CLAIMS

1. A dengue vaccine formulation comprising:
   A. a tetravalent dengue virus composition comprising:
      (i) a live attenuated dengue virus serotype 1,
      (ii) a live attenuated dengue virus serotype 2,
      (iii) a live attenuated dengue virus serotype 3, and
      (iv) a live attenuated dengue virus serotype 4; and
   B. an excipient composition comprising:
      a. trehalose,
      b. poloxamer,
      c. urea,
      d. arginine hydrochloride,
      e. tromethamine, and
      f. human serum albumin.

2. The dengue vaccine formulation according to claim 1, wherein the ratio of the amount of arginine hydrochloride to the amount of human serum albumin contained in the dengue vaccine formulation is 1:1 to 4:1.

3. The dengue vaccine formulation according to claim 1 or 2, wherein the ratio of the amount of urea to the amount of human serum albumin contained in the dengue vaccine formulation is 1:1 to 5:1 and/or wherein the ratio of the amount of urea to the amount of poloxamer contained in the dengue vaccine formulation is 1:1 to 2:1.

4. The dengue vaccine formulation according to any one of claