtggggcgcg aaaggttatc aagaatggcc 9540

atcagtggag atgattgtgt tgtgaaacct ttagatgaca ggttcgcaag cgctttaaca 9600

gctctaaatg acatgggaaa gattaggaaa gacatacaac aatgggaacc ttcaagagga 9660

tggaatgatt ggacacaagt gcccttctgt tcacaccatt tccatgagtt aatcatgaaa 9720

gacggtcgcg tactcgttgt tccatgtaga aaccaagatg aactgattgg cagagcccga 9780

atctcccaag gagcagggtg gtctttgcgg gagacggcct gtttggggaa gtcttacgcc 9840

caaatgtgga gcttgatgta cttccacaga cgcgacctca ggctggcggc aaatgctatt 9900

tgctcggcag taccatcaca ttgggttcca acaagtcgaa caacctggtc catacatgct 9960

aaacatgaat ggatgacaac ggaagacatg ctgacagtct ggaacagggt gtggattcaa 10020

gaaaacccat ggatggaaga caaaactcca gtggaatcat gggaggaaat cccatacttg 10080

gggaaaagag aagaccaatg gtgcggctca ttgattgggt taacaagcag ggccacctgg 10140

gcaaagaaca tccaagcagc aataaatcaa gttagatccc ttataggcaa tgaagaatac 10200

acagattaca tgccatccat gaaaagattc agaagagaag aggaagaagc aggagttctg 10260

tggtagaaag caaaactaac atgaaacaag gctagaagtc aggtcggatt aagccatagt 10320

acggaaaaaa ctatgctacc tgtgagcccc gtccaaggac gttaaaagaa gtcaggccat 10380

cataaatgcc atagcttgag taaactatgc agcctgtagc tccacctgag aaggtgtaaa 10440

aaatccggga ggccacaaac catggaagct gtacgcatgg cgtagtggac tagcggttag 10500

aggagacccc tcccttacaa atcgcagcaa caatgggggc ccaaggcgag atgaagctgt 10560

agtctcgctg gaaggactag aggttagagg agacccccc gaaacaaaaa acagcatatt 10620

gacgctggga aagaccagag atcctgctgt ctcctcagca tcattccagg cacagaacgc 10680

cagaaaatgg aatggtgctg ttgaatcaac aggttct 10717

<210> 6
<211> 3389
<212> PRT
<213> chimeric dengue serotype 2/3 (MVS)

<400> 6

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Arg Met Ile Val
115 120 125

Gly Lys Asn Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ala Ser Gly
130 135 140

Ile Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Asp
145 150 155 160

Asp Thr Val Thr Tyr Lys Cys Pro His Ile Thr Glu Val Glu Pro Glu
165 170 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Thr Tyr Gly
180 185 190

Thr Cys Asn Gln Ala Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
195 200 205

```
Leu Ala Pro His Val Gly Met Gly Leu Asp Thr Arg Thr Gln Thr Trp
    210                 215                 220

Met Ser Ala Glu Gly Ala Trp Arg Gln Val Glu Lys Val Glu Thr Trp
225                 230                 235                 240

Ala Leu Arg His Pro Gly Phe Thr Ile Leu Ala Leu Phe Leu Ala His
                245                 250                 255

Tyr Ile Gly Thr Ser Leu Thr Gln Lys Val Val Ile Phe Ile Leu Leu
            260                 265                 270

Met Leu Val Thr Pro Ser Met Thr Met Arg Cys Val Gly Val Gly Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
    290                 295                 300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Ile Glu Leu Gln Lys Thr Glu Ala Thr Gln Leu Ala Thr Leu
                325                 330                 335

Arg Lys Leu Cys Ile Glu Gly Lys Ile Thr Asn Ile Thr Thr Asp Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Ile Leu Pro Glu Glu Gln Asp Gln
        355                 360                 365

Asn Tyr Val Cys Lys His Thr Tyr Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Gln
385                 390                 395                 400

Cys Leu Glu Ser Ile Glu Gly Lys Val Val Gln His Glu Asn Leu Lys
                405                 410                 415

Tyr Thr Val Ile Ile Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Gln Gly Val Thr Ala Glu Ile Thr Pro Gln Ala Ser Thr
        435                 440                 445

Ala Glu Ala Ile Leu Pro Glu Tyr Gly Thr Leu Gly Leu Glu Cys Ser
    450                 455                 460

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Ile Ser Leu Thr Met Lys
465                 470                 475                 480
```

```
Asn Lys Ala Trp Met Val His Arg Gln Trp Phe Phe Asp Leu Pro Leu
                485                 490                 495

Pro Trp Thr Ser Gly Ala Ser Ala Glu Thr Pro Thr Trp Asn Arg Lys
            500                 505                 510

Glu Leu Leu Val Thr Phe Lys Asn Ala His Ala Lys Lys Gln Glu Val
        515                 520                 525

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
    530                 535                 540

Ala Thr Glu Ile Gln Thr Ser Gly Gly Thr Ser Ile Phe Ala Gly His
545                 550                 555                 560

Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Glu Leu Lys Gly Met Ser
                565                 570                 575

Tyr Ala Met Cys Leu Ser Ser Phe Val Leu Lys Lys Glu Val Ser Glu
            580                 585                 590

Thr Gln His Gly Thr Ile Leu Ile Lys Val Glu Tyr Lys Gly Glu Asp
        595                 600                 605

Ala Pro Cys Lys Ile Pro Phe Ser Thr Glu Asp Gly Gln Gly Lys Ala
    610                 615                 620

Leu Asn Gly Arg Leu Ile Thr Ala Asn Pro Val Val Thr Lys Lys Glu
625                 630                 635                 640

Glu Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser Asn Ile
                645                 650                 655

Val Ile Gly Ile Gly Asp Lys Ala Leu Lys Ile Asn Trp Tyr Lys Lys
            660                 665                 670

Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala Arg
        675                 680                 685

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly
    690                 695                 700

Gly Val Leu Asn Ser Leu Gly Lys Met Val His Gln Ile Phe Gly Ser
705                 710                 715                 720

Ala Tyr Thr Ala Leu Phe Gly Gly Val Ser Trp Met Met Lys Ile Gly
                725                 730                 735

Ile Gly Val Leu Leu Thr Trp Ile Gly Leu Asn Ser Lys Asn Thr Ser
            740                 745                 750
```

```
Met Ser Phe Ser Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr Leu Gly
        755                 760                 765


Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys Asn Lys
    770                 775                 780


Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val His Thr
785                 790                 795                 800


Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys Leu Ala
                805                 810                 815


Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile Arg Ser
            820                 825                 830


Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro Glu Leu
        835                 840                 845


Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met Thr Gly
    850                 855                 860


Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg Pro Gln
865                 870                 875                 880


Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala Lys Met
                885                 890                 895


Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly Pro Glu
            900                 905                 910


Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu Glu Val
        915                 920                 925


Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu Lys Leu
    930                 935                 940


Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser Ala Ala
945                 950                 955                 960


Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp Ile Glu
                965                 970                 975


Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe Ile Glu
            980                 985                 990


Val Lys Asn Cys His Trp Pro Lys  Ser His Thr Leu Trp  Ser Asn Gly
        995                 1000                1005


Val Leu  Glu Ser Glu Met Ile  Ile Pro Lys Asn Leu  Ala Gly Pro
    1010                1015                1020


Val Ser  Gln His Asn Tyr Arg  Pro Gly Tyr His Thr  Gln Ile Thr
```

1025 1030 1035

Gly Pro Trp His Leu Gly Lys Leu Glu Met Asp Phe Asp Phe Cys
1040 1045 1050

Asp Gly Thr Thr Val Val Val Thr Glu Asp Cys Gly Asn Arg Gly
1055 1060 1065

Pro Ser Leu Arg Thr Thr Thr Ala Ser Gly Lys Leu Ile Thr Glu
1070 1075 1080

Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro Leu Arg Tyr Arg Gly
1085 1090 1095

Glu Asp Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Leu Lys Glu
1100 1105 1110

Lys Glu Glu Asn Leu Val Asn Ser Leu Val Thr Ala Gly His Gly
1115 1120 1125

Gln Val Asp Asn Phe Ser Leu Gly Val Leu Gly Met Ala Leu Phe
1130 1135 1140

Leu Glu Glu Met Leu Arg Thr Arg Val Gly Thr Lys His Ala Ile
1145 1150 1155

Leu Leu Val Ala Val Ser Phe Val Thr Leu Ile Thr Gly Asn Met
1160 1165 1170

Ser Phe Arg Asp Leu Gly Arg Val Met Val Met Val Gly Ala Thr
1175 1180 1185

Met Thr Asp Asp Ile Gly Met Gly Val Thr Tyr Leu Ala Leu Leu
1190 1195 1200

Ala Ala Phe Lys Val Arg Pro Thr Phe Ala Ala Gly Leu Leu Leu
1205 1210 1215

Arg Lys Leu Thr Ser Lys Glu Leu Met Met Thr Thr Ile Gly Ile
1220 1225 1230

Val Leu Leu Ser Gln Ser Thr Ile Pro Glu Thr Ile Leu Glu Leu
1235 1240 1245

Thr Asp Ala Leu Ala Leu Gly Met Met Val Leu Lys Met Val Arg
1250 1255 1260

Asn Met Glu Lys Tyr Gln Leu Ala Val Thr Ile Met Ala Ile Leu
1265 1270 1275

Cys Val Pro Asn Ala Val Ile Leu Gln Asn Ala Trp Lys Val Ser
1280 1285 1290

```
Cys Thr  Ile Leu Ala Val Val  Ser Val Ser Pro Leu  Phe Leu Thr
    1295                 1300                 1305

Ser Ser  Gln Gln Lys Thr Asp  Trp Ile Pro Leu Ala  Leu Thr Ile
    1310                 1315                 1320

Lys Gly  Leu Asn Pro Thr Ala  Ile Phe Leu Thr Thr  Leu Ser Arg
    1325                 1330                 1335

Thr Ser  Lys Lys Arg Ser Trp  Pro Leu Asn Glu Ala  Ile Met Ala
    1340                 1345                 1350

Val Gly  Met Val Ser Ile Leu  Ala Ser Ser Leu Leu  Lys Asn Asp
    1355                 1360                 1365

Ile Pro  Met Thr Gly Pro Leu  Val Ala Gly Gly Leu  Leu Thr Val
    1370                 1375                 1380

Cys Tyr  Val Leu Thr Gly Arg  Ser Ala Asp Leu Glu  Leu Glu Arg
    1385                 1390                 1395

Ala Ala  Asp Val Lys Trp Glu  Asp Gln Ala Glu Ile  Ser Gly Ser
    1400                 1405                 1410

Ser Pro  Ile Leu Ser Ile Thr  Ile Ser Glu Asp Gly  Ser Met Ser
    1415                 1420                 1425

Ile Lys  Asn Glu Glu Glu Glu  Gln Thr Leu Thr Ile  Leu Ile Arg
    1430                 1435                 1440

Thr Gly  Leu Leu Val Ile Ser  Gly Leu Phe Pro Val  Ser Ile Pro
    1445                 1450                 1455

Ile Thr  Ala Ala Ala Trp Tyr  Leu Trp Glu Val Lys  Lys Gln Arg
    1460                 1465                 1470

Ala Gly  Val Leu Trp Asp Val  Pro Ser Pro Pro Pro  Met Gly Lys
    1475                 1480                 1485

Ala Glu  Leu Glu Asp Gly Ala  Tyr Arg Ile Lys Gln  Lys Gly Ile
    1490                 1495                 1500

Leu Gly  Tyr Ser Gln Ile Gly  Ala Gly Val Tyr Lys  Glu Gly Thr
    1505                 1510                 1515

Phe His  Thr Met Trp His Val  Thr Arg Gly Ala Val  Leu Met His
    1520                 1525                 1530

Lys Gly  Lys Arg Ile Glu Pro  Ser Trp Ala Asp Val  Lys Lys Asp
    1535                 1540                 1545
```

```
Leu Ile  Ser Tyr Gly Gly Gly  Trp Lys Leu Glu Gly  Glu Trp Lys
    1550                 1555                 1560

Glu Gly  Glu Glu Val Gln Val  Leu Ala Leu Glu Pro  Gly Lys Asn
    1565                 1570                 1575

Pro Arg  Ala Val Gln Thr Lys  Pro Gly Leu Phe Lys  Thr Asn Ala
    1580                 1585                 1590

Gly Thr  Ile Gly Ala Val Ser  Leu Asp Phe Ser Pro  Gly Thr Ser
    1595                 1600                 1605

Gly Ser  Pro Ile Ile Asp Lys  Lys Gly Lys Val Val  Gly Leu Tyr
    1610                 1615                 1620

Gly Asn  Gly Val Val Thr Arg  Ser Gly Ala Tyr Val  Ser Ala Ile
    1625                 1630                 1635

Ala Gln  Thr Glu Lys Ser Ile  Glu Asp Asn Pro Glu  Ile Glu Asp
    1640                 1645                 1650

Asp Ile  Phe Arg Lys Arg Arg  Leu Thr Ile Met Asp  Leu His Pro
    1655                 1660                 1665

Gly Ala  Gly Lys Thr Lys Arg  Tyr Leu Pro Ala Ile  Val Arg Glu
    1670                 1675                 1680

Ala Ile  Lys Arg Gly Leu Arg  Thr Leu Ile Leu Ala  Pro Thr Arg
    1685                 1690                 1695

Val Val  Ala Ala Glu Met Glu  Glu Ala Leu Arg Gly  Leu Pro Ile
    1700                 1705                 1710

Arg Tyr  Gln Thr Pro Ala Ile  Arg Ala Val His Thr  Gly Arg Glu
    1715                 1720                 1725

Ile Val  Asp Leu Met Cys His  Ala Thr Phe Thr Met  Arg Leu Leu
    1730                 1735                 1740

Ser Pro  Val Arg Val Pro Asn  Tyr Asn Leu Ile Ile  Met Asp Glu
    1745                 1750                 1755

Ala His  Phe Thr Asp Pro Ala  Ser Ile Ala Ala Arg  Gly Tyr Ile
    1760                 1765                 1770

Ser Thr  Arg Val Glu Met Gly  Glu Ala Ala Gly Ile  Phe Met Thr
    1775                 1780                 1785

Ala Thr  Pro Pro Gly Ser Arg  Asp Pro Phe Pro Gln  Ser Asn Ala
    1790                 1795                 1800
```

```
Pro Ile  Ile Asp Glu Glu Arg  Glu Ile Pro Glu Arg  Ser Trp Asn
    1805                 1810                 1815

Ser Gly  His Glu Trp Val Thr  Asp Phe Lys Gly Lys  Thr Val Trp
    1820                 1825                 1830

Phe Val  Pro Ser Ile Lys Ala  Gly Asn Asp Ile Ala  Ala Cys Leu
    1835                 1840                 1845

Arg Lys  Asn Gly Lys Lys Val  Ile Gln Leu Ser Arg  Lys Thr Phe
    1850                 1855                 1860

Asp Ser  Glu Tyr Val Lys Thr  Arg Thr Asn Asp Trp  Asp Phe Val
    1865                 1870                 1875

Val Thr  Thr Asp Ile Ser Glu  Met Gly Ala Asn Phe  Lys Ala Glu
    1880                 1885                 1890

Arg Val  Ile Asp Pro Arg Arg  Cys Met Lys Pro Val  Ile Leu Thr
    1895                 1900                 1905

Asp Gly  Glu Glu Arg Val Ile  Leu Ala Gly Pro Met  Pro Val Thr
    1910                 1915                 1920

His Ser  Ser Ala Ala Gln Arg  Arg Gly Arg Ile Gly  Arg Asn Pro
    1925                 1930                 1935

Lys Asn  Glu Asn Asp Gln Tyr  Ile Tyr Met Gly Glu  Pro Leu Glu
    1940                 1945                 1950

Asn Asp  Glu Asp Cys Ala His  Trp Lys Glu Ala Lys  Met Leu Leu
    1955                 1960                 1965

Asp Asn  Ile Asn Thr Pro Glu  Gly Ile Ile Pro Ser  Met Phe Glu
    1970                 1975                 1980

Pro Glu  Arg Glu Lys Val Asp  Ala Ile Asp Gly Glu  Tyr Arg Leu
    1985                 1990                 1995

Arg Gly  Glu Ala Arg Lys Thr  Phe Val Asp Leu Met  Arg Arg Gly
    2000                 2005                 2010

Asp Leu  Pro Val Trp Leu Ala  Tyr Arg Val Ala Ala  Glu Gly Ile
    2015                 2020                 2025

Asn Tyr  Ala Asp Arg Arg Trp  Cys Phe Asp Gly Val  Lys Asn Asn
    2030                 2035                 2040

Gln Ile  Leu Glu Glu Asn Val  Glu Val Glu Ile Trp  Thr Lys Glu
    2045                 2050                 2055

Gly Glu  Arg Lys Lys Leu Lys  Pro Arg Trp Leu Asp  Ala Arg Ile
```

2060 2065 2070

Tyr Ser Asp Pro Leu Ala Leu Lys Glu Phe Lys Glu Phe Ala Ala
2075 2080 2085

Gly Arg Lys Ser Leu Thr Leu Asn Leu Ile Thr Glu Met Gly Arg
2090 2095 2100

Leu Pro Thr Phe Met Thr Gln Lys Ala Arg Asp Ala Leu Asp Asn
2105 2110 2115

Leu Ala Val Leu His Thr Ala Glu Ala Gly Gly Arg Ala Tyr Asn
2120 2125 2130

His Ala Leu Ser Glu Leu Pro Glu Thr Leu Glu Thr Leu Leu Leu
2135 2140 2145

Leu Thr Leu Leu Ala Thr Val Thr Gly Gly Ile Phe Leu Phe Leu
2150 2155 2160

Met Ser Ala Arg Gly Ile Gly Lys Met Thr Leu Gly Met Cys Cys
2165 2170 2175

Ile Ile Thr Ala Ser Ile Leu Leu Trp Tyr Ala Gln Ile Gln Pro
2180 2185 2190

His Trp Ile Ala Ala Ser Ile Ile Leu Glu Phe Phe Leu Ile Val
2195 2200 2205

Leu Leu Ile Pro Glu Pro Glu Lys Gln Arg Thr Pro Gln Asp Asn
2210 2215 2220

Gln Leu Thr Tyr Val Val Ile Ala Ile Leu Thr Val Val Ala Ala
2225 2230 2235

Thr Met Ala Asn Glu Met Gly Phe Leu Glu Lys Thr Lys Lys Asp
2240 2245 2250

Leu Gly Leu Gly Ser Ile Ala Thr Gln Gln Pro Glu Ser Asn Ile
2255 2260 2265

Leu Asp Ile Asp Leu Arg Pro Ala Ser Ala Trp Thr Leu Tyr Ala
2270 2275 2280

Val Ala Thr Thr Phe Val Thr Pro Met Leu Arg His Ser Ile Glu
2285 2290 2295

Asn Ser Ser Val Asn Val Ser Leu Thr Ala Ile Ala Asn Gln Ala
2300 2305 2310

Thr Val Leu Met Gly Leu Gly Lys Gly Trp Pro Leu Ser Lys Met
2315 2320 2325

```
Asp Ile  Gly Val Pro Leu Leu  Ala Ile Gly Cys Tyr  Ser Gln Val
    2330                 2335                 2340

Asn Pro  Ile Thr Leu Thr Ala  Ala Leu Phe Leu Leu  Val Ala His
    2345                 2350                 2355

Tyr Ala  Ile Ile Gly Pro Gly  Leu Gln Ala Lys Ala  Thr Arg Glu
    2360                 2365                 2370

Ala Gln  Lys Arg Ala Ala Ala  Gly Ile Met Lys Asn  Pro Thr Val
    2375                 2380                 2385

Asp Gly  Ile Thr Val Ile Asp  Leu Asp Pro Ile Pro  Tyr Asp Pro
    2390                 2395                 2400

Lys Phe  Glu Lys Gln Leu Gly  Gln Val Met Leu Leu  Val Leu Cys
    2405                 2410                 2415

Val Thr  Gln Val Leu Met Met  Arg Thr Thr Trp Ala  Leu Cys Glu
    2420                 2425                 2430

Ala Leu  Thr Leu Ala Thr Gly  Pro Ile Ser Thr Leu  Trp Glu Gly
    2435                 2440                 2445

Asn Pro  Gly Arg Phe Trp Asn  Thr Thr Ile Ala Val  Ser Met Ala
    2450                 2455                 2460

Asn Ile  Phe Arg Gly Ser Tyr  Leu Ala Gly Ala Gly  Leu Leu Phe
    2465                 2470                 2475

Ser Ile  Met Lys Asn Thr Thr  Asn Thr Arg Arg Gly  Thr Gly Asn
    2480                 2485                 2490

Ile Gly  Glu Thr Leu Gly Glu  Lys Trp Lys Ser Arg  Leu Asn Ala
    2495                 2500                 2505

Leu Gly  Lys Ser Glu Phe Gln  Ile Tyr Lys Lys Ser  Gly Ile Gln
    2510                 2515                 2520

Glu Val  Asp Arg Thr Leu Ala  Lys Glu Gly Ile Lys  Arg Gly Glu
    2525                 2530                 2535

Thr Asp  His His Ala Val Ser  Arg Gly Ser Ala Lys  Leu Arg Trp
    2540                 2545                 2550

Phe Val  Glu Arg Asn Met Val  Thr Pro Glu Gly Lys  Val Val Asp
    2555                 2560                 2565

Leu Gly  Cys Gly Arg Gly Gly  Trp Ser Tyr Tyr Cys  Gly Gly Leu
    2570                 2575                 2580
```

```
Lys Asn  Val Arg Glu Val Lys  Gly Leu Thr Lys Gly  Gly Pro Gly
    2585                 2590                 2595

His Glu  Glu Pro Ile Pro Met  Ser Thr Tyr Gly Trp  Asn Leu Val
    2600                 2605                 2610

Arg Leu  Gln Ser Gly Val Asp  Val Phe Phe Ile Pro  Pro Glu Lys
    2615                 2620                 2625

Cys Asp  Thr Leu Leu Cys Asp  Ile Gly Glu Ser Ser  Pro Asn Pro
    2630                 2635                 2640

Thr Val  Glu Ala Gly Arg Thr  Leu Arg Val Leu Asn  Leu Val Glu
    2645                 2650                 2655

Asn Trp  Leu Asn Asn Asn Thr  Gln Phe Cys Ile Lys  Val Leu Asn
    2660                 2665                 2670

Pro Tyr  Met Pro Ser Val Ile  Glu Lys Met Glu Ala  Leu Gln Arg
    2675                 2680                 2685

Lys Tyr  Gly Gly Ala Leu Val  Arg Asn Pro Leu Ser  Arg Asn Ser
    2690                 2695                 2700

Thr His  Glu Met Tyr Trp Val  Ser Asn Ala Ser Gly  Asn Ile Val
    2705                 2710                 2715

Ser Ser  Val Asn Met Ile Ser  Arg Met Leu Ile Asn  Arg Phe Thr
    2720                 2725                 2730

Met Arg  Tyr Lys Lys Ala Thr  Tyr Glu Pro Asp Val  Asp Leu Gly
    2735                 2740                 2745

Ser Gly  Thr Arg Asn Ile Gly  Ile Glu Ser Glu Ile  Pro Asn Leu
    2750                 2755                 2760

Asp Ile  Ile Gly Lys Arg Ile  Glu Lys Ile Lys Gln  Glu His Glu
    2765                 2770                 2775

Thr Ser  Trp His Tyr Asp Gln  Asp His Pro Tyr Lys  Thr Trp Ala
    2780                 2785                 2790

Tyr His  Gly Ser Tyr Glu Thr  Lys Gln Thr Gly Ser  Ala Ser Ser
    2795                 2800                 2805

Met Val  Asn Gly Val Val Arg  Leu Leu Thr Lys Pro  Trp Asp Val
    2810                 2815                 2820

Val Pro  Met Val Thr Gln Met  Ala Met Thr Asp Thr  Thr Pro Phe
    2825                 2830                 2835
```

```
Gly Gln  Gln Arg Val Phe Lys  Glu Lys Val Asp Thr  Arg Thr Gln
    2840                 2845                 2850

Glu Pro  Lys Glu Gly Thr Lys  Lys Leu Met Lys Ile  Thr Ala Glu
    2855                 2860                 2865

Trp Leu  Trp Lys Glu Leu Gly  Lys Lys Lys Thr Pro  Arg Met Cys
    2870                 2875                 2880

Thr Arg  Glu Glu Phe Thr Arg  Lys Val Arg Ser Asn  Ala Ala Leu
    2885                 2890                 2895

Gly Ala  Ile Phe Thr Asp Glu  Asn Lys Trp Lys Ser  Ala Arg Glu
    2900                 2905                 2910

Ala Val  Glu Asp Ser Arg Phe  Trp Glu Leu Val Asp  Lys Glu Arg
    2915                 2920                 2925

Asn Leu  His Leu Glu Gly Lys  Cys Glu Thr Cys Val  Tyr Asn Met
    2930                 2935                 2940

Met Gly  Lys Arg Glu Lys Lys  Leu Gly Glu Phe Gly  Lys Ala Lys
    2945                 2950                 2955

Gly Ser  Arg Ala Ile Trp Tyr  Met Trp Leu Gly Ala  Arg Phe Leu
    2960                 2965                 2970

Glu Phe  Glu Ala Leu Gly Phe  Leu Asn Glu Asp His  Trp Phe Ser
    2975                 2980                 2985

Arg Glu  Asn Ser Leu Ser Gly  Val Glu Gly Glu Gly  Leu His Lys
    2990                 2995                 3000

Leu Gly  Tyr Ile Leu Arg Asp  Val Ser Lys Lys Glu  Gly Gly Ala
    3005                 3010                 3015

Met Tyr  Ala Asp Asp Thr Ala  Gly Trp Asp Thr Arg  Ile Thr Leu
    3020                 3025                 3030

Glu Asp  Leu Lys Asn Glu Glu  Met Val Thr Asn His  Met Glu Gly
    3035                 3040                 3045

Glu His  Lys Lys Leu Ala Glu  Ala Ile Phe Lys Leu  Thr Tyr Gln
    3050                 3055                 3060

Asn Lys  Val Val Arg Val Gln  Arg Pro Thr Pro Arg  Gly Thr Val
    3065                 3070                 3075

Met Asp  Ile Ile Ser Arg Arg  Asp Gln Arg Gly Ser  Gly Gln Val
    3080                 3085                 3090

Gly Thr  Tyr Gly Leu Asn Thr  Phe Thr Asn Met Glu  Ala Gln Leu
```

3095 3100 3105

Ile Arg Gln Met Glu Gly Glu Gly Val Phe Lys Ser Ile Gln His
3110 3115 3120

Leu Thr Ile Thr Glu Glu Ile Ala Val Gln Asn Trp Leu Ala Arg
3125 3130 3135

Val Gly Arg Glu Arg Leu Ser Arg Met Ala Ile Ser Gly Asp Asp
3140 3145 3150

Cys Val Val Lys Pro Leu Asp Asp Arg Phe Ala Ser Ala Leu Thr
3155 3160 3165

Ala Leu Asn Asp Met Gly Lys Ile Arg Lys Asp Ile Gln Gln Trp
3170 3175 3180

Glu Pro Ser Arg Gly Trp Asn Asp Trp Thr Gln Val Pro Phe Cys
3185 3190 3195

Ser His His Phe His Glu Leu Ile Met Lys Asp Gly Arg Val Leu
3200 3205 3210

Val Val Pro Cys Arg Asn Gln Asp Glu Leu Ile Gly Arg Ala Arg
3215 3220 3225

Ile Ser Gln Gly Ala Gly Trp Ser Leu Arg Glu Thr Ala Cys Leu
3230 3235 3240

Gly Lys Ser Tyr Ala Gln Met Trp Ser Leu Met Tyr Phe His Arg
3245 3250 3255

Arg Asp Leu Arg Leu Ala Ala Asn Ala Ile Cys Ser Ala Val Pro
3260 3265 3270

Ser His Trp Val Pro Thr Ser Arg Thr Thr Trp Ser Ile His Ala
3275 3280 3285

Lys His Glu Trp Met Thr Thr Glu Asp Met Leu Thr Val Trp Asn
3290 3295 3300

Arg Val Trp Ile Gln Glu Asn Pro Trp Met Glu Asp Lys Thr Pro
3305 3310 3315

Val Glu Ser Trp Glu Glu Ile Pro Tyr Leu Gly Lys Arg Glu Asp
3320 3325 3330

Gln Trp Cys Gly Ser Leu Ile Gly Leu Thr Ser Arg Ala Thr Trp
3335 3340 3345

Ala Lys Asn Ile Gln Ala Ala Ile Asn Gln Val Arg Ser Leu Ile
3350 3355 3360

Gly Asn Glu Glu Tyr Thr Asp Tyr Met Pro Ser Met Lys Arg Phe
3365 3370 3375

Arg Arg Glu Glu Glu Glu Ala Gly Val Leu Trp
3380 3385

<210> 7
<211> 10723
<212> DNA
<213> chimeric dengue seroytpe 2/4 (MVS)

<220>
<221> r
<222> (3773)..(3773)
<223> a or g

<400> 7
agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gacctttaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagctctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgatg gcgaacccct catgatagtg 480

gcaaaacatg aaaggggggag acctctcttg tttaagacaa cagaggggat caacaaatgc 540

actctcattg ccatggactt gggtgaaatg tgtgaggaca ctgtcacgta taaatgcccc 600

ttactggtca ataccgaacc tgaagacatt gattgctggt gcaatctcac gtctacctgg 660

gtcatgtatg ggacatgcac ccagagcgga gaacggagac gagagaagcg ctcagtagct 720

ttaacaccac attcaggaat gggattggaa acaagagctg agacatggat gtcatcggaa 780

ggggcttgga agcatgctca gagagtagag agctggatac tcagaaaccc aggattcgcg 840

ctcttggcag gatttatggc ttatatgatt gggcaaacag gaatccagcg aactgtcttc 900

tttgtcctaa tgatgctggt cgccccatcc tacggaatgc gatgcgtagg agtaggaaac 960

agagactttg tggaaggagt ctcaggtgga gcatgggtcg atctggtgct agaacatgga 1020

ggatgcgtca caaccatggc ccagggaaaa ccaaccttgg attttgaact gactaagaca 1080

acagccaagg aagtggctct gttaagaacc tattgcattg aagcctcaat atcaaacata 1140

accacggcaa caagatgtcc aacgcaagga gagccttatc taaaagagga acaagaccaa 1200

cagtacattt gccggagaga tgtggtagac agagggtggg gcaatggctg tggcttgttt 1260

ggaaaaggag gagttgtgac atgtgcgaag ttttcatgtt cggggaagat aacaggcaat 1320

ttggtccaaa ttgagaacct tgaatacaca gtggttgtaa cagtccacaa tggagacacc 1380

catgcagtag gaaatgacac gtccaatcat ggagttacag ccacgataac tcccaggtca 1440

ccatcggtgg aagtcaaatt gccggactat ggagaactaa cactcgattg tgaacccagg 1500

tctggaattg actttaatga gatgattctg atgaaaatga aaaagaaaac atggcttgtg 1560

cataagcaat ggtttttgga tctacctcta ccatggacag caggagcaga cacatcagag 1620

gttcactgga attacaaaga gagaatggtg acatttaagg ttcctcatgc caagagacag 1680

gatgtgacag tgctgggatc tcaggaagga gccatgcatt ctgccctcgc tggagccaca 1740

gaagtggact ccggtgatgg aaatcacatg tttgcaggac atctcaagtg caaagtccgt 1800

atggagaaat tgagaatcaa gggaatgtca tacacgatgt gttcaggaaa gttctcaatt 1860

gacaaagaga tggcagaaac acagcatggg acaacagtgg tgaaagtcaa gtatgaaggt 1920

gctggagctc cgtgtaaagt ccccatagag ataagagatg tgaacaagga aaaagtggtt 1980

gggcgtatca tctcatccac ccctttggct gagaatacca acagtgtaac caacatagag 2040

ttagaacccc cctttgggga cagctacata gtgataggtg ttggaaacag tgcattaaca 2100

ctccattggt tcaggaaagg gagttccatt ggcaagatgt ttgagtccac atacagaggt 2160

gcaaaacgaa tggccattct aggtgaaaca gcttgggatt ttggttccgt tggtggactg 2220

ttcacatcat tgggaaaggc tgtgcaccag gtttttggaa gtgtgtatac aaccctgttt 2280

ggaggagtct catggatgat tagaatccta attgggttcc tagtgttgtg gattggcacg 2340

aactcaagga acacttcaat ggctatgacg tgcatagctg ccggcattgt gacactgtat 2400

ttgggggtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cgggtgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc carggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga tccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctcccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggtaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagccatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttccc tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> 8
<211> 3391
<212> PRT
<213> chimeric dengue serotype 2/4 (MVS)

<220>
<221> Xaa
<222> (1226)..(1226)
<223> Arg or Lys

<400> 8

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Ser Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Leu Met Ile Val
115 120 125

Ala Lys His Glu Arg Gly Arg Pro Leu Leu Phe Lys Thr Thr Glu Gly
130 135 140

Ile Asn Lys Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Glu
145 150 155 160

Asp Thr Val Thr Tyr Lys Cys Pro Leu Leu Val Asn Thr Glu Pro Glu
165 170 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Met Tyr Gly
180 185 190

Thr Cys Thr Gln Ser Gly Glu Arg Arg Arg Glu Lys Arg Ser Val Ala
195 200 205

Leu Thr Pro His Ser Gly Met Gly Leu Glu Thr Arg Ala Glu Thr Trp
210 215 220

Met Ser Ser Glu Gly Ala Trp Lys His Ala Gln Arg Val Glu Ser Trp
225 230 235 240

Ile Leu Arg Asn Pro Gly Phe Ala Leu Leu Ala Gly Phe Met Ala Tyr
245 250 255

Met Ile Gly Gln Thr Gly Ile Gln Arg Thr Val Phe Phe Val Leu Met
260 265 270

Met Leu Val Ala Pro Ser Tyr Gly Met Arg Cys Val Gly Val Gly Asn
275 280 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ala Trp Val Asp Leu Val
290 295 300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Gln Gly Lys Pro Thr
305 310 315 320

Leu Asp Phe Glu Leu Thr Lys Thr Thr Ala Lys Glu Val Ala Leu Leu
325 330 335

Arg Thr Tyr Cys Ile Glu Ala Ser Ile Ser Asn Ile Thr Thr Ala Thr
340 345 350

Arg Cys Pro Thr Gln Gly Glu Pro Tyr Leu Lys Glu Glu Gln Asp Gln

```
        355                 360                 365

Gln Tyr Ile Cys Arg Arg Asp Val Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Gly Val Val Thr Cys Ala Lys Phe Ser
385                 390                 395                 400

Cys Ser Gly Lys Ile Thr Gly Asn Leu Val Gln Ile Glu Asn Leu Glu
                405                 410                 415

Tyr Thr Val Val Val Thr Val His Asn Gly Asp Thr His Ala Val Gly
            420                 425                 430

Asn Asp Thr Ser Asn His Gly Val Thr Ala Thr Ile Thr Pro Arg Ser
        435                 440                 445

Pro Ser Val Glu Val Lys Leu Pro Asp Tyr Gly Glu Leu Thr Leu Asp
    450                 455                 460

Cys Glu Pro Arg Ser Gly Ile Asp Phe Asn Glu Met Ile Leu Met Lys
465                 470                 475                 480

Met Lys Lys Lys Thr Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Thr Ala Gly Ala Asp Thr Ser Glu Val His Trp Asn
            500                 505                 510

Tyr Lys Glu Arg Met Val Thr Phe Lys Val Pro His Ala Lys Arg Gln
        515                 520                 525

Asp Val Thr Val Leu Gly Ser Gln Glu Gly Ala Met His Ser Ala Leu
    530                 535                 540

Ala Gly Ala Thr Glu Val Asp Ser Gly Asp Gly Asn His Met Phe Ala
545                 550                 555                 560

Gly His Leu Lys Cys Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly
                565                 570                 575

Met Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly
        595                 600                 605

Ala Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys
    610                 615                 620

Glu Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn
625                 630                 635                 640
```

```
Thr Asn Ser Val Thr Asn Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser
                645                 650                 655

Tyr Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe
            660                 665                 670

Arg Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly
        675                 680                 685

Ala Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Val Gly Gly Leu Phe Thr Ser Leu Gly Lys Ala Val His Gln Val Phe
705                 710                 715                 720

Gly Ser Val Tyr Thr Thr Leu Phe Gly Gly Val Ser Trp Met Ile Arg
                725                 730                 735

Ile Leu Ile Gly Phe Leu Val Leu Trp Ile Gly Thr Asn Ser Arg Asn
            740                 745                 750

Thr Ser Met Ala Met Thr Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
        755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910
```

```
Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925


Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940


Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960


Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975


Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990


Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005


Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020


Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035


Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050


Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065


Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080


Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095


Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110


Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125


His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140


Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155


Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170
```

```
Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                 1180                 1185


Ala Thr  Met Thr Gly Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                 1195                 1200


Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                 1210                 1215


Leu Leu  Arg Lys Leu Thr Ser  Xaa Glu Leu Met Met  Thr Thr Ile
    1220                 1225                 1230


Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                 1240                 1245


Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                 1255                 1260


Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                 1270                 1275


Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                 1285                 1290


Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                 1300                 1305


Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                 1315                 1320


Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                 1330                 1335


Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                 1345                 1350


Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                 1360                 1365


Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                 1375                 1380


Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                 1390                 1395


Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                 1405                 1410


Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                 1420                 1425


Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
```

1430 1435 1440

Ile Arg Thr Gly Leu Leu Val Ile Ser Gly Leu Phe Pro Val Ser
1445 1450 1455

Ile Pro Ile Thr Ala Ala Ala Trp Tyr Leu Trp Glu Val Lys Lys
1460 1465 1470

Gln Arg Ala Gly Val Leu Trp Asp Val Pro Ser Pro Pro Pro Met
1475 1480 1485

Gly Lys Ala Glu Leu Glu Asp Gly Ala Tyr Arg Ile Lys Gln Lys
1490 1495 1500

Gly Ile Leu Gly Tyr Ser Gln Ile Gly Ala Gly Val Tyr Lys Glu
1505 1510 1515

Gly Thr Phe His Thr Met Trp His Val Thr Arg Gly Ala Val Leu
1520 1525 1530

Met His Lys Gly Lys Arg Ile Glu Pro Ser Trp Ala Asp Val Lys
1535 1540 1545

Lys Asp Leu Ile Ser Tyr Gly Gly Gly Trp Lys Leu Glu Gly Glu
1550 1555 1560

Trp Lys Glu Gly Glu Glu Val Gln Val Leu Ala Leu Glu Pro Gly
1565 1570 1575

Lys Asn Pro Arg Ala Val Gln Thr Lys Pro Gly Leu Phe Lys Thr
1580 1585 1590

Asn Ala Gly Thr Ile Gly Ala Val Ser Leu Asp Phe Ser Pro Gly
1595 1600 1605

Thr Ser Gly Ser Pro Ile Ile Asp Lys Lys Gly Lys Val Val Gly
1610 1615 1620

Leu Tyr Gly Asn Gly Val Val Thr Arg Ser Gly Ala Tyr Val Ser
1625 1630 1635

Ala Ile Ala Gln Thr Glu Lys Ser Ile Glu Asp Asn Pro Glu Ile
1640 1645 1650

Glu Asp Asp Ile Phe Arg Lys Arg Arg Leu Thr Ile Met Asp Leu
1655 1660 1665

His Pro Gly Ala Gly Lys Thr Lys Arg Tyr Leu Pro Ala Ile Val
1670 1675 1680

Arg Glu Ala Ile Lys Arg Gly Leu Arg Thr Leu Ile Leu Ala Pro
1685 1690 1695

```
Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                1720                1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950
```

```
Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                 1960                 1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                 1975                 1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                 1990                 1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                 2005                 2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                 2020                 2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                 2035                 2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                 2050                 2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                 2065                 2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                 2080                 2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                 2095                 2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Val Arg  Asp Ala Leu
    2105                 2110                 2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                 2125                 2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                 2140                 2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                 2155                 2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                 2170                 2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                 2185                 2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                 2200                 2205
```

```
Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                2215                2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                2230                2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                2245                2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                2260                2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                2275                2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                2290                2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                2305                2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                2320                2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                2335                2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Leu Phe  Leu Leu Val
    2345                2350                2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                2365                2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                2380                2385

Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                2395                2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                2410                2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                2425                2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                2440                2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                2455                2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
```

2465 2470 2475

Leu Phe Ser Ile Met Lys Asn Thr Thr Asn Thr Arg Arg Gly Thr
2480 2485 2490

Gly Asn Ile Gly Glu Thr Leu Gly Glu Lys Trp Lys Ser Arg Leu
2495 2500 2505

Asn Ala Leu Gly Lys Ser Glu Phe Gln Ile Tyr Lys Lys Ser Gly
2510 2515 2520

Ile Gln Glu Val Asp Arg Thr Leu Ala Lys Glu Gly Ile Lys Arg
2525 2530 2535

Gly Glu Thr Asp His His Ala Val Ser Arg Gly Ser Ala Lys Leu
2540 2545 2550

Arg Trp Phe Val Glu Arg Asn Met Val Thr Pro Glu Gly Lys Val
2555 2560 2565

Val Asp Leu Gly Cys Gly Arg Gly Gly Trp Ser Tyr Tyr Cys Gly
2570 2575 2580

Gly Leu Lys Asn Val Arg Glu Val Lys Gly Leu Thr Lys Gly Gly
2585 2590 2595

Pro Gly His Glu Glu Pro Ile Pro Met Ser Thr Tyr Gly Trp Asn
2600 2605 2610

Leu Val Arg Leu Gln Ser Gly Val Asp Val Phe Phe Ile Pro Pro
2615 2620 2625

Glu Lys Cys Asp Thr Leu Leu Cys Asp Ile Gly Glu Ser Ser Pro
2630 2635 2640

Asn Pro Thr Val Glu Ala Gly Arg Thr Leu Arg Val Leu Asn Leu
2645 2650 2655

Val Glu Asn Trp Leu Asn Asn Asn Thr Gln Phe Cys Ile Lys Val
2660 2665 2670

Leu Asn Pro Tyr Met Pro Ser Val Ile Glu Lys Met Glu Ala Leu
2675 2680 2685

Gln Arg Lys Tyr Gly Gly Ala Leu Val Arg Asn Pro Leu Ser Arg
2690 2695 2700

Asn Ser Thr His Glu Met Tyr Trp Val Ser Asn Ala Ser Gly Asn
2705 2710 2715

Ile Val Ser Ser Val Asn Met Ile Ser Arg Met Leu Ile Asn Arg
2720 2725 2730

```
Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                 2740                 2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                 2755                 2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                 2770                 2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                 2785                 2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                 2800                 2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                 2815                 2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                 2830                 2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                 2845                 2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                 2860                 2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                 2875                 2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                 2890                 2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                 2905                 2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                 2920                 2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                 2935                 2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                 2950                 2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                 2965                 2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                 2980                 2985
```

```
Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                 2995                 3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                 3010                 3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                 3025                 3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                 3040                 3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                 3055                 3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                 3070                 3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                 3085                 3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                 3100                 3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                 3145                 3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                 3160                 3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                 3175                 3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                 3190                 3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                 3205                 3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                 3220                 3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                 3235                 3240
```

```
Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                 3250                 3255


His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                 3265                 3270


Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                 3280                 3285


His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                 3295                 3300


Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                 3310                 3315


Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                 3325                 3330


Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                 3340                 3345


Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                 3355                 3360


Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                 3370                 3375


Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                 3385                 3390


<210>  9
<211>  10735
<212>  DNA
<213>  dengue virus

<400>  9
agttgttagt ctacgtggac cgacaagaac agtttcgaat cggaagcttg cttaacgtag       60

ttctaacagt tttttattag agagcagatc tctgatgatc aaccaacgaa aaaagacggg      120

tcgaccgtct ttcaatatgc tgaaacgcgc gagaaaccgc gtgtcaactg tttcacagtt      180

ggcgaagaga ttctcaaaag gattgctctc aggccaagga cccatgaaat tggtgatggc      240

tttcatagca ttcttaagat ttctagccat acccccaaca gcaggaattt tggctagatg      300

gggctcattc aagaagaatg gagcgattaa agtgttacgg ggtttcaaga gagaaatctc      360

aaacatgcta aacataatga acaggaggaa aagatccgtg accatgctcc ttatgctgct      420

gcccacagcc ctggcgttcc atctgacgac acgaggggga gagccgcata tgatagttag      480

caagcaggaa agaggaaagt cacttttgtt caagacctct gcaggtgtca acatgtgcac      540

cctcattgcg atggatttgg gagagttgtg tgaggacacg atgacctaca aatgcccccg      600

gatcactgag gcggaaccag atgacgttga ctgttggtgc aatgccacgg acacatgggt      660

gacctatgga acgtgctctc aaactggcga acaccgacga gacaaacgtt ccgtcgcatt      720
```

ggccccacac gtggggcttg gcctagaaac aagagccgaa acgtggatgt cctctgaagg 780

tgcttggaaa cagatacaaa aagtagagac ttgggctctg agacatccag gattcacggt 840

gatagccctt tttctagcac atgccatagg aacatccatc acccagaaag ggatcatttt 900

cattttgctg atgctggtaa caccatctat ggccatgcga tgcgtgggaa taggcaacag 960

agacttcgtg gaaggactgt caggagcaac atgggtggat gtggtactgg agcatggaag 1020

ttgcgtcacc accatggcaa aaaacaaacc aacactggac attgaactct tgaagacgga 1080

ggtcacaaac cctgcagttc tgcgtaaatt gtgcattgaa gctaaaatat caaacaccac 1140

caccgattcg agatgtccaa cacaaggaga agccacactg gtggaagaac aagacgcgaa 1200

ctttgtgtgc cgacgaacgt tcgtggacag aggctggggc aatggctgtg ggctattcgg 1260

aaaaggtagt ctaataacgt gtgccaagtt taagtgtgtg acaaaactag aaggaaagat 1320

agttcaatat gaaaacctaa aatattcagt gatagtcacc gtccacactg gagatcagca 1380

ccaggtggga aatgagacta cagaacatgg aacaactgca accataacac ctcaagctcc 1440

tacgtcggaa atacagctga ccgactacgg aacccttaca ttagattgtt cacctaggac 1500

agggctagat tttaacgaga tggtgttgct gacaatgaaa gaaagatcat ggcttgtcca 1560

caaacaatgg tttctagact taccactgcc ttggacctct ggggcttcaa catcccaaga 1620

gacttggaac agacaagatt tactggtcac atttaagaca gctcatgcaa agaagcagga 1680

agtagtcgta ctaggatcac aagaaggagc aatgcacact gcgctgactg gagcgacaga 1740

aatccaaacg tcaggaacga caacaatttt cgcaggacac ctaaaatgca gactaaaaat 1800

ggacaaacta actttaaaag ggatgtcata tgtgatgtgc acaggctcat tcaagttaga 1860

gaaagaagtg gctgagaccc agcatggaac tgttctggtg caggttaaat atgaaggaac 1920

agacgcacca tgcaagattc ccttttcgac ccaagatgag aaaggagcaa cccagaatgg 1980

gagattaata acagccaacc ccatagtcac tgacaaagaa aaaccagtca atattgaggc 2040

agaaccaccc tttggtgaga gctacatcgt ggtaggagca ggtgaaaaag ctttgaaact 2100

aagctggttc aagaaaggaa gcagcatagg gaaaatgttt gaagcaactg cccgaggagc 2160

acgaaggatg gccattctgg gagacaccgc atgggacttc ggttctatag gaggagtgtt 2220

cacgtctatg ggaaaactgg tacaccaggt ttttggaact gcatatggag ttttgtttag 2280

cggagtttct tggaccatga aaataggaat agggattctg ctgacatggc taggattaaa 2340

ttcaaggaac acgtcccttt cgatgatgtg catcgcagtt ggcatggtca cactgtacct 2400

aggagtcatg gttcaggcag attcgggatg tgtaatcaac tggaaaggca gagaacttaa 2460

atgtggaagc ggcatttttg tcactaatga agttcacact tggacagagc aatacaaatt 2520

ccaggctgac tcccccaaga gactatcagc agccattggg aaggcatggg aggagggtgt 2580

gtgtggaatc cgatcagcca ctcgtctcga gaacatcatg tggaaacaaa tatcaaatga 2640

attgaaccac atcctacttg aaaatgacat gaaatttaca gtggtcgtgg gagatgttag 2700

tggaatcttg gcccaaggga aaaaaatgat taggccacaa cccatggaac acaaatactc 2760

gtggaaaagc tggggaaaag ccaaaatcat aggagcggat gtacagaaca ccaccttcat 2820

catcgacggc ccaaacaccc cagaatgccc tgacaatcaa agagcatgga atatttggga 2880

agtagaggac tatggatttg ggattttcac gacaaacata tggttgaaat tgcgtgactc 2940

ctacacccaa gtatgtgacc accggctgat gtcagctgcc attaaggaca gcaaggcagt 3000

ccatgctgac atggggtact ggatagaaag tgaaaagaac gagacatgga agttggcgag 3060

agcctccttt atagaagtta agacatgcat ctggccaaaa tcccacactc tatggagcaa 3120

tggagttctg gaaagtgaaa tgataattcc aaagatatat ggaggaccaa tatctcagca 3180

caactacaga ccaggatatt tcacacaaac agcagggccg tggcacctag gcaagttgga 3240

actagatttc gatttttgtg aaggtaccac agttgttgtg gatgaacatt gtggaaatcg 3300

aggaccatct ctcagaacca caacagtcac aggaaagata atccatgaat ggtgctgcag 3360

atcttgtacg ctacccccccc tacgtttcaa aggggaagac gggtgttggt acggcatgga 3420

aatcagacca gtgaaggaca aggaagagaa cctggtcaag tcaatggtct ctgcagggtc 3480

aggagaagtg gacagctttt cactaggact gctatgcata tcaataatga ttgaagaagt 3540

gatgagatcc agatggagca aaaaaatgct gatgactgga acactggctg tgttcctcct 3600

tcttataatg ggacaattga catggagtga tctgatcagg ttatgtatta tggttggagc 3660

caacgcttca gacaagatgg ggatgggaac aacgtaccta gctttaatgg ccactttcaa 3720

aatgagacca atgttcgccg tcgggctatt atttcgcaga ctaacatcta gagaagttct 3780

tcttcttaca attggcttga gcctggtggc atccgtggag ctaccaagtt ccctagagga 3840

gctgggggat ggacttgcaa taggcatcat gatgttgaaa ttattgactg attttcagtc 3900

acaccagcta tgggctactc tgctatcctt gacatttatt aaaacaactt tttcattgca 3960

ctatgcatgg aagacaatgg ctatggtact gtcaattgta tctctcttcc ctttatgcct 4020

gtccacgacc tctcaaaaaa caacatggct tccggtgctg ttgggatctc ttggatgcaa 4080

accactaccc atgtttctta taacagaaaa caaaatctgg ggaaggaaga gttggcccct 4140

caatgaagga attatggctg ttggaatagt tagtattcta ctaagttcac ttttaaaaaa 4200

tgatgtgccg ctagccggcc cattaatagc tggaggcatg ctaatagcat gttatgtcat 4260

atccggaagc tcagctgatt tatcactgga gaaagcggct gaggtctcct gggaggaaga 4320

agcagaacac tcaggcgcct cacacaacat actagtagag gttcaagatg atggaaccat 4380

gaagataaaa gatgaagaga gagatgacac gctcaccatt ctccttaaag caactctgct 4440

ggcagtctca ggggtgtacc caatgtcaat accagcgacc ctttttgtgt ggtatttttg 4500

gcagaaaaag aaacagagat caggagtgct atgggacaca cccagccccc cagaagtgga 4560

aagagcagtt cttgatgatg gcatctatag aattttgcaa agaggactgt tgggcaggtc 4620

ccaagtagga gtaggagttt tccaagaagg cgtgttccac acaatgtggc acgtcactag 4680

gggagctgtc ctcatgtatc aaggaaaaag gctggaacca agctgggcca gtgtcaaaaa 4740

agacttgatc tcatatggag gaggttggag gtttcaagga tcctggaaca cgggagaaga 4800

agtacaggtg attgctgttg aaccgggaaa aaaccccaaa aatgtacaaa caacgccggg 4860

taccttcaag acccctgaag gcgaagttgg agccatagcc ttagacttta aacctggcac 4920

atctggatct cccatcgtaa acagagaggg aaaaatagta ggtctttatg gaaatggagt 4980

ggtgacaaca agcggaactt acgttagtgc catagctcaa gctaaggcat cacaagaagg 5040

gcctctacca gagattgagg acgaggtgtt taggaaaaga aacttaacaa taatggacct 5100

acatccagga tcgggaaaaa caagaagata ccttccagcc atagtccgtg aggccataaa 5160

aaggaagctg cgcacgctaa tcctagctcc cacaagagtt gtcgcttctg aaatggcaga 5220

ggcactcaag ggagtgccaa taaggtatca gacaacagca gtgaagagtg aacacacagg 5280

aaaggagata gttgacctta tgtgccacgc cactttcacc atgcgcctcc tgtctcccgt 5340

gagagttccc aattataaca tgattatcat ggatgaagca cacttcaccg atccagccag 5400

catagcagcc agagggtaca tctcaacccg agtgggtatg ggtgaagcag ctgcgatctt 5460

tatgacagcc actcccccag gatcggtgga ggcctttcca cagagcaatg caattatcca 5520

agatgaggaa agagacattc ctgagagatc atggaactca ggctatgact ggatcactga 5580

ttttccaggt aaaacagtct ggtttgttcc aagcatcaaa tcaggaaatg acattgccaa 5640

ctgtttaaga aaaaacggga aacgggtgat ccaattgagc agaaaaacct ttgacactga 5700

gtaccagaaa acaaaaaaca acgactggga ctatgtcgtc acaacagaca tttccgaaat 5760

gggagcaaat ttccgggccg acagggtaat agacccaagg cggtgtctga aaccggtaat 5820

actaaaagat ggtccagagc gcgtcattct agccggaccg atgccagtga ctgtggccag 5880

tgccgcccag aggagaggaa gaattggaag gaaccaaaac aaggaaggtg atcagtatat 5940

ttacatggga cagcctttaa acaatgatga ggaccacgct cattggacag aagcaaagat 6000

gctccttgac aatataaaca caccagaagg gattatccca gccctctatg agccggagag 6060

agaaaagagt gcagctatag acggggaata cagactgcgg ggtgaagcaa ggaaaacgtt 6120

cgtggagctc atgagaagag gggatctacc agtctggcta tcctacaaag ttgcctcaga 6180

aggcttccag tactccgaca gaaggtggtg cttcgatggg gaaaggaaca accaggtgtt 6240

ggaggagaac atggacgtgg agatctggac aaaagaagga gaaagaaaga aactacgacc 6300

tcgctggttg gacgccagaa catactctga cccactggct ctgcgcgagt ttaaagagtt 6360

tgcagcagga agaagaagcg tctcaggtga cctaatatta gaaataggga aacttccaca 6420

acatttgacg caaagggccc agaatgcttt ggacaacttg gtcatgttgc acaattccga 6480

acaaggagga aaagcctata gacatgctat ggaagaactg ccagacacaa tagaaacgtt 6540

gatgctccta gccttgatag ctgtgttgac tggtggagtg acgctgttct tcctatcagg 6600

aagaggtcta ggaaaaacat ctatcggctt actctgcgtg atggcctcaa gcgcactgtt 6660

atggatggcc agtgtggagc cccattggat agcggcctcc atcatactgg agttctttct 6720

gatggtactg cttattccag agccagacag acagcgcact ccacaggaca accagctagc 6780

atatgtggtg ataggtctgt tattcatgat attgacagtg gcagccaatg agatgggatt 6840

attggaaacc acaaagaaag acctggggat tggccatgta gctgctgaaa accaccacca 6900

tgctacaatg ctggacgtag acctacatcc agcttcagcc tggaccctct atgcagtggc 6960

cacaacaatc atcactccta tgatgagaca cacaattgaa aacacaacgg caaatatttc 7020

cctgacagcc atcgcaaacc aagcagctat attgatggga cttgacaagg gatggccaat 7080

atcgaagatg gacataggag ttccacttct cgccttgggg tgctattccc aagtgaatcc 7140

gctgacactg atagcggcag tattgatgct agtagctcat tacgccataa ttggacctgg 7200

actgcaagca aaagctacta gagaagctca aaaaagaaca gcggctggaa taatgaaaaa 7260

tccaactgtc gacgggattg ttgcaataga cttagatccc gtggtttacg atgcaaaatt 7320

tgaaaaacaa ctaggccaaa taatgttgtt gatactttgc acatcacaga ttcttttgat 7380

gcggactaca tgggccttgt gtgaatccat cacattggct actggacctc tgaccactct 7440

ttgggaggga tctccaggaa aattctggaa caccacaata gcggtatcca tggcaaacat 7500

tttcaggggg agttatctag caggagcagg tctggccttc tcattaatga aatctctagg 7560

aggaggtagg agaggcacgg gagcccaagg ggaaacactg ggagaaaaat ggaaaagaca 7620

actaaaccaa ctgagcaagt cagaattcaa tacttacaag aggagtggga ttatggaggt 7680

ggatagatcc gaagccaaag agggactgaa aagaggagaa acaaccaaac acgcagtatc 7740

gagaggaacg gccaaactga ggtggttcgt ggagaggaac cttgtgaaac cagaagggaa 7800

agtcatagac ctcggttgtg gaagaggtgg ctggtcatat tattgcgctg ggctgaagaa 7860

agtcacagaa gtgaaaggat acacaaaagg aggacctgga catgaggaac caatcccaat 7920

ggcgacctat ggatggaacc tagtaaagct gcactccgga aaagatgtat tttttatacc 7980

acctgagaaa tgtgacaccc ttttgtgtga tattggtgag tcctctccga acccaactat 8040

agaggaagga agaacgttac gtgttctgaa aatggtggaa ccatggctca gaggaaacca 8100

attttgcata aaaattctaa atccctatat gccgagcgtg gtagaaactc tggaacaaat 8160

gcaaagaaaa catggaggaa tgctagtgcg aaacccactc tcaagaaatt ccacccatga 8220

aatgtactgg gtttcatgtg gaacaggaaa cattgtgtca gcagtaaaca tgacatctag 8280

aatgttgcta aatcggttca caatggctca caggaagcca acatatgaaa gagacgtgga 8340

cttaggcgct ggaacaagac atgtggcagt agaaccagag gtagccaacc tagatatcat 8400

tggccagagg atagagaata taaaaaatga acataagtca acatggcatt atgatgagga 8460

caatccatac aaaacatggg cctatcatgg atcatatgag gttaagccat caggatcggc 8520

ctcatccatg gtcaatggcg tggtgagatt gctcaccaaa ccatgggatg ttatccccat 8580

ggtcacacaa atagccatga ctgataccac acccttgga caacagaggg tgtttaaaga 8640

gaaagttgac acgcgcacac caaaagcaaa acgtggcaca gcacaaatta tggaagtgac 8700

agccaggtgg ttatggggtt tcctttctag aaacaaaaaa cccagaattt gcacaagaga 8760

ggagtttaca agaaaagtta ggtcaaacgc agctattgga gcagtgttcg ttgatgaaaa 8820

tcaatggaac tcggcaaaag aagcagtgga agacgaacgg ttctgggaac ttgtccacag 8880

agagagggag cttcataaac aggggaaatg tgccacgtgt gtctacaata tgatggggaa 8940

gagagagaaa aaattaggag agttcggaaa ggcaaaagga agtcgtgcaa tatggtacat 9000

gtggttggga gcacgcttcc tagagtttga agcccttggt ttcatgaatg aagatcactg 9060

gttcagtaga gagaattcac tcagtggagt ggaaggagaa ggactccaca aacttggata 9120

catactcaga gacatatcaa ggattccagg ggggaacatg tatgcagatg acacagccgg 9180

atgggacaca agaataacag aggatgatct ccagaatgag gctaaaatca ctgacatcat 9240

ggagcccgaa catgccctgc tggctacgtc aatctttaag ctgacctacc aaaataaggt 9300

ggtaagggtg cagagaccag caaaaaatgg aaccgtgatg gatgttatat ccagacgtga 9360

ccagagaggc agtggacagg ttggaactta tggcttaaac actttcacca acatggaggc 9420

ccaactgata agacaaatgg agtccgaggg aatcttttta cccagcgaat tggaaacccc 9480

aaatctagcc ggaagagttc tcgactggtt ggaaaaatat ggtgtcgaaa ggctgaaaag 9540

aatggcaatc agcggagatg actgtgtggt gaaaccaatt gatgacaggt tcgcaacagc 9600

cttaacagct ttgaatgaca tgggaaaagt aagaaaagac ataccacaat gggaaccttc 9660

aaaaggatgg aatgattggc aacaagtgcc tttctgttca caccacttcc accagctaat 9720

tatgaaggat gggagggaga tagtggtgcc atgccgcaac caagatgaac ttgtggggag 9780

ggccagagta tcacaaggcg ccggatggag cctgagagaa accgcatgcc taggcaagtc 9840

atatgcacaa atgtggcagc tgatgtattt ccacaggaga gacctgagac tggcggctaa 9900

cgctatttgt tcagccgttc cagttgattg ggtcccaacc agccgcacca cctggtcgat 9960

ccatgcccat caccaatgga tgacaacaga agacatgtta tcagtatgga atagggtctg 10020

gatagaggaa aacccatgga tggaggataa gactcatgtg tccagttggg aagaagttcc 10080

atacctagga aagagggaag atcagtggtg tggatccctg ataggcttaa cagcaagggc 10140

cacctgggcc actaatatac aagtggccat aaaccaagtg agaaggctca ttgggaatga 10200

gaattatcta gattacatga catcaatgaa gagattcaag aatgagagtg atcccgaagg 10260

ggcactctgg taagtcaaca cattcacaaa ataaaggaaa ataaaaaatc aaatgaggca 10320

agaagtcagg ccagattaag ccatagtacg gtaagagcta tgctgcctgt gagccccgtc 10380

caaggacgta aaatgaagtc aggccgaaag ccacggtttg agcaagccgt gctgcctgtg 10440

gctccatcgt ggggatgtaa aaacccggga ggctgcaacc catggaagct gtacgcatgg 10500

ggtagcagac tagtggttag aggagacccc tcccaagaca caacgcagca gcggggccca 10560

acaccagggg aagctgtacc ctggtggtaa ggactagagg ttagaggaga ccccccgcgt 10620

aacaataaac agcatattga cgctgggaga gaccagagat cctgctgtct ctacagcatc 10680

attccaggca cagaacgcca gaaaatggaa tggtgctgtt gaatcaacag gttct 10735

<210> 10
<211> 3392
<212> PRT
<213> dengue virus

<400> 10

Met Ile Asn Gln Arg Lys Lys Thr Gly Arg Pro Ser Phe Asn Met Leu
1 5 10 15

```
Lys Arg Ala Arg Asn Arg Val Ser Thr Val Ser Gln Leu Ala Lys Arg
            20                  25                  30

Phe Ser Lys Gly Leu Leu Ser Gly Gln Gly Pro Met Lys Leu Val Met
        35                  40                  45

Ala Phe Ile Ala Phe Leu Arg Phe Leu Ala Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Ala Arg Trp Gly Ser Phe Lys Lys Asn Gly Ala Ile Lys Val
65                  70                  75                  80

Leu Arg Gly Phe Lys Arg Glu Ile Ser Asn Met Leu Asn Ile Met Asn
                85                  90                  95

Arg Arg Lys Arg Ser Val Thr Met Leu Leu Met Leu Leu Pro Thr Ala
            100                 105                 110

Leu Ala Phe His Leu Thr Thr Arg Gly Gly Glu Pro His Met Ile Val
        115                 120                 125

Ser Lys Gln Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ser Ala Gly
    130                 135                 140

Val Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Leu Cys Glu
145                 150                 155                 160

Asp Thr Met Thr Tyr Lys Cys Pro Arg Ile Thr Glu Ala Glu Pro Asp
                165                 170                 175

Asp Val Asp Cys Trp Cys Asn Ala Thr Asp Thr Trp Val Thr Tyr Gly
            180                 185                 190

Thr Cys Ser Gln Thr Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
        195                 200                 205

Leu Ala Pro His Val Gly Leu Gly Leu Glu Thr Arg Ala Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys Gln Ile Gln Lys Val Glu Thr Trp
225                 230                 235                 240

Ala Leu Arg His Pro Gly Phe Thr Val Ile Ala Leu Phe Leu Ala His
                245                 250                 255

Ala Ile Gly Thr Ser Ile Thr Gln Lys Gly Ile Ile Phe Ile Leu Leu
            260                 265                 270

Met Leu Val Thr Pro Ser Met Ala Met Arg Cys Val Gly Ile Gly Asn
        275                 280                 285
```

```
Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
    290                 295                 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Ile Glu Leu Leu Lys Thr Glu Val Thr Asn Pro Ala Val Leu
                325                 330                 335

Arg Lys Leu Cys Ile Glu Ala Lys Ile Ser Asn Thr Thr Thr Asp Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Thr Leu Val Glu Glu Gln Asp Ala
        355                 360                 365

Asn Phe Val Cys Arg Arg Thr Phe Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Ile Thr Cys Ala Lys Phe Lys
385                 390                 395                 400

Cys Val Thr Lys Leu Glu Gly Lys Ile Val Gln Tyr Glu Asn Leu Lys
                405                 410                 415

Tyr Ser Val Ile Val Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Thr Glu His Gly Thr Thr Ala Thr Ile Thr Pro Gln Ala
        435                 440                 445

Pro Thr Ser Glu Ile Gln Leu Thr Asp Tyr Gly Thr Leu Thr Leu Asp
    450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Thr
465                 470                 475                 480

Met Lys Glu Arg Ser Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Thr Ser Gly Ala Ser Thr Ser Gln Glu Thr Trp Asn
            500                 505                 510

Arg Gln Asp Leu Leu Val Thr Phe Lys Thr Ala His Ala Lys Lys Gln
        515                 520                 525

Glu Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Thr Ser Gly Thr Thr Thr Ile Phe Ala
545                 550                 555                 560
```

```
Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Thr Leu Lys Gly
                565                 570                 575


Met Ser Tyr Val Met Cys Thr Gly Ser Phe Lys Leu Glu Lys Glu Val
            580                 585                 590


Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val Lys Tyr Glu Gly
        595                 600                 605


Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly
    610                 615                 620


Ala Thr Gln Asn Gly Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp
625                 630                 635                 640


Lys Glu Lys Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser
                645                 650                 655


Tyr Ile Val Val Gly Ala Gly Glu Lys Ala Leu Lys Leu Ser Trp Phe
            660                 665                 670


Lys Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly
        675                 680                 685


Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700


Ile Gly Gly Val Phe Thr Ser Met Gly Lys Leu Val His Gln Val Phe
705                 710                 715                 720


Gly Thr Ala Tyr Gly Val Leu Phe Ser Gly Val Ser Trp Thr Met Lys
                725                 730                 735


Ile Gly Ile Gly Ile Leu Leu Thr Trp Leu Gly Leu Asn Ser Arg Asn
            740                 745                 750


Thr Ser Leu Ser Met Met Cys Ile Ala Val Gly Met Val Thr Leu Tyr
        755                 760                 765


Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Ile Asn Trp Lys
    770                 775                 780


Gly Arg Glu Leu Lys Cys Gly Ser Gly Ile Phe Val Thr Asn Glu Val
785                 790                 795                 800


His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Ala Asp Ser Pro Lys Arg
                805                 810                 815


Leu Ser Ala Ala Ile Gly Lys Ala Trp Glu Glu Gly Val Cys Gly Ile
            820                 825                 830


Arg Ser Ala Thr Arg Leu Glu Asn Ile Met Trp Lys Gln Ile Ser Asn
```

835 840 845

Glu Leu Asn His Ile Leu Leu Glu Asn Asp Met Lys Phe Thr Val Val
850 855 860

Val Gly Asp Val Ser Gly Ile Leu Ala Gln Gly Lys Lys Met Ile Arg
865 870 875 880

Pro Gln Pro Met Glu His Lys Tyr Ser Trp Lys Ser Trp Gly Lys Ala
885 890 895

Lys Ile Ile Gly Ala Asp Val Gln Asn Thr Thr Phe Ile Ile Asp Gly
900 905 910

Pro Asn Thr Pro Glu Cys Pro Asp Asn Gln Arg Ala Trp Asn Ile Trp
915 920 925

Glu Val Glu Asp Tyr Gly Phe Gly Ile Phe Thr Thr Asn Ile Trp Leu
930 935 940

Lys Leu Arg Asp Ser Tyr Thr Gln Val Cys Asp His Arg Leu Met Ser
945 950 955 960

Ala Ala Ile Lys Asp Ser Lys Ala Val His Ala Asp Met Gly Tyr Trp
965 970 975

Ile Glu Ser Glu Lys Asn Glu Thr Trp Lys Leu Ala Arg Ala Ser Phe
980 985 990

Ile Glu Val Lys Thr Cys Ile Trp Pro Lys Ser His Thr Leu Trp Ser
995 1000 1005

Asn Gly Val Leu Glu Ser Glu Met Ile Ile Pro Lys Ile Tyr Gly
1010 1015 1020

Gly Pro Ile Ser Gln His Asn Tyr Arg Pro Gly Tyr Phe Thr Gln
1025 1030 1035

Thr Ala Gly Pro Trp His Leu Gly Lys Leu Glu Leu Asp Phe Asp
1040 1045 1050

Phe Cys Glu Gly Thr Thr Val Val Val Asp Glu His Cys Gly Asn
1055 1060 1065

Arg Gly Pro Ser Leu Arg Thr Thr Thr Val Thr Gly Lys Ile Ile
1070 1075 1080

His Glu Trp Cys Cys Arg Ser Cys Thr Leu Pro Pro Leu Arg Phe
1085 1090 1095

Lys Gly Glu Asp Gly Cys Trp Tyr Gly Met Glu Ile Arg Pro Val
1100 1105 1110

```
Lys Asp  Lys Glu Glu Asn Leu  Val Lys Ser Met Val  Ser Ala Gly
    1115                1120                1125

Ser Gly  Glu Val Asp Ser Phe  Ser Leu Gly Leu Leu  Cys Ile Ser
    1130                1135                1140

Ile Met  Ile Glu Glu Val Met  Arg Ser Arg Trp Ser  Lys Lys Met
    1145                1150                1155

Leu Met  Thr Gly Thr Leu Ala  Val Phe Leu Leu Leu  Ile Met Gly
    1160                1165                1170

Gln Leu  Thr Trp Ser Asp Leu  Ile Arg Leu Cys Ile  Met Val Gly
    1175                1180                1185

Ala Asn  Ala Ser Asp Lys Met  Gly Met Gly Thr Thr  Tyr Leu Ala
    1190                1195                1200

Leu Met  Ala Thr Phe Lys Met  Arg Pro Met Phe Ala  Val Gly Leu
    1205                1210                1215

Leu Phe  Arg Arg Leu Thr Ser  Arg Glu Val Leu Leu  Leu Thr Ile
    1220                1225                1230

Gly Leu  Ser Leu Val Ala Ser  Val Glu Leu Pro Ser  Ser Leu Glu
    1235                1240                1245

Glu Leu  Gly Asp Gly Leu Ala  Ile Gly Ile Met Met  Leu Lys Leu
    1250                1255                1260

Leu Thr  Asp Phe Gln Ser His  Gln Leu Trp Ala Thr  Leu Leu Ser
    1265                1270                1275

Leu Thr  Phe Ile Lys Thr Thr  Phe Ser Leu His Tyr  Ala Trp Lys
    1280                1285                1290

Thr Met  Ala Met Val Leu Ser  Ile Val Ser Leu Phe  Pro Leu Cys
    1295                1300                1305

Leu Ser  Thr Thr Ser Gln Lys  Thr Thr Trp Leu Pro  Val Leu Leu
    1310                1315                1320

Gly Ser  Leu Gly Cys Lys Pro  Leu Pro Met Phe Leu  Ile Thr Glu
    1325                1330                1335

Asn Lys  Ile Trp Gly Arg Lys  Ser Trp Pro Leu Asn  Glu Gly Ile
    1340                1345                1350

Met Ala  Val Gly Ile Val Ser  Ile Leu Leu Ser Ser  Leu Leu Lys
    1355                1360                1365
```

```
Asn Asp  Val Pro Leu Ala Gly  Pro Leu Ile Ala Gly  Gly Met Leu
    1370                 1375                 1380

Ile Ala  Cys Tyr Val Ile Ser  Gly Ser Ser Ala Asp  Leu Ser Leu
    1385                 1390                 1395

Glu Lys  Ala Ala Glu Val Ser  Trp Glu Glu Glu Ala  Glu His Ser
    1400                 1405                 1410

Gly Ala  Ser His Asn Ile Leu  Val Glu Val Gln Asp  Asp Gly Thr
    1415                 1420                 1425

Met Lys  Ile Lys Asp Glu Glu  Arg Asp Asp Thr Leu  Thr Ile Leu
    1430                 1435                 1440

Leu Lys  Ala Thr Leu Leu Ala  Val Ser Gly Val Tyr  Pro Met Ser
    1445                 1450                 1455

Ile Pro  Ala Thr Leu Phe Val  Trp Tyr Phe Trp Gln  Lys Lys Lys
    1460                 1465                 1470

Gln Arg  Ser Gly Val Leu Trp  Asp Thr Pro Ser Pro  Pro Glu Val
    1475                 1480                 1485

Glu Arg  Ala Val Leu Asp Asp  Gly Ile Tyr Arg Ile  Leu Gln Arg
    1490                 1495                 1500

Gly Leu  Leu Gly Arg Ser Gln  Val Gly Val Gly Val  Phe Gln Glu
    1505                 1510                 1515

Gly Val  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                 1525                 1530

Met Tyr  Gln Gly Lys Arg Leu  Glu Pro Ser Trp Ala  Ser Val Lys
    1535                 1540                 1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Arg Phe  Gln Gly Ser
    1550                 1555                 1560

Trp Asn  Thr Gly Glu Glu Val  Gln Val Ile Ala Val  Glu Pro Gly
    1565                 1570                 1575

Lys Asn  Pro Lys Asn Val Gln  Thr Thr Pro Gly Thr  Phe Lys Thr
    1580                 1585                 1590

Pro Glu  Gly Glu Val Gly Ala  Ile Ala Leu Asp Phe  Lys Pro Gly
    1595                 1600                 1605

Thr Ser  Gly Ser Pro Ile Val  Asn Arg Glu Gly Lys  Ile Val Gly
    1610                 1615                 1620
```

```
Leu Tyr  Gly Asn Gly Val Val  Thr Thr Ser Gly Thr  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Ala Lys Ala  Ser Gln Glu Gly Pro  Leu Pro Glu
    1640                1645                1650

Ile Glu  Asp Glu Val Phe Arg  Lys Arg Asn Leu Thr  Ile Met Asp
    1655                1660                1665

Leu His  Pro Gly Ser Gly Lys  Thr Arg Arg Tyr Leu  Pro Ala Ile
    1670                1675                1680

Val Arg  Glu Ala Ile Lys Arg  Lys Leu Arg Thr Leu  Ile Leu Ala
    1685                1690                1695

Pro Thr  Arg Val Val Ala Ser  Glu Met Ala Glu Ala  Leu Lys Gly
    1700                1705                1710

Val Pro  Ile Arg Tyr Gln Thr  Thr Ala Val Lys Ser  Glu His Thr
    1715                1720                1725

Gly Lys  Glu Ile Val Asp Leu  Met Cys His Ala Thr  Phe Thr Met
    1730                1735                1740

Arg Leu  Leu Ser Pro Val Arg  Val Pro Asn Tyr Asn  Met Ile Ile
    1745                1750                1755

Met Asp  Glu Ala His Phe Thr  Asp Pro Ala Ser Ile  Ala Ala Arg
    1760                1765                1770

Gly Tyr  Ile Ser Thr Arg Val  Gly Met Gly Glu Ala  Ala Ala Ile
    1775                1780                1785

Phe Met  Thr Ala Thr Pro Pro  Gly Ser Val Glu Ala  Phe Pro Gln
    1790                1795                1800

Ser Asn  Ala Ile Ile Gln Asp  Glu Glu Arg Asp Ile  Pro Glu Arg
    1805                1810                1815

Ser Trp  Asn Ser Gly Tyr Asp  Trp Ile Thr Asp Phe  Pro Gly Lys
    1820                1825                1830

Thr Val  Trp Phe Val Pro Ser  Ile Lys Ser Gly Asn  Asp Ile Ala
    1835                1840                1845

Asn Cys  Leu Arg Lys Asn Gly  Lys Arg Val Ile Gln  Leu Ser Arg
    1850                1855                1860

Lys Thr  Phe Asp Thr Glu Tyr  Gln Lys Thr Lys Asn  Asn Asp Trp
    1865                1870                1875

Asp Tyr  Val Val Thr Thr Asp  Ile Ser Glu Met Gly  Ala Asn Phe
```

1880 1885 1890

Arg Ala Asp Arg Val Ile Asp Pro Arg Arg Cys Leu Lys Pro Val
1895 1900 1905

Ile Leu Lys Asp Gly Pro Glu Arg Val Ile Leu Ala Gly Pro Met
1910 1915 1920

Pro Val Thr Val Ala Ser Ala Ala Gln Arg Arg Gly Arg Ile Gly
1925 1930 1935

Arg Asn Gln Asn Lys Glu Gly Asp Gln Tyr Ile Tyr Met Gly Gln
1940 1945 1950

Pro Leu Asn Asn Asp Glu Asp His Ala His Trp Thr Glu Ala Lys
1955 1960 1965

Met Leu Leu Asp Asn Ile Asn Thr Pro Glu Gly Ile Ile Pro Ala
1970 1975 1980

Leu Tyr Glu Pro Glu Arg Glu Lys Ser Ala Ala Ile Asp Gly Glu
1985 1990 1995

Tyr Arg Leu Arg Gly Glu Ala Arg Lys Thr Phe Val Glu Leu Met
2000 2005 2010

Arg Arg Gly Asp Leu Pro Val Trp Leu Ser Tyr Lys Val Ala Ser
2015 2020 2025

Glu Gly Phe Gln Tyr Ser Asp Arg Arg Trp Cys Phe Asp Gly Glu
2030 2035 2040

Arg Asn Asn Gln Val Leu Glu Glu Asn Met Asp Val Glu Ile Trp
2045 2050 2055

Thr Lys Glu Gly Glu Arg Lys Lys Leu Arg Pro Arg Trp Leu Asp
2060 2065 2070

Ala Arg Thr Tyr Ser Asp Pro Leu Ala Leu Arg Glu Phe Lys Glu
2075 2080 2085

Phe Ala Ala Gly Arg Arg Ser Val Ser Gly Asp Leu Ile Leu Glu
2090 2095 2100

Ile Gly Lys Leu Pro Gln His Leu Thr Gln Arg Ala Gln Asn Ala
2105 2110 2115

Leu Asp Asn Leu Val Met Leu His Asn Ser Glu Gln Gly Gly Lys
2120 2125 2130

Ala Tyr Arg His Ala Met Glu Glu Leu Pro Asp Thr Ile Glu Thr
2135 2140 2145

```
Leu Met  Leu Leu Ala Leu Ile  Ala Val Leu Thr Gly  Gly Val Thr
    2150                 2155                 2160

Leu Phe  Phe Leu Ser Gly Arg  Gly Leu Gly Lys Thr  Ser Ile Gly
    2165                 2170                 2175

Leu Leu  Cys Val Met Ala Ser  Ser Ala Leu Leu Trp  Met Ala Ser
    2180                 2185                 2190

Val Glu  Pro His Trp Ile Ala  Ala Ser Ile Ile Leu  Glu Phe Phe
    2195                 2200                 2205

Leu Met  Val Leu Leu Ile Pro  Glu Pro Asp Arg Gln  Arg Thr Pro
    2210                 2215                 2220

Gln Asp  Asn Gln Leu Ala Tyr  Val Val Ile Gly Leu  Leu Phe Met
    2225                 2230                 2235

Ile Leu  Thr Val Ala Ala Asn  Glu Met Gly Leu Leu  Glu Thr Thr
    2240                 2245                 2250

Lys Lys  Asp Leu Gly Ile Gly  His Val Ala Ala Glu  Asn His His
    2255                 2260                 2265

His Ala  Thr Met Leu Asp Val  Asp Leu His Pro Ala  Ser Ala Trp
    2270                 2275                 2280

Thr Leu  Tyr Ala Val Ala Thr  Thr Ile Ile Thr Pro  Met Met Arg
    2285                 2290                 2295

His Thr  Ile Glu Asn Thr Thr  Ala Asn Ile Ser Leu  Thr Ala Ile
    2300                 2305                 2310

Ala Asn  Gln Ala Ala Ile Leu  Met Gly Leu Asp Lys  Gly Trp Pro
    2315                 2320                 2325

Ile Ser  Lys Met Asp Ile Gly  Val Pro Leu Leu Ala  Leu Gly Cys
    2330                 2335                 2340

Tyr Ser  Gln Val Asn Pro Leu  Thr Leu Ile Ala Ala  Val Leu Met
    2345                 2350                 2355

Leu Val  Ala His Tyr Ala Ile  Ile Gly Pro Gly Leu  Gln Ala Lys
    2360                 2365                 2370

Ala Thr  Arg Glu Ala Gln Lys  Arg Thr Ala Ala Gly  Ile Met Lys
    2375                 2380                 2385

Asn Pro  Thr Val Asp Gly Ile  Val Ala Ile Asp Leu  Asp Pro Val
    2390                 2395                 2400
```

```
Val Tyr  Asp Ala Lys Phe Glu  Lys Gln Leu Gly Gln  Ile Met Leu
    2405                 2410                 2415

Leu Ile  Leu Cys Thr Ser Gln  Ile Leu Leu Met Arg  Thr Thr Trp
    2420                 2425                 2430

Ala Leu  Cys Glu Ser Ile Thr  Leu Ala Thr Gly Pro  Leu Thr Thr
    2435                 2440                 2445

Leu Trp  Glu Gly Ser Pro Gly  Lys Phe Trp Asn Thr  Thr Ile Ala
    2450                 2455                 2460

Val Ser  Met Ala Asn Ile Phe  Arg Gly Ser Tyr Leu  Ala Gly Ala
    2465                 2470                 2475

Gly Leu  Ala Phe Ser Leu Met  Lys Ser Leu Gly Gly  Gly Arg Arg
    2480                 2485                 2490

Gly Thr  Gly Ala Gln Gly Glu  Thr Leu Gly Glu Lys  Trp Lys Arg
    2495                 2500                 2505

Gln Leu  Asn Gln Leu Ser Lys  Ser Glu Phe Asn Thr  Tyr Lys Arg
    2510                 2515                 2520

Ser Gly  Ile Met Glu Val Asp  Arg Ser Glu Ala Lys  Glu Gly Leu
    2525                 2530                 2535

Lys Arg  Gly Glu Thr Thr Lys  His Ala Val Ser Arg  Gly Thr Ala
    2540                 2545                 2550

Lys Leu  Arg Trp Phe Val Glu  Arg Asn Leu Val Lys  Pro Glu Gly
    2555                 2560                 2565

Lys Val  Ile Asp Leu Gly Cys  Gly Arg Gly Gly Trp  Ser Tyr Tyr
    2570                 2575                 2580

Cys Ala  Gly Leu Lys Lys Val  Thr Glu Val Lys Gly  Tyr Thr Lys
    2585                 2590                 2595

Gly Gly  Pro Gly His Glu Glu  Pro Ile Pro Met Ala  Thr Tyr Gly
    2600                 2605                 2610

Trp Asn  Leu Val Lys Leu His  Ser Gly Lys Asp Val  Phe Phe Ile
    2615                 2620                 2625

Pro Pro  Glu Lys Cys Asp Thr  Leu Leu Cys Asp Ile  Gly Glu Ser
    2630                 2635                 2640

Ser Pro  Asn Pro Thr Ile Glu  Glu Gly Arg Thr Leu  Arg Val Leu
    2645                 2650                 2655
```

```
Lys Met  Val Glu Pro Trp Leu  Arg Gly Asn Gln Phe  Cys Ile Lys
    2660                 2665                 2670


Ile Leu  Asn Pro Tyr Met Pro  Ser Val Val Glu Thr  Leu Glu Gln
    2675                 2680                 2685


Met Gln  Arg Lys His Gly Gly  Met Leu Val Arg Asn  Pro Leu Ser
    2690                 2695                 2700


Arg Asn  Ser Thr His Glu Met  Tyr Trp Val Ser Cys  Gly Thr Gly
    2705                 2710                 2715


Asn Ile  Val Ser Ala Val Asn  Met Thr Ser Arg Met  Leu Leu Asn
    2720                 2725                 2730


Arg Phe  Thr Met Ala His Arg  Lys Pro Thr Tyr Glu  Arg Asp Val
    2735                 2740                 2745


Asp Leu  Gly Ala Gly Thr Arg  His Val Ala Val Glu  Pro Glu Val
    2750                 2755                 2760


Ala Asn  Leu Asp Ile Ile Gly  Gln Arg Ile Glu Asn  Ile Lys Asn
    2765                 2770                 2775


Glu His  Lys Ser Thr Trp His  Tyr Asp Glu Asp Asn  Pro Tyr Lys
    2780                 2785                 2790


Thr Trp  Ala Tyr His Gly Ser  Tyr Glu Val Lys Pro  Ser Gly Ser
    2795                 2800                 2805


Ala Ser  Ser Met Val Asn Gly  Val Val Arg Leu Leu  Thr Lys Pro
    2810                 2815                 2820


Trp Asp  Val Ile Pro Met Val  Thr Gln Ile Ala Met  Thr Asp Thr
    2825                 2830                 2835


Thr Pro  Phe Gly Gln Gln Arg  Val Phe Lys Glu Lys  Val Asp Thr
    2840                 2845                 2850


Arg Thr  Pro Lys Ala Lys Arg  Gly Thr Ala Gln Ile  Met Glu Val
    2855                 2860                 2865


Thr Ala  Arg Trp Leu Trp Gly  Phe Leu Ser Arg Asn  Lys Lys Pro
    2870                 2875                 2880


Arg Ile  Cys Thr Arg Glu Glu  Phe Thr Arg Lys Val  Arg Ser Asn
    2885                 2890                 2895


Ala Ala  Ile Gly Ala Val Phe  Val Asp Glu Asn Gln  Trp Asn Ser
    2900                 2905                 2910


Ala Lys  Glu Ala Val Glu Asp  Glu Arg Phe Trp Glu  Leu Val His
```

```
     2915                2920                2925

 Arg Glu  Arg Glu Leu His Lys  Gln Gly Lys Cys Ala  Thr Cys Val
     2930                2935                2940

 Tyr Asn  Met Met Gly Lys Arg  Glu Lys Lys Leu Gly  Glu Phe Gly
     2945                2950                2955

 Lys Ala  Lys Gly Ser Arg Ala  Ile Trp Tyr Met Trp  Leu Gly Ala
     2960                2965                2970

 Arg Phe  Leu Glu Phe Glu Ala  Leu Gly Phe Met Asn  Glu Asp His
     2975                2980                2985

 Trp Phe  Ser Arg Glu Asn Ser  Leu Ser Gly Val Glu  Gly Glu Gly
     2990                2995                3000

 Leu His  Lys Leu Gly Tyr Ile  Leu Arg Asp Ile Ser  Arg Ile Pro
     3005                3010                3015

 Gly Gly  Asn Met Tyr Ala Asp  Asp Thr Ala Gly Trp  Asp Thr Arg
     3020                3025                3030

 Ile Thr  Glu Asp Asp Leu Gln  Asn Glu Ala Lys Ile  Thr Asp Ile
     3035                3040                3045

 Met Glu  Pro Glu His Ala Leu  Leu Ala Thr Ser Ile  Phe Lys Leu
     3050                3055                3060

 Thr Tyr  Gln Asn Lys Val Val  Arg Val Gln Arg Pro  Ala Lys Asn
     3065                3070                3075

 Gly Thr  Val Met Asp Val Ile  Ser Arg Arg Asp Gln  Arg Gly Ser
     3080                3085                3090

 Gly Gln  Val Gly Thr Tyr Gly  Leu Asn Thr Phe Thr  Asn Met Glu
     3095                3100                3105

 Ala Gln  Leu Ile Arg Gln Met  Glu Ser Glu Gly Ile  Phe Leu Pro
     3110                3115                3120

 Ser Glu  Leu Glu Thr Pro Asn  Leu Ala Gly Arg Val  Leu Asp Trp
     3125                3130                3135

 Leu Glu  Lys Tyr Gly Val Glu  Arg Leu Lys Arg Met  Ala Ile Ser
     3140                3145                3150

 Gly Asp  Asp Cys Val Val Lys  Pro Ile Asp Asp Arg  Phe Ala Thr
     3155                3160                3165

 Ala Leu  Thr Ala Leu Asn Asp  Met Gly Lys Val Arg  Lys Asp Ile
     3170                3175                3180
```

```
Pro Gln  Trp Glu Pro Ser Lys  Gly Trp Asn Asp Trp  Gln Gln Val
    3185                 3190                 3195

Pro Phe  Cys Ser His His Phe  His Gln Leu Ile Met  Lys Asp Gly
    3200                 3205                 3210

Arg Glu  Ile Val Val Pro Cys  Arg Asn Gln Asp Glu  Leu Val Gly
    3215                 3220                 3225

Arg Ala  Arg Val Ser Gln Gly  Ala Gly Trp Ser Leu  Arg Glu Thr
    3230                 3235                 3240

Ala Cys  Leu Gly Lys Ser Tyr  Ala Gln Met Trp Gln  Leu Met Tyr
    3245                 3250                 3255

Phe His  Arg Arg Asp Leu Arg  Leu Ala Ala Asn Ala  Ile Cys Ser
    3260                 3265                 3270

Ala Val  Pro Val Asp Trp Val  Pro Thr Ser Arg Thr  Thr Trp Ser
    3275                 3280                 3285

Ile His  Ala His His Gln Trp  Met Thr Thr Glu Asp  Met Leu Ser
    3290                 3295                 3300

Val Trp  Asn Arg Val Trp Ile  Glu Glu Asn Pro Trp  Met Glu Asp
    3305                 3310                 3315

Lys Thr  His Val Ser Ser Trp  Glu Glu Val Pro Tyr  Leu Gly Lys
    3320                 3325                 3330

Arg Glu  Asp Gln Trp Cys Gly  Ser Leu Ile Gly Leu  Thr Ala Arg
    3335                 3340                 3345

Ala Thr  Trp Ala Thr Asn Ile  Gln Val Ala Ile Asn  Gln Val Arg
    3350                 3355                 3360

Arg Leu  Ile Gly Asn Glu Asn  Tyr Leu Asp Tyr Met  Thr Ser Met
    3365                 3370                 3375

Lys Arg  Phe Lys Asn Glu Ser  Asp Pro Glu Gly Ala  Leu Trp
    3380                 3385                 3390
```

<210> 11
<211> 10723
<212> DNA
<213> dengue virus

<400> 11

```
agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaacgta       60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg      120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag      180
```

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acacgtaacg gagaaccaca catgatcgtc 480

agcagacaag agaaagggaa aagtcttctg tttaaaacag aggatggcgt gaacatgtgt 540

accctcatgg ccatggacct tggtgaattg tgtgaagaca caatcacgta caagtgtccc 600

cttctcaggc agaatgagcc agaagacata gactgttggt gcaactctac gtccacgtgg 660

gtaacttatg ggacgtgtac caccatggga gaacatagaa gagaaaaaag atcagtggca 720

ctcgttccac atgtgggaat gggactggag acacgaactg aaacatggat gtcatcagaa 780

ggggcctgga aacatgtcca gagaattgaa acttggatct tgagacatcc aggcttcacc 840

atgatggcag caatcctggc atacaccata ggaacgacac atttccaaag agccctgatt 900

ttcatcttac tgacagctgt cactccttca atgacaatgc gttgcatagg aatgtcaaat 960

agagactttg tggaaggggt ttcaggagga agctgggttg acatagtctt agaacatgga 1020

agctgtgtga cgacgatggc aaaaaacaaa ccaacattgg attttgaact gataaaaaca 1080

gaagccaaac agcctgccac cctaaggaag tactgtatag aggcaaagct aaccaacaca 1140

acaacagaat ctcgctgccc aacacaaggg gaacccagcc taaatgaaga gcaggacaaa 1200

aggttcgtct gcaaacactc catggtagac agaggatggg gaaatggatg tggactattt 1260

ggaaagggag gcattgtgac ctgtgctatg ttcagatgca aaaagaacat ggaaggaaaa 1320

gttgtgcaac cagaaaactt ggaatacacc attgtgataa cacctcactc aggggaagag 1380

catgcagtcg gaaatgacac aggaaaacat ggcaaggaaa tcaaaataac accacagagt 1440

tccatcacag aagcagaatt gacaggttat ggcactgtca caatggagtg ctctccaaga 1500

acgggcctcg acttcaatga gatggtgttg ctgcagatgg aaaataaagc ttggctggtg 1560

cacaggcaat ggttcctaga cctgccgtta ccatggttgc ccggagcgga cacacaaggg 1620

tcaaattgga tacagaaaga gacattggtc actttcaaaa atccccatgc gaagaaacag 1680

gatgttgttg ttttaggatc ccaagaaggg gccatgcaca cagcacttac aggggccaca 1740

gaaatccaaa tgtcatcagg aaacttactc ttcacaggac atctcaagtg caggctgaga 1800

atggacaagc tacagctcaa aggaatgtca tactctatgt gcacaggaaa gtttaaagtt 1860

gtgaaggaaa tagcagaaac acaacatgga acaatagtta tcagagtgca atatgaaggg 1920

gacggctctc catgcaagat ccctttgag ataatggatt tggaaaaaag acatgtctta 1980

ggtcgcctga ttacagtcaa cccaattgtg acagaaaaag atagcccagt caacatagaa 2040

gcagaacctc cattcggaga cagctacatc atcataggag tagagccggg acaactgaag 2100

ctcaactggt ttaagaaagg aagttctatc ggccaaatgt ttgagacaac aatgaggggg 2160

gcgaagagaa tggccatttt aggtgacaca gcctgggatt ttggatcctt gggaggagtg 2220

tttacatcta taggaaaggc tctccaccaa gtctttggag caatctatgg agctgccttc 2280

agtggggttt catggactat gaaaatcctc ataggagtca ttatcacatg gataggaatg 2340

aattcacgca gcacctcact gtctgtgaca ctagtattgg tgggaattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagagggc 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgctc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctga gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctcccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgttcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgga 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag ctggagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> 12
<211> 3391
<212> PRT
<213> dengue virus

<400> 12

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val
115 120 125

Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Asp Gly
130 135 140

Val Asn Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu
145 150 155 160

Asp Thr Ile Thr Tyr Lys Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu
165 170 175

Asp Ile Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly
180 185 190

Thr Cys Thr Thr Met Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala
195 200 205

Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp

210 215 220

Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp
225 230 235 240

Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile Leu Ala Tyr
245 250 255

Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu
260 265 270

Thr Ala Val Thr Pro Ser Met Thr Met Arg Cys Ile Gly Met Ser Asn
275 280 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val
290 295 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305 310 315 320

Leu Asp Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu
325 330 335

Arg Lys Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser
340 345 350

Arg Cys Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys
355 360 365

Arg Phe Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly
370 375 380

Cys Gly Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Arg
385 390 395 400

Cys Lys Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu
405 410 415

Tyr Thr Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly
420 425 430

Asn Asp Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser
435 440 445

Ser Ile Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu
450 455 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln
465 470 475 480

Met Glu Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu
485 490 495

```
Pro Leu Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile
            500                 505                 510

Gln Lys Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln
        515                 520                 525

Asp Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr
545                 550                 555                 560

Gly His Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly
                565                 570                 575

Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly
        595                 600                 605

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys
    610                 615                 620

Arg His Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu
625                 630                 635                 640

Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser
                645                 650                 655

Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe
            660                 665                 670

Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly
        675                 680                 685

Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Leu Gly Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe
705                 710                 715                 720

Gly Ala Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys
                725                 730                 735

Ile Leu Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser
            740                 745                 750

Thr Ser Leu Ser Val Thr Leu Val Leu Val Gly Ile Val Thr Leu Tyr
        755                 760                 765
```

```
Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Gly Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035
```

```
Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Asp Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Lys Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                1240                1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                1255                1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                1270                1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                1285                1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Leu
```

```
    1295                1300                1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                1315                1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                1330                1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                1345                1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                1360                1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                1375                1380

Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                1390                1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                1405                1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                1420                1425

Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
    1430                1435                1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                1450                1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                1465                1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                1480                1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                1495                1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                1510                1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                1525                1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                1540                1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                1555                1560
```

```
Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                1570                1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                1585                1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                1615                1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                1690                1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Glu  His Thr Gly
    1715                1720                1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815
```

```
Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                 1825                 1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                 1840                 1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                 1855                 1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                 1870                 1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                 1885                 1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                 1900                 1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                 1915                 1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                 1930                 1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                 1945                 1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                 1960                 1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                 1975                 1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                 1990                 1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                 2005                 2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                 2020                 2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                 2035                 2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                 2050                 2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                 2065                 2070
```

```
Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                 2080                 2085


Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                 2095                 2100


Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                 2110                 2115


Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                 2125                 2130


Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                 2140                 2145


Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                 2155                 2160


Phe Leu  Met Ser Gly Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                 2170                 2175


Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                 2185                 2190


Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                 2200                 2205


Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                 2215                 2220


Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                 2230                 2235


Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                 2245                 2250


Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                 2260                 2265


Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                 2275                 2280


Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                 2290                 2295


Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                 2305                 2310


Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                 2320                 2325


Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
```

2330 2335 2340

Gln Val Asn Pro Ile Thr Leu Thr Ala Ala Leu Phe Leu Leu Val
2345 2350 2355

Ala His Tyr Ala Ile Ile Gly Pro Gly Leu Gln Ala Lys Ala Thr
2360 2365 2370

Arg Glu Ala Gln Lys Arg Ala Ala Ala Gly Ile Met Lys Asn Pro
2375 2380 2385

Thr Val Asp Gly Ile Thr Val Ile Asp Leu Asp Pro Ile Pro Tyr
2390 2395 2400

Asp Pro Lys Phe Glu Lys Gln Leu Gly Gln Val Met Leu Leu Val
2405 2410 2415

Leu Cys Val Thr Gln Val Leu Met Met Arg Thr Thr Trp Ala Leu
2420 2425 2430

Cys Glu Ala Leu Thr Leu Ala Thr Gly Pro Ile Ser Thr Leu Trp
2435 2440 2445

Glu Gly Asn Pro Gly Arg Phe Trp Asn Thr Thr Ile Ala Val Ser
2450 2455 2460

Met Ala Asn Ile Phe Arg Gly Ser Tyr Leu Ala Gly Ala Gly Leu
2465 2470 2475

Leu Phe Ser Ile Met Lys Asn Thr Thr Asn Thr Arg Arg Gly Thr
2480 2485 2490

Gly Asn Ile Gly Glu Thr Leu Gly Glu Lys Trp Lys Ser Arg Leu
2495 2500 2505

Asn Ala Leu Gly Lys Ser Glu Phe Gln Ile Tyr Lys Lys Ser Gly
2510 2515 2520

Ile Gln Glu Val Asp Arg Thr Leu Ala Lys Glu Gly Ile Lys Arg
2525 2530 2535

Gly Glu Thr Asp His His Ala Val Ser Arg Gly Ser Ala Lys Leu
2540 2545 2550

Arg Trp Phe Val Glu Arg Asn Met Val Thr Pro Glu Gly Lys Val
2555 2560 2565

Val Asp Leu Gly Cys Gly Arg Gly Gly Trp Ser Tyr Tyr Cys Gly
2570 2575 2580

Gly Leu Lys Asn Val Arg Glu Val Lys Gly Leu Thr Lys Gly Gly
2585 2590 2595

```
Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                 2605                 2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                 2620                 2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                 2635                 2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                 2650                 2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                 2665                 2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                 2680                 2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                 2695                 2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                 2710                 2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                 2725                 2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                 2740                 2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                 2755                 2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                 2770                 2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                 2785                 2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                 2800                 2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                 2815                 2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                 2830                 2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                 2845                 2850
```

```
Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                 2860                 2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                 2875                 2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                 2890                 2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                 2905                 2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                 2920                 2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                 2935                 2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                 2950                 2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                 2965                 2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                 2980                 2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                 2995                 3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                 3010                 3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                 3025                 3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                 3040                 3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                 3055                 3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                 3070                 3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                 3085                 3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                 3100                 3105
```

```
Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120


Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135


Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                 3145                 3150


Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                 3160                 3165


Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                 3175                 3180


Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                 3190                 3195


Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                 3205                 3210


Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                 3220                 3225


Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                 3235                 3240


Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                 3250                 3255


His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                 3265                 3270


Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                 3280                 3285


His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                 3295                 3300


Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                 3310                 3315


Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                 3325                 3330


Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                 3340                 3345


Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                 3355                 3360


Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
```

3365 3370 3375

Arg Phe Arg Arg Glu Glu Glu Glu Ala Gly Val Leu Trp
3380 3385 3390

<210> 13
<211> 10696
<212> DNA
<213> dengue virus

<400> 13
agttgttagt ctacgtggac cgacaagaac agtttcgact cggaagcttg cttaacgtag 60

tgctgacagt tttttattag agagcagatc tctgatgaac aaccaacgga aaaagacggg 120

aaaaccgtct atcaatatgc tgaaacgcgt gagaaaccgt gtgtcaactg gatcacagtt 180

ggcgaagaga ttctcaagag gattgctgaa cggccaagga ccaatgaaat tggttatggc 240

atttatagct ttcctcagat ttctagccat tccaccgaca gcaggagtct tggctagatg 300

gggtaccttt aagaagtcgg gggctattaa ggtcttaaaa ggcttcaaga aggagatctc 360

aaacatgctg agcattatca acaaacggaa aaagacatcg ctctgtctca tgatgatgtt 420

accagcaaca cttgctttcc acttaacttc acgagatgga gagccgcgca tgattgtggg 480

gaagaatgaa agaggaaaat ccctactttt caagacagcc tctggaatca acatgtgcac 540

actcatagcc atggatctgg gagagatgtg tgatgacacg gtcacttaca aatgccccca 600

cattaccgaa gtggagcctg aagacattga ctgctggtgc aaccttacat cgacatgggt 660

gacttatgga acatgcaatc aagctggaga gcatagacgc gataagagat cagtggcgtt 720

agctccccat gttggcatgg gactggacac acgcactcaa acctggatgt cggctgaagg 780

agcttggaga caagtcgaga aggtagagac atgggccctt aggcacccag ggtttaccat 840

actagcccta tttcttgccc attacatagg cacttccttg acccagaaag tggttatttt 900

tatactatta atgctggtta ccccatccat gacaatgaga tgtgtaggag taggaaacag 960

agattttgtg gaaggcctat cgggagctac gtgggttgac gtggtgctcg agcacggtgg 1020

gtgtgtgact accatggcta agaacaagcc cacgctggac atagagcttc agaagaccga 1080

ggccacccaa ctggcgaccc taaggaagct atgcattgag ggaaaaatta ccaacataac 1140

aaccgactca agatgtccca cccaagggga agcgatttta cctgaggagc aggaccagaa 1200

ctacgtgtgt aagcatacat acgtggacag aggctgggga aacggttgtg gtttgtttgg 1260

caagggaagc ttggtgacat gcgcgaaatt tcaatgttta gaatcaatag agggaaaagt 1320

ggtgcaacat gagaacctca aatacaccgt catcatcaca gtgcacacag gagaccaaca 1380

ccaggtggga aatgaaacgc agggagtcac ggctgagata acaccccagg catcaaccgc 1440

tgaagtcatt ttacctgaat atggaaccct cgggctagaa tgctcaccac ggacaggttt 1500

ggatttcaat gaaatgatct cattgacaat gaagaacaaa gcatggatgg tacatagaca 1560

atggttcttt gacttacccc taccatggac atcaggagct acagcagaaa caccaacttg 1620

gaacaggaaa gagcttcttg tgacatttaa aaatgcacat gcaaaaaagc aagaagtagt 1680

tgttcttgga tcacaagagg gagcaatgca tacagcactg acaggagcta cagagatcca 1740

aacctcagga ggcacaagta tctttgcggg gcacttaaaa tgtagactca agatggacaa 1800

attggaactc aaggggatga gctatgcaat gtgcttgagt agctttgtgt tgaagaaaga 1860

agtctccgaa acgcagcatg ggacaatact cattaaggtt gagtacaaag gggaagatgc 1920

accctgcaag attcctttct ccacggagga tggacaagga aaagctcaca atggcagact 1980

gatcacagcc aatccagtgg tgaccaagaa ggaggagcct gtcaacattg aggctgaacc 2040

tccttttgga gaaagtaaca tagtaattgg aattggagac aaagccctga aaatcaactg 2100

gtacaagaag ggaagctcga ttgggaagat gttcgaggcc actgccagag gtgcaaggcg 2160

catggccatc ttgggagaca cagcctggga ctttggatca gtgggtggtg ttttgaattc 2220

attagggaaa atggtccacc aaatatttgg gagtgcttac acagccctat ttggtggagt 2280

ctcctggatg atgaaaattg gaataggtgt cctcttaacc tggatagggt tgaactcaaa 2340

aaatacttct atgtcatttt catgcatcgc gataggaatc attacactct atctgggagc 2400

cgtggtgcaa gctgacatgg ggtgtgtcat aaactggaaa ggcaaagaac tcaaatgtgg 2460

aagtggaatt ttcgtcacta atgaggtcca cacctggaca gagcaataca aatttcaagc 2520

agactccccc aagagactgg caacagccat tgcaggcgct tgggaaaatg gagtgtgcgg 2580

aattaggtca acaaccagaa tggagaacct cttgtggaag caaatagcca atgaactgaa 2640

ttacatatta tgggaaaaca acattaaatt aacggtagtt gtaggcgaca taactggggt 2700

cttagagcaa gggaaaagaa cactaacacc acaacccatg gagctaaaat attcttggaa 2760

aacatgggga aaggcaaaaa tagtgacagc tgaaacacaa aattcctctt tcataataga 2820

tgggccaagc acaccggagt gtccaagtgc ctcaagagca tggaatgtgt gggaggtgga 2880

ggattacggg ttcggagttt tcacaaccaa catatggctg aaactccgag aggtgtacac 2940

ccaactatgt gaccataggc taatgtcggc agccgtcaag gatgagaggg ctgtacatgc 3000

cgacatgggc tattggatag aaagccaaaa gaatgggagt tggaagctag aaaaagcatc 3060

cttcatagag gtgaaaacct gcacatggcc aaaatcacac actctctgga gcaatggtgt 3120

gctagagagt gacatgatta tcccaaagag tctagctggt cccatttcgc aacacaacca 3180

caggcccggg taccacaccc aaacggcagg accctggcac ttaggaaaat tggagctgga 3240

cttcaactat tgtgaaggaa caacagttgt catctcagaa aactgtggga caagaggccc 3300

atcattgaga acaacaacgg tgtcagggaa gttgatacac gaatggtgct gccgctcgtg 3360

cacacttcct cccctacgat acatgggaga agacggctgc tggtatggca tggaaatcag 3420

acccattaat gagaaagaag agaatatggt aaagtctcta gcctcagcag ggagtggaaa 3480

ggtggacaac ttcacaatgg gtgtcttgtg tttggcaatc ctctttgaag aggtgatgag 3540

aggaaaattt gggaaaaaac acatgattgc aggggttctc ttcacgtttg tgctcctcct 3600

ctcagggcaa ataacatgga gagacatggc gcacacactc ataatgattg ggtccaacgc 3660

ctctgacaga atggggatgg gcgtcactta cctagctcta attgcaacat ttaaaattca 3720

gccattcctg gctttgggat tcttcctgag gaaactgaca tctagagaaa atttattgct 3780

gggagttggg ttggccatgg cagcaacgtt acgactgcca gaggacattg aacagatggc 3840

gaatggaatt gctttggggc tcatggctct taaactgata acacaatttg aaacatacca 3900

actatggacg gcattagttt ccctaacgtg ttcaaataca attttcacgt tgactgttgc 3960

ctggagaaca gccactctga ttttagccgg aatttcgctt ttgccagtgt gccagtcttc 4020

gagcatgagg aaaacagatt ggctcccaat gactgtggca gctatgggag ctcaacccct 4080

accacttttt attttcagtc tgaaagatac actcaaaagg agaagctggc cactgaatga 4140

gggggtgatg gcagttggac ttgtgagcat tctagctagt tctctcctta ggaatgatgt 4200

gcctatggct ggaccattag tggctggggg cttgctgata gcgtgctacg tcataactgg 4260

cacgtcagca gacctcactg tagaaaaagc agcagatgta acatgggagg aagaggccga 4320

gcaaacagga gtgtcccaca atttaatggt cacagttgat gatgatggaa caatgagaat 4380

aaaagatgac gagactgaga acatcttaac agtgctttta aaaacagcac tactaatagt 4440

atcaggcatc tttccatact ccatacccgc aacactgttg gtctggcata cttggcaaaa 4500

gcaaacccaa agatccggcg tcctatggga cgtacccagc cccccagaga cacagaaagc 4560

ggaactggaa gaaggggtct ataggatcaa acagcaagga atttttggga aaacccaagt 4620

gggggttgga gtacagaaag aaggagtttt ccacaccatg tggcatgtca caagaggggc 4680

agtgttgaca cacaatggga aaagactgga accaaactgg gctagcgtga aaaaagatct 4740

gatttcatac ggaggaggat ggagattgag tgcacaatgg caaaaggggg aggaggtgca 4800

ggttattgcc gtagagcctg ggaagaaccc aaagaacttt caaaccatgc caggcatttt 4860

tcagacaaca acaggggaaa taggagcaat tgcactggat ttcaagcctg gaacttcagg 4920

atctcccatc ataaacagag agggaaaggt agtgggactg tatggcaatg gagtggttac 4980

aaagaatgga ggctatgtca gtggaatagc gcaaacaaat gcagaaccag atggaccgac 5040

accagagttg gaagaagaga tgttcaaaaa gcgaaatcta accataatgg atctccatcc 5100

tgggtcagga aagacgcgga aatatcttcc agctattgtt agagaggcaa tcaagagacg 5160

cttaaggact ctaattttgg caccaacaag ggtagttgca gctgagatgg aagaagcatt 5220

gaaagggctc ccaataaggt atcaaacaac tgcaacaaaa tctgaacaca caggaagaga 5280

gattgttgat ctaatgtgtc acgcaacgtt cacaatgcgc ttgctgtcac cagtcagggt 5340

tccaaactac aacttgataa taatggatga ggcccatttc acagacccag ccagtatagc 5400

ggctagaggg tacatatcaa ctcgtgtagg aatgggagag gcagccgcaa ttttcatgac 5460

agcaacaccc cctggaacag ctgatgcctt tcctcagagc aacgctccaa ttcaagatga 5520

agagagagac ataccggaac gctcatggaa ttcaggcaat gaatggatta ctgactttgt 5580

tgggaagaca gtgtggtttg tccctagcat caaagccgga aatgacatag caaactgctt 5640

gcggaaaaat ggaaaaaagg ttattcaact cagcaggaag acctttgaca cagaatatca 5700

aaagaccaaa ctgaatgatt gggactttgt ggtgacaaca gacatttcag aaatgggagc 5760

caatttcaaa gcagatagag tgatcgaccc aagaagatgt ctcaagccgg tgattttgac 5820

agatggaccc gagcgggtga tcctggctgg accaatgcca gtcaccgtag cgagcgctgc 5880

gcaaaggaga gggagagttg gcaggaaccc acaaaaagaa aatgaccagt acatattcat 5940

gggccagcct ctcaacaatg atgaagacca tgctcactgg acagaagcaa aaatgctgct 6000

ggacaacatc aacacaccag aagggattat accagctctc tttgaaccag aaagggagaa 6060

gtcagccgcc atagacggcg aataccgcct gaagggtgag tccaggaaga ctttcgtgga 6120

actcatgagg aggggtgacc tcccagtttg gctagcccat aaagtagcat cagaagggat 6180

caaatataca gatagaaaat ggtgctttga tggagaacgt aataatcaaa ttttagagga 6240

gaatatggat gtggaaatct ggacaaagga aggagaaaag aaaaaactga gacctaggtg 6300

gcttgatgcc cgcacttatt cagatccttt agcactcaaa gaattcaagg attttgcagc 6360

tggcagaaag tcaatcgccc ttgatcttgt gacagaaata ggaagagtgc cttcacactt 6420

agcccacaga acgagaaacg ccctggataa tttggtgatg ctgcacacgt cagaacatgg 6480

cggtagggcc tacaggcatg cagtggagga actaccagaa acgatggaaa cactcttact 6540

cctgggactg atgatcttgt taacaggtgg agcaatgctc ttcttgatat caggtaaagg 6600

gattggaaag acttcaatag gactcatttg tgtaattgct tccagcggca tgttatggat 6660

ggctgatgtc ccactccaat ggatcgcatc ggctatagtc ctggagtttt ttatgatggt 6720

gttgctcata ccagaaccag aaaagcagag aactccccaa gacaaccaac tcgcatatgt 6780

cgtgataggc atacttacat tggctgcaat agtagcggcc aatgaaatgg gactgttgga 6840

aactacaaag agagatttag gaatgtctaa agaaccaggt gttgtttctc caaccagcta 6900

tttggatgtg gacttgcacc cagcatcagc ctggacattg tacgccgtgg ccacaacagt 6960

aataacacca atgttgagac acaccataga gaattccaca gcaaatgtgt ctctggcagc 7020

catagctaac caggcagtgg tcctgatggg tttagacaaa ggatggccga tatcgaaaat 7080

ggacttgggc gtaccactat tggcactggg ttgctattca caagtgaacc cactaactct 7140

tgcagcggca gtacttttgc tagtcacaca ttatgcaatt ataggtccag gattgcaggc 7200

aaaagccacc cgtgaagctc agaaaaggac agctgctgga ataatgaaga atccaacggt 7260

ggatggaata atgacaatag acctagatcc tgtaatatat gattcaaaat ttgaaaagca 7320

actaggacag gtcatgctcc tggttctgtg tgcagtccaa cttttattga tgagaacatc 7380

atgggccttg tgtgaagttc taaccctagc cacaggacca ataacaacac tctgggaagg 7440

atcacctggg aagttctgga acaccacgat agctgtttcc atggcgaaca tctttagagg 7500

gagctattta gcaggagctg ggcttgcttt ttctatcatg aaatcagttg gaacaggaaa 7560

gagaggaaca gggtcacaag gtgaaacctt aggagaaaag tggaaaaaga aattaaatca 7620

gttatcccgg aaagagtttg acctttacaa gaaatccgga atcaccgaag tggatagaac 7680

agaagccaaa gaagggttaa aaagaggaga aataacacac catgccgtgt ccagaggcag 7740

cgcaaaactt caatggttcg tggagagaaa catggtcatt cctgaaggaa gagtcataga 7800

cctaggctgt ggaagaggag gctggtcata ttactgtgca ggactgaaaa aagttacaga 7860

agtgcgagga tacacaaaag gcggcccagg acacgaagaa ccagtaccta tgtctacata 7920

cggatggaac atagtcaagt taatgagtgg aaaggatgtt ttttatctgc cacctgaaaa 7980

gtgtgatacc ctattgtgtg acattggaga atcttcacca agcccaacag tggaagaaag 8040

cagaaccata agagttttga agatggttga accatggcta aagaacaacc agttttgcat 8100

taaagtattg aacccataca tgccaactgt gattgagcac ttagaaagac tacaaaggaa 8160

acatggagga atgcttgtga gaaatccact ctcacgaaac tccacgcacg aaatgtattg 8220

gatatccaat ggtacaggca atatcgtctc ttcagtcaac atggtatcca gattgctact 8280

gaacagattc acaatgacac acaggagacc caccatagag aaagatgtgg atctaggagc 8340

aggaacccga catgtcaatg cggaaccaga aacacccaac atggatgtca ttggggaaag 8400

aataaaaagg atcaaagagg agcatagttc aacatggcac tatgatgatg aaaatcctta 8460

caaaacgtgg gcttaccatg gatcctatga agtaaaagcc acaggctcag cctcctccat 8520

gataaatgga gtcgtgaaac tcctcacaaa accatgggat gtggtgccca tggtgacaca 8580

gatggcaatg acagatacaa ctccattcgg ccagcaaaga gtttttaaag agaaagtgga 8640

caccaggaca cctaggccca tgccaggaac aagaaaggtt atggagatca cagcggagtg 8700

gctttggagg accctgggaa ggaacaaaag acccagatta tgcacaaggg aggaattcac 8760

aaagaaggtc agaaccaacg cagctatggg cgctgtcttc acagaagaga accaatggga 8820

cagtgcgaga gctgctgttg aggacgaaga attttggaaa cttgtggaca gagaacgtga 8880

actccacaaa ctgggcaagt gtggaagctg cgtttacaac atgatgggca agagagagaa 8940

aaaacttgga gagtttggta aagcaaaagg cagtagggct atatggtaca tgtggttggg 9000

agccaggtac cttgagttcg aggcgctcgg attcctcaat gaagaccact ggttctcgcg 9060

tgaaaactct tacagtggag tagaaggaga aggactgcac aagctgggat acatcttgag 9120

agatatttcc aagatacccg gaggagccat gtatgctgat gacacagccg gttgggacac 9180

aagaataaca gaagatgacc tgcacaatga ggaaaaaatc acacagcaga tggaccctga 9240

acacaggcag ctagcgaacg ctatattcaa gctcacatac caaaacaaag tggtcaaagt 9300

ccaacgacca actccaaagg gcacggtaat ggacatcata tctaggaaag accaaagagg 9360

cagtggacag gtgggaactt atggtctgaa cacattcacc aacatggaag cccagctaat 9420

cagacaaatg gaaggagaag gcgtgttgtc aaaggcagac ctcgagaacc cccatccgct 9480

agagaagaaa attacacaat ggttggaaac taaaggagtg gaaaggttaa aaagaatggc 9540

catcagcggg gatgattgcg ttgtgaaacc aatcgacgac agattcgcca atgccctgct 9600

tgccctgaac gatatgggaa aggttagaaa ggacatacct caatggcagc catcaaaggg 9660

atggcatgat tggcaacagg tccccttctg ctcccaccac tttcatgaat tgatcatgaa 9720

agatggaaga aagttggtag ttccctgcag accccaggac gaactaatag gaagagcgag 9780

aatctcccaa ggagcaggat ggagccttag agaaactgca tgtctaggga aagcctacgc 9840

tcaaatgtgg gctctcatgt attttcacag aagagatctt agactagcat ccaacgccat 9900

atgttcagca gtaccagtcc actgggtccc cacgagcaga acgacatggt ctattcatgc 9960

tcaccatcag tggatgacta cagaagacat gcttactgtc tggaacaggg tgtggataga 10020

ggacaatcca tggatggaag acaaaactcc agtcacaacg tgggaagatg ttccatatct 10080

agggaagaga gaagaccaat ggtgcggatc actcataggt ctcacttcca gagcaacctg 10140

ggcccagaac atactcacag caatccaaca ggtgagaagc ctcataggca atgaagagtt 10200

tctggactac atgccttcga tgaagagatt caggaaggag gaggagtcag agggagccat 10260

ttggtaaaag caggaggtaa actgtcaggc cacattaagc cacagtacgg aagaagctgt 10320

gcagcctgtg agccccgtcc aaggacgtta aaagaagaag tcaggcccaa aagccacggt 10380

ttgagcaaac cgtgctgcct gtagctccgt cgtggggacg taaagcctgg gaggctgcaa 10440

accgtggaag ctgtacgcac ggtgtagcag actagtggtt agaggagacc cctcccatga 10500

cacaacgcag cagcggggcc cgagcactga gggaagctgt acctccttgc aaaggactag 10560

aggttagagg agacccccg caaacaaaaa cagcatattg acgctgggag agaccagaga 10620

tcctgctgtc tcctcagcat cattccaggc acagaacgcc agaaaatgga atggtgctgt 10680

tgaatcaaca ggttct 10696

<210> 14
<211> 3390
<212> PRT
<213> dengue virus

<400> 14

Met Asn Asn Gln Arg Lys Lys Thr Gly Lys Pro Ser Ile Asn Met Leu
1 5 10 15

Lys Arg Val Arg Asn Arg Val Ser Thr Gly Ser Gln Leu Ala Lys Arg
20 25 30

Phe Ser Arg Gly Leu Leu Asn Gly Gln Gly Pro Met Lys Leu Val Met
35 40 45

Ala Phe Ile Ala Phe Leu Arg Phe Leu Ala Ile Pro Pro Thr Ala Gly
50 55 60

Val Leu Ala Arg Trp Gly Thr Phe Lys Lys Ser Gly Ala Ile Lys Val
65 70 75 80

Leu Lys Gly Phe Lys Lys Glu Ile Ser Asn Met Leu Ser Ile Ile Asn
85 90 95

Lys Arg Lys Lys Thr Ser Leu Cys Leu Met Met Met Leu Pro Ala Thr
100 105 110

Leu Ala Phe His Leu Thr Ser Arg Asp Gly Glu Pro Arg Met Ile Val
115 120 125

Gly Lys Asn Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ala Ser Gly
130 135 140

```
Ile Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Asp
145                 150                 155                 160

Asp Thr Val Thr Tyr Lys Cys Pro His Ile Thr Glu Val Glu Pro Glu
                165                 170                 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Thr Tyr Gly
            180                 185                 190

Thr Cys Asn Gln Ala Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
        195                 200                 205

Leu Ala Pro His Val Gly Met Gly Leu Asp Thr Arg Thr Gln Thr Trp
    210                 215                 220

Met Ser Ala Glu Gly Ala Trp Arg Gln Val Glu Lys Val Glu Thr Trp
225                 230                 235                 240

Ala Leu Arg His Pro Gly Phe Thr Ile Leu Ala Leu Phe Leu Ala His
                245                 250                 255

Tyr Ile Gly Thr Ser Leu Thr Gln Lys Val Val Ile Phe Ile Leu Leu
            260                 265                 270

Met Leu Val Thr Pro Ser Met Thr Met Arg Cys Val Gly Val Gly Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
    290                 295                 300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Ile Glu Leu Gln Lys Thr Glu Ala Thr Gln Leu Ala Thr Leu
                325                 330                 335

Arg Lys Leu Cys Ile Glu Gly Lys Ile Thr Asn Ile Thr Thr Asp Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Ile Leu Pro Glu Glu Gln Asp Gln
        355                 360                 365

Asn Tyr Val Cys Lys His Thr Tyr Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Gln
385                 390                 395                 400

Cys Leu Glu Ser Ile Glu Gly Lys Val Val Gln His Glu Asn Leu Lys
                405                 410                 415
```

```
Tyr Thr Val Ile Ile Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Gln Gly Val Thr Ala Glu Ile Thr Pro Gln Ala Ser Thr
        435                 440                 445

Ala Glu Val Ile Leu Pro Glu Tyr Gly Thr Leu Gly Leu Glu Cys Ser
    450                 455                 460

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Ile Ser Leu Thr Met Lys
465                 470                 475                 480

Asn Lys Ala Trp Met Val His Arg Gln Trp Phe Phe Asp Leu Pro Leu
                485                 490                 495

Pro Trp Thr Ser Gly Ala Thr Ala Glu Thr Pro Thr Trp Asn Arg Lys
            500                 505                 510

Glu Leu Leu Val Thr Phe Lys Asn Ala His Ala Lys Lys Gln Glu Val
        515                 520                 525

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
    530                 535                 540

Ala Thr Glu Ile Gln Thr Ser Gly Gly Thr Ser Ile Phe Ala Gly His
545                 550                 555                 560

Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Glu Leu Lys Gly Met Ser
                565                 570                 575

Tyr Ala Met Cys Leu Ser Ser Phe Val Leu Lys Lys Glu Val Ser Glu
            580                 585                 590

Thr Gln His Gly Thr Ile Leu Ile Lys Val Glu Tyr Lys Gly Glu Asp
        595                 600                 605

Ala Pro Cys Lys Ile Pro Phe Ser Thr Glu Asp Gly Gln Gly Lys Ala
    610                 615                 620

His Asn Gly Arg Leu Ile Thr Ala Asn Pro Val Val Thr Lys Lys Glu
625                 630                 635                 640

Glu Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser Asn Ile
                645                 650                 655

Val Ile Gly Ile Gly Asp Lys Ala Leu Lys Ile Asn Trp Tyr Lys Lys
            660                 665                 670

Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala Arg
        675                 680                 685

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly
```

690 695 700

Gly Val Leu Asn Ser Leu Gly Lys Met Val His Gln Ile Phe Gly Ser
705 710 715 720

Ala Tyr Thr Ala Leu Phe Gly Gly Val Ser Trp Met Met Lys Ile Gly
725 730 735

Ile Gly Val Leu Leu Thr Trp Ile Gly Leu Asn Ser Lys Asn Thr Ser
740 745 750

Met Ser Phe Ser Cys Ile Ala Ile Gly Ile Ile Thr Leu Tyr Leu Gly
755 760 765

Ala Val Val Gln Ala Asp Met Gly Cys Val Ile Asn Trp Lys Gly Lys
770 775 780

Glu Leu Lys Cys Gly Ser Gly Ile Phe Val Thr Asn Glu Val His Thr
785 790 795 800

Trp Thr Glu Gln Tyr Lys Phe Gln Ala Asp Ser Pro Lys Arg Leu Ala
805 810 815

Thr Ala Ile Ala Gly Ala Trp Glu Asn Gly Val Cys Gly Ile Arg Ser
820 825 830

Thr Thr Arg Met Glu Asn Leu Leu Trp Lys Gln Ile Ala Asn Glu Leu
835 840 845

Asn Tyr Ile Leu Trp Glu Asn Asn Ile Lys Leu Thr Val Val Val Gly
850 855 860

Asp Ile Thr Gly Val Leu Glu Gln Gly Lys Arg Thr Leu Thr Pro Gln
865 870 875 880

Pro Met Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala Lys Ile
885 890 895

Val Thr Ala Glu Thr Gln Asn Ser Ser Phe Ile Ile Asp Gly Pro Ser
900 905 910

Thr Pro Glu Cys Pro Ser Ala Ser Arg Ala Trp Asn Val Trp Glu Val
915 920 925

Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu Lys Leu
930 935 940

Arg Glu Val Tyr Thr Gln Leu Cys Asp His Arg Leu Met Ser Ala Ala
945 950 955 960

Val Lys Asp Glu Arg Ala Val His Ala Asp Met Gly Tyr Trp Ile Glu
965 970 975

```
Ser Gln Lys Asn Gly Ser Trp Lys Leu Glu Lys Ala Ser Phe Ile Glu
            980                 985                 990


Val Lys Thr Cys Thr Trp Pro Lys  Ser His Thr Leu Trp  Ser Asn Gly
        995                 1000                1005


Val Leu  Glu Ser Asp Met Ile  Ile Pro Lys Ser Leu  Ala Gly Pro
    1010                1015                1020


Ile Ser  Gln His Asn His Arg  Pro Gly Tyr His Thr  Gln Thr Ala
    1025                1030                1035


Gly Pro  Trp His Leu Gly Lys  Leu Glu Leu Asp Phe  Asn Tyr Cys
    1040                1045                1050


Glu Gly  Thr Thr Val Val Ile  Ser Glu Asn Cys Gly  Thr Arg Gly
    1055                1060                1065


Pro Ser  Leu Arg Thr Thr Thr  Val Ser Gly Lys Leu  Ile His Glu
    1070                1075                1080


Trp Cys  Cys Arg Ser Cys Thr  Leu Pro Pro Leu Arg  Tyr Met Gly
    1085                1090                1095


Glu Asp  Gly Cys Trp Tyr Gly  Met Glu Ile Arg Pro  Ile Asn Glu
    1100                1105                1110


Lys Glu  Glu Asn Met Val Lys  Ser Leu Ala Ser Ala  Gly Ser Gly
    1115                1120                1125


Lys Val  Asp Asn Phe Thr Met  Gly Val Leu Cys Leu  Ala Ile Leu
    1130                1135                1140


Phe Glu  Glu Val Met Arg Gly  Lys Phe Gly Lys Lys  His Met Ile
    1145                1150                1155


Ala Gly  Val Leu Phe Thr Phe  Val Leu Leu Leu Ser  Gly Gln Ile
    1160                1165                1170


Thr Trp  Arg Asp Met Ala His  Thr Leu Ile Met Ile  Gly Ser Asn
    1175                1180                1185


Ala Ser  Asp Arg Met Gly Met  Gly Val Thr Tyr Leu  Ala Leu Ile
    1190                1195                1200


Ala Thr  Phe Lys Ile Gln Pro  Phe Leu Ala Leu Gly  Phe Phe Leu
    1205                1210                1215


Arg Lys  Leu Thr Ser Arg Glu  Asn Leu Leu Leu Gly  Val Gly Leu
    1220                1225                1230
```

```
Ala Met  Ala Ala Thr Leu Arg  Leu Pro Glu Asp Ile  Glu Gln Met
    1235                 1240                 1245

Ala Asn  Gly Ile Ala Leu Gly  Leu Met Ala Leu Lys  Leu Ile Thr
    1250                 1255                 1260

Gln Phe  Glu Thr Tyr Gln Leu  Trp Thr Ala Leu Val  Ser Leu Thr
    1265                 1270                 1275

Cys Ser  Asn Thr Ile Phe Thr  Leu Thr Val Ala Trp  Arg Thr Ala
    1280                 1285                 1290

Thr Leu  Ile Leu Ala Gly Ile  Ser Leu Leu Pro Val  Cys Gln Ser
    1295                 1300                 1305

Ser Ser  Met Arg Lys Thr Asp  Trp Leu Pro Met Thr  Val Ala Ala
    1310                 1315                 1320

Met Gly  Ala Gln Pro Leu Pro  Leu Phe Ile Phe Ser  Leu Lys Asp
    1325                 1330                 1335

Thr Leu  Lys Arg Arg Ser Trp  Pro Leu Asn Glu Gly  Val Met Ala
    1340                 1345                 1350

Val Gly  Leu Val Ser Ile Leu  Ala Ser Ser Leu Leu  Arg Asn Asp
    1355                 1360                 1365

Val Pro  Met Ala Gly Pro Leu  Val Ala Gly Gly Leu  Leu Ile Ala
    1370                 1375                 1380

Cys Tyr  Val Ile Thr Gly Thr  Ser Ala Asp Leu Thr  Val Glu Lys
    1385                 1390                 1395

Ala Ala  Asp Val Thr Trp Glu  Glu Glu Ala Glu Gln  Thr Gly Val
    1400                 1405                 1410

Ser His  Asn Leu Met Val Thr  Val Asp Asp Asp Gly  Thr Met Arg
    1415                 1420                 1425

Ile Lys  Asp Asp Glu Thr Glu  Asn Ile Leu Thr Val  Leu Leu Lys
    1430                 1435                 1440

Thr Ala  Leu Leu Ile Val Ser  Gly Ile Phe Pro Tyr  Ser Ile Pro
    1445                 1450                 1455

Ala Thr  Leu Leu Val Trp His  Thr Trp Gln Lys Gln  Thr Gln Arg
    1460                 1465                 1470

Ser Gly  Val Leu Trp Asp Val  Pro Ser Pro Pro Glu  Thr Gln Lys
    1475                 1480                 1485
```

```
Ala Glu  Leu Glu Glu Gly Val  Tyr Arg Ile Lys Gln  Gln Gly Ile
    1490                 1495                 1500


Phe Gly  Lys Thr Gln Val Gly  Val Gly Val Gln Lys  Glu Gly Val
    1505                 1510                 1515


Phe His  Thr Met Trp His Val  Thr Arg Gly Ala Val  Leu Thr His
    1520                 1525                 1530


Asn Gly  Lys Arg Leu Glu Pro  Asn Trp Ala Ser Val  Lys Lys Asp
    1535                 1540                 1545


Leu Ile  Ser Tyr Gly Gly Gly  Trp Arg Leu Ser Ala  Gln Trp Gln
    1550                 1555                 1560


Lys Gly  Glu Glu Val Gln Val  Ile Ala Val Glu Pro  Gly Lys Asn
    1565                 1570                 1575


Pro Lys  Asn Phe Gln Thr Met  Pro Gly Ile Phe Gln  Thr Thr Thr
    1580                 1585                 1590


Gly Glu  Ile Gly Ala Ile Ala  Leu Asp Phe Lys Pro  Gly Thr Ser
    1595                 1600                 1605


Gly Ser  Pro Ile Ile Asn Arg  Glu Gly Lys Val Val  Gly Leu Tyr
    1610                 1615                 1620


Gly Asn  Gly Val Val Thr Lys  Asn Gly Gly Tyr Val  Ser Gly Ile
    1625                 1630                 1635


Ala Gln  Thr Asn Ala Glu Pro  Asp Gly Pro Thr Pro  Glu Leu Glu
    1640                 1645                 1650


Glu Glu  Met Phe Lys Lys Arg  Asn Leu Thr Ile Met  Asp Leu His
    1655                 1660                 1665


Pro Gly  Ser Gly Lys Thr Arg  Lys Tyr Leu Pro Ala  Ile Val Arg
    1670                 1675                 1680


Glu Ala  Ile Lys Arg Arg Leu  Arg Thr Leu Ile Leu  Ala Pro Thr
    1685                 1690                 1695


Arg Val  Val Ala Ala Glu Met  Glu Glu Ala Leu Lys  Gly Leu Pro
    1700                 1705                 1710


Ile Arg  Tyr Gln Thr Thr Ala  Thr Lys Ser Glu His  Thr Gly Arg
    1715                 1720                 1725


Glu Ile  Val Asp Leu Met Cys  His Ala Thr Phe Thr  Met Arg Leu
    1730                 1735                 1740


Leu Ser  Pro Val Arg Val Pro  Asn Tyr Asn Leu Ile  Ile Met Asp
```

```
    1745                1750                1755

Glu Ala  His Phe Thr Asp Pro  Ala Ser Ile Ala Ala  Arg Gly Tyr
    1760                1765                1770

Ile Ser  Thr Arg Val Gly Met  Gly Glu Ala Ala Ala  Ile Phe Met
    1775                1780                1785

Thr Ala  Thr Pro Pro Gly Thr  Ala Asp Ala Phe Pro  Gln Ser Asn
    1790                1795                1800

Ala Pro  Ile Gln Asp Glu Glu  Arg Asp Ile Pro Glu  Arg Ser Trp
    1805                1810                1815

Asn Ser  Gly Asn Glu Trp Ile  Thr Asp Phe Val Gly  Lys Thr Val
    1820                1825                1830

Trp Phe  Val Pro Ser Ile Lys  Ala Gly Asn Asp Ile  Ala Asn Cys
    1835                1840                1845

Leu Arg  Lys Asn Gly Lys Lys  Val Ile Gln Leu Ser  Arg Lys Thr
    1850                1855                1860

Phe Asp  Thr Glu Tyr Gln Lys  Thr Lys Leu Asn Asp  Trp Asp Phe
    1865                1870                1875

Val Val  Thr Thr Asp Ile Ser  Glu Met Gly Ala Asn  Phe Lys Ala
    1880                1885                1890

Asp Arg  Val Ile Asp Pro Arg  Arg Cys Leu Lys Pro  Val Ile Leu
    1895                1900                1905

Thr Asp  Gly Pro Glu Arg Val  Ile Leu Ala Gly Pro  Met Pro Val
    1910                1915                1920

Thr Val  Ala Ser Ala Ala Gln  Arg Arg Gly Arg Val  Gly Arg Asn
    1925                1930                1935

Pro Gln  Lys Glu Asn Asp Gln  Tyr Ile Phe Met Gly  Gln Pro Leu
    1940                1945                1950

Asn Asn  Asp Glu Asp His Ala  His Trp Thr Glu Ala  Lys Met Leu
    1955                1960                1965

Leu Asp  Asn Ile Asn Thr Pro  Glu Gly Ile Ile Pro  Ala Leu Phe
    1970                1975                1980

Glu Pro  Glu Arg Glu Lys Ser  Ala Ala Ile Asp Gly  Glu Tyr Arg
    1985                1990                1995

Leu Lys  Gly Glu Ser Arg Lys  Thr Phe Val Glu Leu  Met Arg Arg
    2000                2005                2010
```

```
Gly Asp  Leu Pro Val Trp Leu  Ala His Lys Val Ala  Ser Glu Gly
    2015                 2020                 2025

Ile Lys  Tyr Thr Asp Arg Lys  Trp Cys Phe Asp Gly  Glu Arg Asn
    2030                 2035                 2040

Asn Gln  Ile Leu Glu Glu Asn  Met Asp Val Glu Ile  Trp Thr Lys
    2045                 2050                 2055

Glu Gly  Glu Lys Lys Lys Leu  Arg Pro Arg Trp Leu  Asp Ala Arg
    2060                 2065                 2070

Thr Tyr  Ser Asp Pro Leu Ala  Leu Lys Glu Phe Lys  Asp Phe Ala
    2075                 2080                 2085

Ala Gly  Arg Lys Ser Ile Ala  Leu Asp Leu Val Thr  Glu Ile Gly
    2090                 2095                 2100

Arg Val  Pro Ser His Leu Ala  His Arg Thr Arg Asn  Ala Leu Asp
    2105                 2110                 2115

Asn Leu  Val Met Leu His Thr  Ser Glu His Gly Gly  Arg Ala Tyr
    2120                 2125                 2130

Arg His  Ala Val Glu Glu Leu  Pro Glu Thr Met Glu  Thr Leu Leu
    2135                 2140                 2145

Leu Leu  Gly Leu Met Ile Leu  Leu Thr Gly Gly Ala  Met Leu Phe
    2150                 2155                 2160

Leu Ile  Ser Gly Lys Gly Ile  Gly Lys Thr Ser Ile  Gly Leu Ile
    2165                 2170                 2175

Cys Val  Ile Ala Ser Ser Gly  Met Leu Trp Met Ala  Asp Val Pro
    2180                 2185                 2190

Leu Gln  Trp Ile Ala Ser Ala  Ile Val Leu Glu Phe  Phe Met Met
    2195                 2200                 2205

Val Leu  Leu Ile Pro Glu Pro  Glu Lys Gln Arg Thr  Pro Gln Asp
    2210                 2215                 2220

Asn Gln  Leu Ala Tyr Val Val  Ile Gly Ile Leu Thr  Leu Ala Ala
    2225                 2230                 2235

Ile Val  Ala Ala Asn Glu Met  Gly Leu Leu Glu Thr  Thr Lys Arg
    2240                 2245                 2250

Asp Leu  Gly Met Ser Lys Glu  Pro Gly Val Val Ser  Pro Thr Ser
    2255                 2260                 2265
```

```
Tyr Leu  Asp Val Asp Leu His  Pro Ala Ser Ala Trp  Thr Leu Tyr
    2270                 2275                 2280

Ala Val  Ala Thr Thr Val Ile  Thr Pro Met Leu Arg  His Thr Ile
    2285                 2290                 2295

Glu Asn  Ser Thr Ala Asn Val  Ser Leu Ala Ala Ile  Ala Asn Gln
    2300                 2305                 2310

Ala Val  Val Leu Met Gly Leu  Asp Lys Gly Trp Pro  Ile Ser Lys
    2315                 2320                 2325

Met Asp  Leu Gly Val Pro Leu  Leu Ala Leu Gly Cys  Tyr Ser Gln
    2330                 2335                 2340

Val Asn  Pro Leu Thr Leu Ala  Ala Ala Val Leu Leu  Leu Val Thr
    2345                 2350                 2355

His Tyr  Ala Ile Ile Gly Pro  Gly Leu Gln Ala Lys  Ala Thr Arg
    2360                 2365                 2370

Glu Ala  Gln Lys Arg Thr Ala  Ala Gly Ile Met Lys  Asn Pro Thr
    2375                 2380                 2385

Val Asp  Gly Ile Met Thr Ile  Asp Leu Asp Pro Val  Ile Tyr Asp
    2390                 2395                 2400

Ser Lys  Phe Glu Lys Gln Leu  Gly Gln Val Met Leu  Leu Val Leu
    2405                 2410                 2415

Cys Ala  Val Gln Leu Leu Leu  Met Arg Thr Ser Trp  Ala Leu Cys
    2420                 2425                 2430

Glu Val  Leu Thr Leu Ala Thr  Gly Pro Ile Thr Thr  Leu Trp Glu
    2435                 2440                 2445

Gly Ser  Pro Gly Lys Phe Trp  Asn Thr Thr Ile Ala  Val Ser Met
    2450                 2455                 2460

Ala Asn  Ile Phe Arg Gly Ser  Tyr Leu Ala Gly Ala  Gly Leu Ala
    2465                 2470                 2475

Phe Ser  Ile Met Lys Ser Val  Gly Thr Gly Lys Arg  Gly Thr Gly
    2480                 2485                 2490

Ser Gln  Gly Glu Thr Leu Gly  Glu Lys Trp Lys Lys  Lys Leu Asn
    2495                 2500                 2505

Gln Leu  Ser Arg Lys Glu Phe  Asp Leu Tyr Lys Lys  Ser Gly Ile
    2510                 2515                 2520
```

```
Thr Glu  Val Asp Arg Thr Glu  Ala Lys Glu Gly Leu  Lys Arg Gly
    2525                2530                2535


Glu Ile  Thr His His Ala Val  Ser Arg Gly Ser Ala  Lys Leu Gln
    2540                2545                2550


Trp Phe  Val Glu Arg Asn Met  Val Ile Pro Glu Gly  Arg Val Ile
    2555                2560                2565


Asp Leu  Gly Cys Gly Arg Gly  Gly Trp Ser Tyr Tyr  Cys Ala Gly
    2570                2575                2580


Leu Lys  Lys Val Thr Glu Val  Arg Gly Tyr Thr Lys  Gly Gly Pro
    2585                2590                2595


Gly His  Glu Glu Pro Val Pro  Met Ser Thr Tyr Gly  Trp Asn Ile
    2600                2605                2610


Val Lys  Leu Met Ser Gly Lys  Asp Val Phe Tyr Leu  Pro Pro Glu
    2615                2620                2625


Lys Cys  Asp Thr Leu Leu Cys  Asp Ile Gly Glu Ser  Ser Pro Ser
    2630                2635                2640


Pro Thr  Val Glu Glu Ser Arg  Thr Ile Arg Val Leu  Lys Met Val
    2645                2650                2655


Glu Pro  Trp Leu Lys Asn Asn  Gln Phe Cys Ile Lys  Val Leu Asn
    2660                2665                2670


Pro Tyr  Met Pro Thr Val Ile  Glu His Leu Glu Arg  Leu Gln Arg
    2675                2680                2685


Lys His  Gly Gly Met Leu Val  Arg Asn Pro Leu Ser  Arg Asn Ser
    2690                2695                2700


Thr His  Glu Met Tyr Trp Ile  Ser Asn Gly Thr Gly  Asn Ile Val
    2705                2710                2715


Ser Ser  Val Asn Met Val Ser  Arg Leu Leu Leu Asn  Arg Phe Thr
    2720                2725                2730


Met Thr  His Arg Arg Pro Thr  Ile Glu Lys Asp Val  Asp Leu Gly
    2735                2740                2745


Ala Gly  Thr Arg His Val Asn  Ala Glu Pro Glu Thr  Pro Asn Met
    2750                2755                2760


Asp Val  Ile Gly Glu Arg Ile  Lys Arg Ile Lys Glu  Glu His Ser
    2765                2770                2775


Ser Thr  Trp His Tyr Asp Asp  Glu Asn Pro Tyr Lys  Thr Trp Ala
```

2780 2785 2790

Tyr His Gly Ser Tyr Glu Val Lys Ala Thr Gly Ser Ala Ser Ser
2795 2800 2805

Met Ile Asn Gly Val Val Lys Leu Leu Thr Lys Pro Trp Asp Val
2810 2815 2820

Val Pro Met Val Thr Gln Met Ala Met Thr Asp Thr Thr Pro Phe
2825 2830 2835

Gly Gln Gln Arg Val Phe Lys Glu Lys Val Asp Thr Arg Thr Pro
2840 2845 2850

Arg Pro Met Pro Gly Thr Arg Lys Val Met Glu Ile Thr Ala Glu
2855 2860 2865

Trp Leu Trp Arg Thr Leu Gly Arg Asn Lys Arg Pro Arg Leu Cys
2870 2875 2880

Thr Arg Glu Glu Phe Thr Lys Lys Val Arg Thr Asn Ala Ala Met
2885 2890 2895

Gly Ala Val Phe Thr Glu Glu Asn Gln Trp Asp Ser Ala Arg Ala
2900 2905 2910

Ala Val Glu Asp Glu Glu Phe Trp Lys Leu Val Asp Arg Glu Arg
2915 2920 2925

Glu Leu His Lys Leu Gly Lys Cys Gly Ser Cys Val Tyr Asn Met
2930 2935 2940

Met Gly Lys Arg Glu Lys Lys Leu Gly Glu Phe Gly Lys Ala Lys
2945 2950 2955

Gly Ser Arg Ala Ile Trp Tyr Met Trp Leu Gly Ala Arg Tyr Leu
2960 2965 2970

Glu Phe Glu Ala Leu Gly Phe Leu Asn Glu Asp His Trp Phe Ser
2975 2980 2985

Arg Glu Asn Ser Tyr Ser Gly Val Glu Gly Glu Gly Leu His Lys
2990 2995 3000

Leu Gly Tyr Ile Leu Arg Asp Ile Ser Lys Ile Pro Gly Gly Ala
3005 3010 3015

Met Tyr Ala Asp Asp Thr Ala Gly Trp Asp Thr Arg Ile Thr Glu
3020 3025 3030

Asp Asp Leu His Asn Glu Glu Lys Ile Thr Gln Gln Met Asp Pro
3035 3040 3045

```
Glu His  Arg Gln Leu Ala Asn  Ala Ile Phe Lys Leu  Thr Tyr Gln
    3050                3055                3060

Asn Lys  Val Val Lys Val Gln  Arg Pro Thr Pro Lys  Gly Thr Val
    3065                3070                3075

Met Asp  Ile Ile Ser Arg Lys  Asp Gln Arg Gly Ser  Gly Gln Val
    3080                3085                3090

Gly Thr  Tyr Gly Leu Asn Thr  Phe Thr Asn Met Glu  Ala Gln Leu
    3095                3100                3105

Ile Arg  Gln Met Glu Gly Glu  Gly Val Leu Ser Lys  Ala Asp Leu
    3110                3115                3120

Glu Asn  Pro His Pro Leu Glu  Lys Lys Ile Thr Gln  Trp Leu Glu
    3125                3130                3135

Thr Lys  Gly Val Glu Arg Leu  Lys Arg Met Ala Ile  Ser Gly Asp
    3140                3145                3150

Asp Cys  Val Val Lys Pro Ile  Asp Asp Arg Phe Ala  Asn Ala Leu
    3155                3160                3165

Leu Ala  Leu Asn Asp Met Gly  Lys Val Arg Lys Asp  Ile Pro Gln
    3170                3175                3180

Trp Gln  Pro Ser Lys Gly Trp  His Asp Trp Gln Gln  Val Pro Phe
    3185                3190                3195

Cys Ser  His His Phe His Glu  Leu Ile Met Lys Asp  Gly Arg Lys
    3200                3205                3210

Leu Val  Val Pro Cys Arg Pro  Gln Asp Glu Leu Ile  Gly Arg Ala
    3215                3220                3225

Arg Ile  Ser Gln Gly Ala Gly  Trp Ser Leu Arg Glu  Thr Ala Cys
    3230                3235                3240

Leu Gly  Lys Ala Tyr Ala Gln  Met Trp Ala Leu Met  Tyr Phe His
    3245                3250                3255

Arg Arg  Asp Leu Arg Leu Ala  Ser Asn Ala Ile Cys  Ser Ala Val
    3260                3265                3270

Pro Val  His Trp Val Pro Thr  Ser Arg Thr Thr Trp  Ser Ile His
    3275                3280                3285

Ala His  His Gln Trp Met Thr  Thr Glu Asp Met Leu  Thr Val Trp
    3290                3295                3300
```

```
Asn Arg  Val Trp Ile Glu Asp  Asn Pro Trp Met Glu  Asp Lys Thr
    3305                 3310                 3315


Pro Val  Thr Thr Trp Glu Asp  Val Pro Tyr Leu Gly  Lys Arg Glu
    3320                 3325                 3330


Asp Gln  Trp Cys Gly Ser Leu  Ile Gly Leu Thr Ser  Arg Ala Thr
    3335                 3340                 3345


Trp Ala  Gln Asn Ile Leu Thr  Ala Ile Gln Gln Val  Arg Ser Leu
    3350                 3355                 3360


Ile Gly  Asn Glu Glu Phe Leu  Asp Tyr Met Pro Ser  Met Lys Arg
    3365                 3370                 3375


Phe Arg  Lys Glu Glu Glu Ser  Glu Gly Ala Ile Trp
    3380                 3385                 3390


<210>  15
<211>  10648
<212>  DNA
<213>  dengue virus

<400>  15
agttgttagt ctgtgtggac cgacaaggac agttccaaat cggaagcttg cttaacacag       60

ttctaacagt ttgtttgaat agagagcaga tctctggaaa aatgaaccaa cgaaaaaagg      120

tggttagacc acctttcaat atgctgaaac gcgagagaaa ccgcgtatca acccctcaag      180

ggttggtgaa gagattctca accggacttt tttctgggaa aggaccctta cggatggtgc      240

tagcattcat cacgtttttg cgagtccttt ccatcccacc aacagcaggg attctgaaaa      300

gatggggaca gttgaagaaa aataaggcca tcaggatact gattggattc aggaaggaga      360

taggccgcat gctgaacatc ttgaacggga gaaaaaggtc aacgataaca ttgctgtgct      420

tgattcccac cgtaatggcg tttcacttgt caacaagaga tggcgaaccc ctcatgatag      480

tggcaaaaca tgaaaggggg agacctctct tgtttaagac aacagagggg atcaacaaat      540

gcactctcat tgccatggac ttgggtgaaa tgtgtgagga cactgtcacg tataaatgcc      600

ccttactggt caataccgaa cctgaagaca ttgattgctg gtgcaatctc acgtctacct      660

gggtcatgta tgggacatgc acccagagcg gagaacggag acgagagaag cgctcagtag      720

ctttaacacc acattcagga atgggattgg aaacaagagc tgagacatgg atgtcatcgg      780

aaggggcttg gaagcatgct cagagagtag agagctggat actcagaaac ccaggattcg      840

cgctcttggc aggatttatg gcttatatga ttgggcaaac aggaatccag cgaactgtct      900

tctttgtcct aatgatgctg gtcgccccat cctacggaat gcgatgcgta ggagtaggaa      960

acagagactt tgtggaagga gtctcaggtg gagcatgggt cgatctggtg ctagaacatg     1020

gaggatgcgt cacaaccatg gcccagggaa aaccaacctt ggattttgaa ctgactaaga     1080

caacagccaa ggaagtggct ctgttaagaa cctattgcat tgaagcctca atatcaaaca     1140
```

taaccacggc aacaagatgt ccaacgcaag gagagcctta tctaaaagag gaacaagacc 1200

aacagtacat ttgccggaga gatgtggtag acagagggtg gggcaatggc tgtggcttgt 1260

ttggaaaagg aggagttgtg acatgtgcga agttttcatg ttcggggaag ataacaggca 1320

atttggtcca aattgagaac cttgaataca cagtggttgt aacagtccac aatggagaca 1380

cccatgcagt aggaaatgac acatccaatc atggagttac agccacgata actcccaggt 1440

caccatcggt ggaagtcaaa ttgccggact atggagaact aacactcgat tgtgaaccca 1500

ggtctggaat tgactttaat gagatgattc tgatgaaaat gaaaaagaaa acatggcttg 1560

tgcataagca atggtttttg gatctacctc taccatggac agcaggagca gacacatcag 1620

aggttcactg gaattacaaa gagagaatgg tgacatttaa ggttcctcat gccaagagac 1680

aggatgtgac agtgctggga tctcaggaag gagccatgca ttctgccctc gctggagcca 1740

cagaagtgga ctccggtgat ggaaatcaca tgtttgcagg acatctcaag tgcaaagtcc 1800

gtatggagaa attgagaatc aagggaatgt catacacgat gtgttcagga aagttctcaa 1860

ttgacaaaga gatggcagaa acacagcatg ggacaacagt ggtgaaagtc aagtatgaag 1920

gtgctggagc tccgtgtaaa gtccccatag agataagaga tgtgaacaag gaaaaagtgg 1980

ttgggcgtat catctcatcc acccctttgg ctgagaatac caacagtgca accaacatag 2040

agttagaacc ccccttttggg gacagctaca tagtgatagg tgttggaaac agtgcattaa 2100

cactccattg gttcaggaaa gggagttcca ttggcaagat gtttgagtcc acatacagag 2160

gtgcaaaacg aatggccatt ctaggtgaaa cagcttggga ttttggttcc gttggtggac 2220

tgttcacatc attgggaaag gctgtgcacc aggtttttgg aagtgtgtat acaaccatgt 2280

ttggaggagt ctcatggatg attagaatcc taattgggtt cctagtgttg tggattggca 2340

cgaactcaag gaacacttca atggctatga cgtgcatagc tgttggagga atcactctgt 2400

ttctgggctt cacagttcaa gcagacatgg gttgtgtggt gtcatggagt gggaaagaat 2460

tgaagtgtgg aagcggaatt tttgtggttg acaacgtgca cacttggaca gaacagtaca 2520

aatttcaacc ggagtcccca gcgagactag cgtctgcaat attgaatgcc cacaaagatg 2580

gggtctgtgg aattagatca accacgaggc tggaaaatgt catgtggaag caaataacca 2640

acgagctaaa ttatgttctc tgggaaggag gacatgacct cactgtagtg gctggggatg 2700

tgaagggggt gttgaccaaa ggcaagagag cactcacacc cccagtgaat gatctgaaat 2760

attcatggaa gacatgggga aaagcaaaaa tcttcacccc agaagcaaga aatagcacat 2820

ttttaataga cggaccagac acctccgaat gccccaatga acgaagagca tggaactttc 2880

ttgaggtgga agactatgga tttggcatgt tcacgaccaa catatggatg aaattccgag 2940

aaggaagttc agaagtgtgt gaccacaggt taatgtcagc ggcaattaaa gatcagaaag 3000

ctgtgcatgc tgacatgggt tattggatag agagctcaaa aaaccagacc tggcagatag 3060

agaaagcatc tcttattgaa gtgaaaacat gtctgtggcc caagacccac acattgtgga 3120

gcaatggagt gctggaaagc cagatgctca ttccaaaatc atatgcgggc ccttttttcac 3180

agcacaatta ccgccagggc tatgccacgc aaaccgtggg cccatggcac ttaggcaaat 3240

tagagataga ctttggagaa tgccccggaa caacagtcgc aattcaggag gattgtgacc 3300

atagaggccc atctttgagg accaccactg catctggaaa actagtcacg caatggtgct 3360

gccgctcctg cacgatgcct cccttaaggt tcttgggaga agatgggtgc tggtatggga 3420

tggagattag gcccttgagt gaaaaagaag agaacatggt caaatcacag gtaacggccg 3480

gacagggcac atcagaaact ttttctatgg gtctgttgtg cctgaccttg tttgtggaag 3540

aatgcttgag gagaagagtc actaggaaac acatgatatt ggttgtggtg atcactcttt 3600

gtgccatcat cctaggaggc ctcacatgga tggacttact acgagccctc atcatgttgg 3660

gggacactat gtctggtaga ataggaggac agatccacct agccatcatg gcagtgttca 3720

agatgtcacc aggatacgtg ctgggtgtgt ttttaaggaa actcacttca agagagacag 3780

cactaatggt aataggaatg gccatgacaa cggtgctttc aattccacat gaccttatgg 3840

aactcattga tggaatatca ctggggctaa ttttgctaaa aatagtgaca cattttgaca 3900

acacccaagt gggaacctta gccctttcct tgaccttcat aagatcaaca atgccattgg 3960

tcatggcttg gaggaccatt atggctgtgt tgtttgtggt cacactcatt cctttgtgca 4020

ggacaagctg tcttcaaaaa cagtctcatt gggtagaaat aacagcactc atcctaggag 4080

cccaagctct gccagtgtac ctaatgactc ttatgaaagg agcctcaaga agatcttggc 4140

ctcttaacga gggcataatg gctgtgggtt tggttagtct cttaggaagc gctcttttaa 4200

agaatgatgt ccctttagct ggcccaatgg tggcaggagg cttacttctg gcggcttacg 4260

tgatgagtgg tagctcagca gatctgtcac tagagaaggc cgccaatgtg cagtgggatg 4320

aaatggcaga cataacaggc tcaagcccaa tcatagaagt gaagcaggat gaagatggct 4380

ctttctccat acgggacgtc gaggaaacca atatgataac ccttttggtg aaactggcac 4440

tgataacagt gtcaggtctc tacccccttgg caattccagt cacaatgacc ttatggtaca 4500

tgtggcaagt gaaaacacaa agatcaggag ccctgtggga cgtcccctca cccgctgcca 4560

ctcaaaaagc cgcactgtct gaaggagtgt acaggatcat gcaaagaggg ttatttggga 4620

aaactcaggt tggagtaggg atacacatgg aaggtgtatt tcacacaatg tggcatgtaa 4680

caagaggatc agtgatctgc catgagactg ggagattgga gccatcttgg gctgacgtca 4740

ggaatgacat gatatcatac ggtgggggat ggagacttgg agacaaatgg gacaaagaag 4800

aagatgttca ggtcctcgcc atagaaccag gaaaaaatcc taaacatgtc caaacgaaac 4860

ccggcctttt caagacccta actggagaaa ttggagcagt aacattagat ttcaaacccg 4920

gaacgtctgg ttctcccatc atcaacagga aaggaaaagt catcggactc tatggaaatg 4980

gagtagttac caaatcaggt gattacgtca gtgccataac gcaagccgaa agaattggag 5040

agccagatta tgaagtggat gaggacattt ttcgaaagaa aagattaact ataatggact 5100

tacaccccgg agctggaaag acaaaaagaa ttcttccatc aatagtgaga gaagccttaa 5160

aaaggaggct gcgaaccttg attttggctc ccacgagagt ggtggcggcc gagatggaag 5220

aggccctacg tggactgcca atccgttatc agaccccagc tgtgaaatca gaacacacag 5280

gaagagagat tgtagacctc atgtgtcatg caaccttcac aacaagactt ttgtcatcaa 5340

ccagagttcc aaattacaac ctcatagtga tggatgaagc acatttcacc gatccttcta 5400

gtgtcgcggc tagaggatac atctcgacca gggtggaaat gggagaggca gcagccatct 5460

tcatgaccgc aacccctccc ggagcgacag atccctttcc ccagagcaac agcccaatag 5520

aagacatcga gagggaaatt ccggaaaggt catggaacac agggttcgac tggataacag 5580

actaccaagg gaaaactgtg tggtttgttc ccagcataaa agctggaaat gacattgcaa 5640

attgtttgag aaagtcggga aagaaagtta tccagttgag taggaaaacc tttgatacag 5700

agtatccaaa aacgaaactc acggactggg attttgtggt cactacagac atatctgaaa 5760

tgggggccaa ttttagagct gggagagtga tagaccctag gagatgcctc aagccagtta 5820

tcctaacaga tgggccagag agagtcattt tagcaggtcc tattccagtg actccagcaa 5880

gcgctgctca gagaagaggg cgaataggaa ggaacccagc acaagaagac gaccaatacg 5940

ttttctccgg agacccacta aaaaatgatg aagatcatgc ccactggaca gaagcaaaga 6000

tgctgcttga caatatctac accccagaag ggatcattcc aacattgttt ggtccggaaa 6060

gggaaaaaac ccaagccatt gatggagagt ttcgcctcag aggggaacaa aggaagactt 6120

ttgtggaatt aatgaggaga ggagaccttc cggtgtggct gagctataag gtagcttctg 6180

ctggcatttc ttacaaagat cgggaatggt gcttcacagg ggaaaggaat aaccaaattt 6240

tagaagaaaa catggaggtt gaaatttgga ctagagaggg agaaaagaaa aagctaaggc 6300

caagatggtt agatgcacgt gtatacgctg accccatggc tttgaaggat tttaaggagt 6360

ttgctagtgg aaggaagagc ataactctcg acatcctaac agagattgcc agtttgccaa 6420

cttacctttc ctctagggcc aagctcgccc ttgataacat agtcatgctc cacacaacag 6480

aaagaggagg gagggcctac caacacgccc tgaacgaact cccggagtca ctggaaacac 6540

ttatgcttgt agctttacta ggtgctatga cagcaggtat cttcctgttt ttcatgcaag 6600

ggaaaggaat agggaaattg tcaatgggtt tgataaccat tgcggtggct agtggcttgc 6660

tctgggtagc agaaattcaa ccccagtgga tagcggcctc aatcatacta gagtttttc 6720

tcatggtact gttgataccg gaaccagaaa aacaaaggac cccacaagac aatcaattga 6780

tctacgtcat attgaccatt ctcaccatta ttggtctcat agcagccaac gagatggggc 6840

tgattgaaaa aacaaaaacg gattttgggt tttaccaggt aaaaacagaa accaccatcc 6900

tcgatgtgga cttgagacca gcttcagcat ggacgctcta tgcagtagcc accacaattc 6960

tgactcccat gctgagacac accatagaaa acacgtcggc caacctatct ctagcagcca 7020

ttgccaacca ggcggccgtc ctaatggggc ttggaaaagg atggccgctc cacagaatgg 7080

acctcggtgt gccgctgtta gcaatgggat gctattctca agtgaaccca acaactttga 7140

cagcatcctt agtcatgctt ttagtccatt atgcaataat aggtccagga ttgcaggcaa 7200

aagccacaag agaggcccag aaaaggacag ctgctgggat catgaaaaac cccacggtgg 7260

acgggataac agtaatagat ctagaaccaa tatcctatga cccaaaattt gaaaagcaat 7320

tagggcaggt catgctactc gtcttgtgtg ctggacaact actcttgatg agaacaacat 7380

gggctttctg tgaagtcttg actttggcca caggaccaat cttgaccttg tgggagggca 7440

acccgggaag gttttggaac acgaccatag ccgtatccac cgccaacatt ttcaggggaa 7500

gttacctggc gggagctgga ctggcttttt cactcataaa gaatgtacaa acccctagga 7560

ggggaactgg gaccacagga gagacactgg gagagaagtg gaagagacag ctaaactcat 7620

tagacagaaa agagtttgaa gagtataaaa gaagtggaat actagaagtg gacaggactg 7680

aagccaagtc tgccctgaaa gatgggtcta aaatcaagca tgcagtatct agagggtcca 7740

gtaagattag atggattgtt gagagaggga tggtaaagcc aaaagggaaa gttgtagatc 7800

ttggctgtgg gagaggagga tggtcttatt acatggcgac gctcaagaac gtgactgaag 7860

tgaaagggta tacaaaagga ggtccaggac atgaagaacc gattcccatg gctacttatg 7920

gctggaattt ggtcaaactc cattcagggg ttgacgtgtt ctacaaaccc acagagcaag 7980

tggacaccct gctctgtgat attggggagt catcttctaa tccaacaata gaggaaggaa 8040

gaacattaag agttttgaag atggtggagc catggctctc ttcaaaacct gaattctgca 8100

tcaaagtcct taacccctac atgccaacag tcatagaaga gctggagaaa ctgcagagaa 8160

aacatggtgg gaaccttgtc agatgcccgc tgtccaggaa ctccacccat gagatgtatt 8220

gggtgtcagg agcgtcggga aacattgtga gctctgtgaa cacaacatca aagatgttgt 8280

tgaacaggtt cacaacaagg cataggaaac ccacttatga gaaggacgta gatcttgggg 8340

caggaacgag aagtgtctcc actgaaacag aaaaaccaga catgacaatt attgggagaa 8400

ggcttcagcg attgcaagag gagcacaaag aaacctggca ttatgatcag gaaaacccat 8460

acagaacctg ggcgtatcat ggaagctatg aagctccttc gacaggctct gcatcctcca 8520

tggtgaacgg ggtagtaaaa ctgctaacaa aaccttggga tgtggttcca atggtgaccc 8580

agttagccat gacagacaca acccctttg ggcaacaaag agtgttcaaa gagaaggtgg 8640

ataccagaac accacaacca aaacccggta cacgaatggt tatgaccacg acagccaatt 8700

ggctgtgggc cctccttggg aagaagaaaa atcccagact gtgcacaagg gaagagttca 8760

tctcaaaagt tagatcaaac gcagccatag gcgcagtctt tcaggaagaa cagggatgga 8820

catcagccag tgaagctgtg aatgacagcc ggttttggga actggttgac aaagaaaggg 8880

ccctacacca ggaagggaaa tgtgaatcgt gtgtctacaa catgatggga aaacgtgaga 8940

aaaagttagg agagtttggc agagccaagg gaagccgagc aatctggtac atgtggctgg 9000

gagcgcggtt tctggaattt gaagccctgg gttttttgaa tgaagatcac tggtttggca 9060

gagaaaattc atggagtgga gtggaagggg aaggtctgca cagattggga tatatcctgg 9120

aggagataga caagaaggat ggagacctaa tgtatgctga tgacacagca ggctgggaca 9180

caagaatcac tgaggatgac cttcaaaatg aagaactgat cacggaacag atggcccccc 9240

accacaagat cctagccaaa gccattttca aactaaccta tcaaaacaaa gtggtgaaag 9300

tcctcagacc cacaccgaga ggagcggtga tggatatcat atccaggaaa gaccaaagag 9360

gtagtggaca agttggaaca tatggtttga acacattcac caacatggaa gttcaactca 9420

tccgccaaat ggaagctgaa ggagtcatca cacaagatga catgcagaac ccaaaagggt 9480

tgaaagaaag agttgagaaa tggctgaaag agtgtggtgt cgacaggtta aagaggatgg 9540

caatcagtgg agacgattgc gtggtgaagc ccctggatga gaggtttggc acttccctcc 9600

tcttcttgaa cgacatggga aaggtgagga aagacattcc gcagtgggaa ccatctaagg 9660

gatggaaaaa ctggcaagag gttccttttt gctcccacca ctttcacaag atcttcatga 9720

aggatggccg ctcactagtt gttccatgta gaaaccagga tgaactgata gggagagcca 9780

gaatctcgca gggggctgga tggagcttaa gagaaacagc ctgcctgggc aaagcttacg 9840

cccagatgtg gtcgctcatg tacttccaca gaagggatct gcgtttagcc tccatggcca 9900

tatgctcagc agttccaacg gaatggtttc caacaagcag aacaacatgg tcaatccacg 9960

ctcatcatca gtggatgacc actgaagata tgctcaaagt gtggaacaga gtgtggatag 10020

aagacaaccc taatatgact gacaagactc cagtccattc gtgggaagat ataccttacc 10080

tagggaaaag agaggatttg tggtgtggat ccctgattgg actttcttcc agagccacct 10140

gggcgaagaa cattcacacg gccataaccc aggtcagaaa cctgatcgga aaagaggaat 10200

acgtggatta catgccagta atgaaaagat acagcgctcc ttcagagagt gaaggagttc 10260

tgtaattacc aacaacaaac accaaaggct attgaagtca ggccacttgt gccacggctt 10320

gagcaaaccg tgctgcctgt agctccgcca ataatgggag gcgtgaaatc cctagggagg 10380

ccatgcgcca cggaagctgt acgcgtggca tattggacta gcggttagag gagacccctc 10440

ccatcactga caaaacgcag caaaaggggg cccgaagcca ggaggaagct gtactcctgg 10500

tggaaggact agaggttaga ggagaccccc ccaacacaaa aacagcatat tgacgctggg 10560

aaagaccaga gatcctgctg tctctgcaac atcaatccag gcacagagcg ccgcaagatg 10620

gattggtgtt gttgatccaa caggttct 10648

<210> 16
<211> 3387
<212> PRT
<213> dengue virus

<400> 16

Met Asn Gln Arg Lys Lys Val Val Arg Pro Pro Phe Asn Met Leu Lys
1 5 10 15

Arg Glu Arg Asn Arg Val Ser Thr Pro Gln Gly Leu Val Lys Arg Phe
20 25 30

Ser Thr Gly Leu Phe Ser Gly Lys Gly Pro Leu Arg Met Val Leu Ala
35 40 45

Phe Ile Thr Phe Leu Arg Val Leu Ser Ile Pro Pro Thr Ala Gly Ile
50 55 60

Leu Lys Arg Trp Gly Gln Leu Lys Lys Asn Lys Ala Ile Arg Ile Leu
65 70 75 80

Ile Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn Gly
85 90 95

Arg Lys Arg Ser Thr Ile Thr Leu Leu Cys Leu Ile Pro Thr Val Met
100 105 110

Ala Phe His Leu Ser Thr Arg Asp Gly Glu Pro Leu Met Ile Val Ala
115 120 125

Lys His Glu Arg Gly Arg Pro Leu Leu Phe Lys Thr Thr Glu Gly Ile
130 135 140

Asn Lys Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Glu Asp
145 150 155 160

Thr Val Thr Tyr Lys Cys Pro Leu Leu Val Asn Thr Glu Pro Glu Asp
165 170 175

Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Met Tyr Gly Thr
180 185 190

Cys Thr Gln Ser Gly Glu Arg Arg Arg Glu Lys Arg Ser Val Ala Leu
195 200 205

Thr Pro His Ser Gly Met Gly Leu Glu Thr Arg Ala Glu Thr Trp Met
210 215 220

Ser Ser Glu Gly Ala Trp Lys His Ala Gln Arg Val Glu Ser Trp Ile
225 230 235 240

Leu Arg Asn Pro Gly Phe Ala Leu Leu Ala Gly Phe Met Ala Tyr Met
245 250 255

Ile Gly Gln Thr Gly Ile Gln Arg Thr Val Phe Phe Val Leu Met Met
260 265 270

Leu Val Ala Pro Ser Tyr Gly Met Arg Cys Val Gly Val Gly Asn Arg
275 280 285

Asp Phe Val Glu Gly Val Ser Gly Gly Ala Trp Val Asp Leu Val Leu
290 295 300

Glu His Gly Gly Cys Val Thr Thr Met Ala Gln Gly Lys Pro Thr Leu
305 310 315 320

Asp Phe Glu Leu Thr Lys Thr Thr Ala Lys Glu Val Ala Leu Leu Arg
325 330 335

Thr Tyr Cys Ile Glu Ala Ser Ile Ser Asn Ile Thr Thr Ala Thr Arg
340 345 350

```
Cys Pro Thr Gln Gly Glu Pro Tyr Leu Lys Glu Glu Gln Asp Gln Gln
        355                 360                 365

Tyr Ile Cys Arg Arg Asp Val Val Asp Arg Gly Trp Gly Asn Gly Cys
    370                 375                 380

Gly Leu Phe Gly Lys Gly Gly Val Val Thr Cys Ala Lys Phe Ser Cys
385                 390                 395                 400

Ser Gly Lys Ile Thr Gly Asn Leu Val Gln Ile Glu Asn Leu Glu Tyr
                405                 410                 415

Thr Val Val Val Thr Val His Asn Gly Asp Thr His Ala Val Gly Asn
            420                 425                 430

Asp Thr Ser Asn His Gly Val Thr Ala Thr Ile Thr Pro Arg Ser Pro
        435                 440                 445

Ser Val Glu Val Lys Leu Pro Asp Tyr Gly Glu Leu Thr Leu Asp Cys
    450                 455                 460

Glu Pro Arg Ser Gly Ile Asp Phe Asn Glu Met Ile Leu Met Lys Met
465                 470                 475                 480

Lys Lys Lys Thr Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu Pro
                485                 490                 495

Leu Pro Trp Thr Ala Gly Ala Asp Thr Ser Glu Val His Trp Asn Tyr
            500                 505                 510

Lys Glu Arg Met Val Thr Phe Lys Val Pro His Ala Lys Arg Gln Asp
        515                 520                 525

Val Thr Val Leu Gly Ser Gln Glu Gly Ala Met His Ser Ala Leu Ala
    530                 535                 540

Gly Ala Thr Glu Val Asp Ser Gly Asp Gly Asn His Met Phe Ala Gly
545                 550                 555                 560

His Leu Lys Cys Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly Met
                565                 570                 575

Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met Ala
            580                 585                 590

Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly Ala
        595                 600                 605

Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys Glu
    610                 615                 620

Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn Thr
```

625 630 635 640

Asn Ser Ala Thr Asn Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser Tyr
645 650 655

Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe Arg
660 665 670

Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly Ala
675 680 685

Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser Val
690 695 700

Gly Gly Leu Phe Thr Ser Leu Gly Lys Ala Val His Gln Val Phe Gly
705 710 715 720

Ser Val Tyr Thr Thr Met Phe Gly Gly Val Ser Trp Met Ile Arg Ile
725 730 735

Leu Ile Gly Phe Leu Val Leu Trp Ile Gly Thr Asn Ser Arg Asn Thr
740 745 750

Ser Met Ala Met Thr Cys Ile Ala Val Gly Gly Ile Thr Leu Phe Leu
755 760 765

Gly Phe Thr Val Gln Ala Asp Met Gly Cys Val Val Ser Trp Ser Gly
770 775 780

Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Val Val Asp Asn Val His
785 790 795 800

Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ala Arg Leu
805 810 815

Ala Ser Ala Ile Leu Asn Ala His Lys Asp Gly Val Cys Gly Ile Arg
820 825 830

Ser Thr Thr Arg Leu Glu Asn Val Met Trp Lys Gln Ile Thr Asn Glu
835 840 845

Leu Asn Tyr Val Leu Trp Glu Gly Gly His Asp Leu Thr Val Val Ala
850 855 860

Gly Asp Val Lys Gly Val Leu Thr Lys Gly Lys Arg Ala Leu Thr Pro
865 870 875 880

Pro Val Asn Asp Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala Lys
885 890 895

Ile Phe Thr Pro Glu Ala Arg Asn Ser Thr Phe Leu Ile Asp Gly Pro
900 905 910

```
Asp Thr Ser Glu Cys Pro Asn Glu Arg Arg Ala Trp Asn Phe Leu Glu
        915                 920                 925

Val Glu Asp Tyr Gly Phe Gly Met Phe Thr Thr Asn Ile Trp Met Lys
    930                 935                 940

Phe Arg Glu Gly Ser Ser Glu Val Cys Asp His Arg Leu Met Ser Ala
945                 950                 955                 960

Ala Ile Lys Asp Gln Lys Ala Val His Ala Asp Met Gly Tyr Trp Ile
                965                 970                 975

Glu Ser Ser Lys Asn Gln Thr Trp Gln Ile Glu Lys Ala Ser Leu Ile
            980                 985                 990

Glu Val Lys Thr Cys Leu Trp Pro  Lys Thr His Thr Leu  Trp Ser Asn
        995                 1000                1005

Gly Val  Leu Glu Ser Gln Met  Leu Ile Pro Lys Ser  Tyr Ala Gly
    1010                1015                1020

Pro Phe  Ser Gln His Asn Tyr  Arg Gln Gly Tyr Ala  Thr Gln Thr
    1025                1030                1035

Val Gly  Pro Trp His Leu Gly  Lys Leu Glu Ile Asp  Phe Gly Glu
    1040                1045                1050

Cys Pro  Gly Thr Thr Val Ala  Ile Gln Glu Asp Cys  Asp His Arg
    1055                1060                1065

Gly Pro  Ser Leu Arg Thr Thr  Thr Ala Ser Gly Lys  Leu Val Thr
    1070                1075                1080

Gln Trp  Cys Cys Arg Ser Cys  Thr Met Pro Pro Leu  Arg Phe Leu
    1085                1090                1095

Gly Glu  Asp Gly Cys Trp Tyr  Gly Met Glu Ile Arg  Pro Leu Ser
    1100                1105                1110

Glu Lys  Glu Glu Asn Met Val  Lys Ser Gln Val Thr  Ala Gly Gln
    1115                1120                1125

Gly Thr  Ser Glu Thr Phe Ser  Met Gly Leu Leu Cys  Leu Thr Leu
    1130                1135                1140

Phe Val  Glu Glu Cys Leu Arg  Arg Arg Val Thr Arg  Lys His Met
    1145                1150                1155

Ile Leu  Val Val Val Ile Thr  Leu Cys Ala Ile Ile  Leu Gly Gly
    1160                1165                1170
```

```
Leu Thr  Trp Met Asp Leu Leu  Arg Ala Leu Ile Met  Leu Gly Asp
    1175                1180                1185

Thr Met  Ser Gly Arg Ile Gly  Gly Gln Ile His Leu  Ala Ile Met
    1190                1195                1200

Ala Val  Phe Lys Met Ser Pro  Gly Tyr Val Leu Gly  Val Phe Leu
    1205                1210                1215

Arg Lys  Leu Thr Ser Arg Glu  Thr Ala Leu Met Val  Ile Gly Met
    1220                1225                1230

Ala Met  Thr Thr Val Leu Ser  Ile Pro His Asp Leu  Met Glu Leu
    1235                1240                1245

Ile Asp  Gly Ile Ser Leu Gly  Leu Ile Leu Leu Lys  Ile Val Thr
    1250                1255                1260

His Phe  Asp Asn Thr Gln Val  Gly Thr Leu Ala Leu  Ser Leu Thr
    1265                1270                1275

Phe Ile  Arg Ser Thr Met Pro  Leu Val Met Ala Trp  Arg Thr Ile
    1280                1285                1290

Met Ala  Val Leu Phe Val Val  Thr Leu Ile Pro Leu  Cys Arg Thr
    1295                1300                1305

Ser Cys  Leu Gln Lys Gln Ser  His Trp Val Glu Ile  Thr Ala Leu
    1310                1315                1320

Ile Leu  Gly Ala Gln Ala Leu  Pro Val Tyr Leu Met  Thr Leu Met
    1325                1330                1335

Lys Gly  Ala Ser Arg Arg Ser  Trp Pro Leu Asn Glu  Gly Ile Met
    1340                1345                1350

Ala Val  Gly Leu Val Ser Leu  Leu Gly Ser Ala Leu  Leu Lys Asn
    1355                1360                1365

Asp Val  Pro Leu Ala Gly Pro  Met Val Ala Gly Gly  Leu Leu Leu
    1370                1375                1380

Ala Ala  Tyr Val Met Ser Gly  Ser Ser Ala Asp Leu  Ser Leu Glu
    1385                1390                1395

Lys Ala  Ala Asn Val Gln Trp  Asp Glu Met Ala Asp  Ile Thr Gly
    1400                1405                1410

Ser Ser  Pro Ile Ile Glu Val  Lys Gln Asp Glu Asp  Gly Ser Phe
    1415                1420                1425
```

```
Ser Ile  Arg Asp Val Glu Glu  Thr Asn Met Ile Thr  Leu Leu Val
    1430                 1435                 1440


Lys Leu  Ala Leu Ile Thr Val  Ser Gly Leu Tyr Pro  Leu Ala Ile
    1445                 1450                 1455


Pro Val  Thr Met Thr Leu Trp  Tyr Met Trp Gln Val  Lys Thr Gln
    1460                 1465                 1470


Arg Ser  Gly Ala Leu Trp Asp  Val Pro Ser Pro Ala  Ala Thr Gln
    1475                 1480                 1485


Lys Ala  Ala Leu Ser Glu Gly  Val Tyr Arg Ile Met  Gln Arg Gly
    1490                 1495                 1500


Leu Phe  Gly Lys Thr Gln Val  Gly Val Gly Ile His  Met Glu Gly
    1505                 1510                 1515


Val Phe  His Thr Met Trp His  Val Thr Arg Gly Ser  Val Ile Cys
    1520                 1525                 1530


His Glu  Thr Gly Arg Leu Glu  Pro Ser Trp Ala Asp  Val Arg Asn
    1535                 1540                 1545


Asp Met  Ile Ser Tyr Gly Gly  Gly Trp Arg Leu Gly  Asp Lys Trp
    1550                 1555                 1560


Asp Lys  Glu Glu Asp Val Gln  Val Leu Ala Ile Glu  Pro Gly Lys
    1565                 1570                 1575


Asn Pro  Lys His Val Gln Thr  Lys Pro Gly Leu Phe  Lys Thr Leu
    1580                 1585                 1590


Thr Gly  Glu Ile Gly Ala Val  Thr Leu Asp Phe Lys  Pro Gly Thr
    1595                 1600                 1605


Ser Gly  Ser Pro Ile Ile Asn  Arg Lys Gly Lys Val  Ile Gly Leu
    1610                 1615                 1620


Tyr Gly  Asn Gly Val Val Thr  Lys Ser Gly Asp Tyr  Val Ser Ala
    1625                 1630                 1635


Ile Thr  Gln Ala Glu Arg Ile  Gly Glu Pro Asp Tyr  Glu Val Asp
    1640                 1645                 1650


Glu Asp  Ile Phe Arg Lys Lys  Arg Leu Thr Ile Met  Asp Leu His
    1655                 1660                 1665


Pro Gly  Ala Gly Lys Thr Lys  Arg Ile Leu Pro Ser  Ile Val Arg
    1670                 1675                 1680


Glu Ala  Leu Lys Arg Arg Leu  Arg Thr Leu Ile Leu  Ala Pro Thr
```

```
    1685                1690                1695

Arg Val  Val Ala Ala Glu Met  Glu Glu Ala Leu Arg  Gly Leu Pro
    1700                1705                1710

Ile Arg  Tyr Gln Thr Pro Ala  Val Lys Ser Glu His  Thr Gly Arg
    1715                1720                1725

Glu Ile  Val Asp Leu Met Cys  His Ala Thr Phe Thr  Thr Arg Leu
    1730                1735                1740

Leu Ser  Ser Thr Arg Val Pro  Asn Tyr Asn Leu Ile  Val Met Asp
    1745                1750                1755

Glu Ala  His Phe Thr Asp Pro  Ser Ser Val Ala Ala  Arg Gly Tyr
    1760                1765                1770

Ile Ser  Thr Arg Val Glu Met  Gly Glu Ala Ala Ala  Ile Phe Met
    1775                1780                1785

Thr Ala  Thr Pro Pro Gly Ala  Thr Asp Pro Phe Pro  Gln Ser Asn
    1790                1795                1800

Ser Pro  Ile Glu Asp Ile Glu  Arg Glu Ile Pro Glu  Arg Ser Trp
    1805                1810                1815

Asn Thr  Gly Phe Asp Trp Ile  Thr Asp Tyr Gln Gly  Lys Thr Val
    1820                1825                1830

Trp Phe  Val Pro Ser Ile Lys  Ala Gly Asn Asp Ile  Ala Asn Cys
    1835                1840                1845

Leu Arg  Lys Ser Gly Lys Lys  Val Ile Gln Leu Ser  Arg Lys Thr
    1850                1855                1860

Phe Asp  Thr Glu Tyr Pro Lys  Thr Lys Leu Thr Asp  Trp Asp Phe
    1865                1870                1875

Val Val  Thr Thr Asp Ile Ser  Glu Met Gly Ala Asn  Phe Arg Ala
    1880                1885                1890

Gly Arg  Val Ile Asp Pro Arg  Arg Cys Leu Lys Pro  Val Ile Leu
    1895                1900                1905

Thr Asp  Gly Pro Glu Arg Val  Ile Leu Ala Gly Pro  Ile Pro Val
    1910                1915                1920

Thr Pro  Ala Ser Ala Ala Gln  Arg Arg Gly Arg Ile  Gly Arg Asn
    1925                1930                1935

Pro Ala  Gln Glu Asp Asp Gln  Tyr Val Phe Ser Gly  Asp Pro Leu
    1940                1945                1950
```

```
Lys Asn  Asp Glu Asp His Ala  His Trp Thr Glu Ala  Lys Met Leu
    1955                 1960                 1965

Leu Asp  Asn Ile Tyr Thr Pro  Glu Gly Ile Ile Pro  Thr Leu Phe
    1970                 1975                 1980

Gly Pro  Glu Arg Glu Lys Thr  Gln Ala Ile Asp Gly  Glu Phe Arg
    1985                 1990                 1995

Leu Arg  Gly Glu Gln Arg Lys  Thr Phe Val Glu Leu  Met Arg Arg
    2000                 2005                 2010

Gly Asp  Leu Pro Val Trp Leu  Ser Tyr Lys Val Ala  Ser Ala Gly
    2015                 2020                 2025

Ile Ser  Tyr Lys Asp Arg Glu  Trp Cys Phe Thr Gly  Glu Arg Asn
    2030                 2035                 2040

Asn Gln  Ile Leu Glu Glu Asn  Met Glu Val Glu Ile  Trp Thr Arg
    2045                 2050                 2055

Glu Gly  Glu Lys Lys Lys Leu  Arg Pro Arg Trp Leu  Asp Ala Arg
    2060                 2065                 2070

Val Tyr  Ala Asp Pro Met Ala  Leu Lys Asp Phe Lys  Glu Phe Ala
    2075                 2080                 2085

Ser Gly  Arg Lys Ser Ile Thr  Leu Asp Ile Leu Thr  Glu Ile Ala
    2090                 2095                 2100

Ser Leu  Pro Thr Tyr Leu Ser  Ser Arg Ala Lys Leu  Ala Leu Asp
    2105                 2110                 2115

Asn Ile  Val Met Leu His Thr  Thr Glu Arg Gly Gly  Arg Ala Tyr
    2120                 2125                 2130

Gln His  Ala Leu Asn Glu Leu  Pro Glu Ser Leu Glu  Thr Leu Met
    2135                 2140                 2145

Leu Val  Ala Leu Leu Gly Ala  Met Thr Ala Gly Ile  Phe Leu Phe
    2150                 2155                 2160

Phe Met  Gln Gly Lys Gly Ile  Gly Lys Leu Ser Met  Gly Leu Ile
    2165                 2170                 2175

Thr Ile  Ala Val Ala Ser Gly  Leu Leu Trp Val Ala  Glu Ile Gln
    2180                 2185                 2190

Pro Gln  Trp Ile Ala Ala Ser  Ile Ile Leu Glu Phe  Phe Leu Met
    2195                 2200                 2205
```

```
Val Leu  Leu Ile Pro Glu Pro  Glu Lys Gln Arg Thr  Pro Gln Asp
    2210                 2215                 2220

Asn Gln  Leu Ile Tyr Val Ile  Leu Thr Ile Leu Thr  Ile Ile Gly
    2225                 2230                 2235

Leu Ile  Ala Ala Asn Glu Met  Gly Leu Ile Glu Lys  Thr Lys Thr
    2240                 2245                 2250

Asp Phe  Gly Phe Tyr Gln Val  Lys Thr Glu Thr Thr  Ile Leu Asp
    2255                 2260                 2265

Val Asp  Leu Arg Pro Ala Ser  Ala Trp Thr Leu Tyr  Ala Val Ala
    2270                 2275                 2280

Thr Thr  Ile Leu Thr Pro Met  Leu Arg His Thr Ile  Glu Asn Thr
    2285                 2290                 2295

Ser Ala  Asn Leu Ser Leu Ala  Ala Ile Ala Asn Gln  Ala Ala Val
    2300                 2305                 2310

Leu Met  Gly Leu Gly Lys Gly  Trp Pro Leu His Arg  Met Asp Leu
    2315                 2320                 2325

Gly Val  Pro Leu Leu Ala Met  Gly Cys Tyr Ser Gln  Val Asn Pro
    2330                 2335                 2340

Thr Thr  Leu Thr Ala Ser Leu  Val Met Leu Leu Val  His Tyr Ala
    2345                 2350                 2355

Ile Ile  Gly Pro Gly Leu Gln  Ala Lys Ala Thr Arg  Glu Ala Gln
    2360                 2365                 2370

Lys Arg  Thr Ala Ala Gly Ile  Met Lys Asn Pro Thr  Val Asp Gly
    2375                 2380                 2385

Ile Thr  Val Ile Asp Leu Glu  Pro Ile Ser Tyr Asp  Pro Lys Phe
    2390                 2395                 2400

Glu Lys  Gln Leu Gly Gln Val  Met Leu Leu Val Leu  Cys Ala Gly
    2405                 2410                 2415

Gln Leu  Leu Leu Met Arg Thr  Thr Trp Ala Phe Cys  Glu Val Leu
    2420                 2425                 2430

Thr Leu  Ala Thr Gly Pro Ile  Leu Thr Leu Trp Glu  Gly Asn Pro
    2435                 2440                 2445

Gly Arg  Phe Trp Asn Thr Thr  Ile Ala Val Ser Thr  Ala Asn Ile
    2450                 2455                 2460
```

```
Phe Arg  Gly Ser Tyr Leu Ala  Gly Ala Gly Leu Ala  Phe Ser Leu
    2465                 2470                 2475

Ile Lys  Asn Val Gln Thr Pro  Arg Arg Gly Thr Gly  Thr Thr Gly
    2480                 2485                 2490

Glu Thr  Leu Gly Glu Lys Trp  Lys Arg Gln Leu Asn  Ser Leu Asp
    2495                 2500                 2505

Arg Lys  Glu Phe Glu Glu Tyr  Lys Arg Ser Gly Ile  Leu Glu Val
    2510                 2515                 2520

Asp Arg  Thr Glu Ala Lys Ser  Ala Leu Lys Asp Gly  Ser Lys Ile
    2525                 2530                 2535

Lys His  Ala Val Ser Arg Gly  Ser Ser Lys Ile Arg  Trp Ile Val
    2540                 2545                 2550

Glu Arg  Gly Met Val Lys Pro  Lys Gly Lys Val Val  Asp Leu Gly
    2555                 2560                 2565

Cys Gly  Arg Gly Gly Trp Ser  Tyr Tyr Met Ala Thr  Leu Lys Asn
    2570                 2575                 2580

Val Thr  Glu Val Lys Gly Tyr  Thr Lys Gly Gly Pro  Gly His Glu
    2585                 2590                 2595

Glu Pro  Ile Pro Met Ala Thr  Tyr Gly Trp Asn Leu  Val Lys Leu
    2600                 2605                 2610

His Ser  Gly Val Asp Val Phe  Tyr Lys Pro Thr Glu  Gln Val Asp
    2615                 2620                 2625

Thr Leu  Leu Cys Asp Ile Gly  Glu Ser Ser Ser Asn  Pro Thr Ile
    2630                 2635                 2640

Glu Glu  Gly Arg Thr Leu Arg  Val Leu Lys Met Val  Glu Pro Trp
    2645                 2650                 2655

Leu Ser  Ser Lys Pro Glu Phe  Cys Ile Lys Val Leu  Asn Pro Tyr
    2660                 2665                 2670

Met Pro  Thr Val Ile Glu Glu  Leu Glu Lys Leu Gln  Arg Lys His
    2675                 2680                 2685

Gly Gly  Asn Leu Val Arg Cys  Pro Leu Ser Arg Asn  Ser Thr His
    2690                 2695                 2700

Glu Met  Tyr Trp Val Ser Gly  Ala Ser Gly Asn Ile  Val Ser Ser
    2705                 2710                 2715

Val Asn  Thr Thr Ser Lys Met  Leu Leu Asn Arg Phe  Thr Thr Arg
```

2720 2725 2730

His Arg Lys Pro Thr Tyr Glu Lys Asp Val Asp Leu Gly Ala Gly
2735 2740 2745

Thr Arg Ser Val Ser Thr Glu Thr Glu Lys Pro Asp Met Thr Ile
2750 2755 2760

Ile Gly Arg Arg Leu Gln Arg Leu Gln Glu Glu His Lys Glu Thr
2765 2770 2775

Trp His Tyr Asp Gln Glu Asn Pro Tyr Arg Thr Trp Ala Tyr His
2780 2785 2790

Gly Ser Tyr Glu Ala Pro Ser Thr Gly Ser Ala Ser Ser Met Val
2795 2800 2805

Asn Gly Val Val Lys Leu Leu Thr Lys Pro Trp Asp Val Val Pro
2810 2815 2820

Met Val Thr Gln Leu Ala Met Thr Asp Thr Thr Pro Phe Gly Gln
2825 2830 2835

Gln Arg Val Phe Lys Glu Lys Val Asp Thr Arg Thr Pro Gln Pro
2840 2845 2850

Lys Pro Gly Thr Arg Met Val Met Thr Thr Thr Ala Asn Trp Leu
2855 2860 2865

Trp Ala Leu Leu Gly Lys Lys Lys Asn Pro Arg Leu Cys Thr Arg
2870 2875 2880

Glu Glu Phe Ile Ser Lys Val Arg Ser Asn Ala Ala Ile Gly Ala
2885 2890 2895

Val Phe Gln Glu Glu Gln Gly Trp Thr Ser Ala Ser Glu Ala Val
2900 2905 2910

Asn Asp Ser Arg Phe Trp Glu Leu Val Asp Lys Glu Arg Ala Leu
2915 2920 2925

His Gln Glu Gly Lys Cys Glu Ser Cys Val Tyr Asn Met Met Gly
2930 2935 2940

Lys Arg Glu Lys Lys Leu Gly Glu Phe Gly Arg Ala Lys Gly Ser
2945 2950 2955

Arg Ala Ile Trp Tyr Met Trp Leu Gly Ala Arg Phe Leu Glu Phe
2960 2965 2970

Glu Ala Leu Gly Phe Leu Asn Glu Asp His Trp Phe Gly Arg Glu
2975 2980 2985

```
Asn Ser  Trp Ser Gly Val Glu  Gly Glu Gly Leu His  Arg Leu Gly
    2990                 2995                 3000

Tyr Ile  Leu Glu Glu Ile Asp  Lys Lys Asp Gly Asp  Leu Met Tyr
    3005                 3010                 3015

Ala Asp  Asp Thr Ala Gly Trp  Asp Thr Arg Ile Thr  Glu Asp Asp
    3020                 3025                 3030

Leu Gln  Asn Glu Glu Leu Ile  Thr Glu Gln Met Ala  Pro His His
    3035                 3040                 3045

Lys Ile  Leu Ala Lys Ala Ile  Phe Lys Leu Thr Tyr  Gln Asn Lys
    3050                 3055                 3060

Val Val  Lys Val Leu Arg Pro  Thr Pro Arg Gly Ala  Val Met Asp
    3065                 3070                 3075

Ile Ile  Ser Arg Lys Asp Gln  Arg Gly Ser Gly Gln  Val Gly Thr
    3080                 3085                 3090

Tyr Gly  Leu Asn Thr Phe Thr  Asn Met Glu Val Gln  Leu Ile Arg
    3095                 3100                 3105

Gln Met  Glu Ala Glu Gly Val  Ile Thr Gln Asp Asp  Met Gln Asn
    3110                 3115                 3120

Pro Lys  Gly Leu Lys Glu Arg  Val Glu Lys Trp Leu  Lys Glu Cys
    3125                 3130                 3135

Gly Val  Asp Arg Leu Lys Arg  Met Ala Ile Ser Gly  Asp Asp Cys
    3140                 3145                 3150

Val Val  Lys Pro Leu Asp Glu  Arg Phe Gly Thr Ser  Leu Leu Phe
    3155                 3160                 3165

Leu Asn  Asp Met Gly Lys Val  Arg Lys Asp Ile Pro  Gln Trp Glu
    3170                 3175                 3180

Pro Ser  Lys Gly Trp Lys Asn  Trp Gln Glu Val Pro  Phe Cys Ser
    3185                 3190                 3195

His His  Phe His Lys Ile Phe  Met Lys Asp Gly Arg  Ser Leu Val
    3200                 3205                 3210

Val Pro  Cys Arg Asn Gln Asp  Glu Leu Ile Gly Arg  Ala Arg Ile
    3215                 3220                 3225

Ser Gln  Gly Ala Gly Trp Ser  Leu Arg Glu Thr Ala  Cys Leu Gly
    3230                 3235                 3240
```

```
Lys Ala  Tyr Ala Gln Met Trp  Ser Leu Met Tyr Phe  His Arg Arg
    3245                 3250                 3255

Asp Leu  Arg Leu Ala Ser Met  Ala Ile Cys Ser Ala  Val Pro Thr
    3260                 3265                 3270

Glu Trp  Phe Pro Thr Ser Arg  Thr Thr Trp Ser Ile  His Ala His
    3275                 3280                 3285

His Gln  Trp Met Thr Thr Glu  Asp Met Leu Lys Val  Trp Asn Arg
    3290                 3295                 3300

Val Trp  Ile Glu Asp Asn Pro  Asn Met Thr Asp Lys  Thr Pro Val
    3305                 3310                 3315

His Ser  Trp Glu Asp Ile Pro  Tyr Leu Gly Lys Arg  Glu Asp Leu
    3320                 3325                 3330

Trp Cys  Gly Ser Leu Ile Gly  Leu Ser Ser Arg Ala  Thr Trp Ala
    3335                 3340                 3345

Lys Asn  Ile His Thr Ala Ile  Thr Gln Val Arg Asn  Leu Ile Gly
    3350                 3355                 3360

Lys Glu  Glu Tyr Val Asp Tyr  Met Pro Val Met Lys  Arg Tyr Ser
    3365                 3370                 3375

Ala Pro  Ser Glu Ser Glu Gly  Val Leu
    3380                 3385
```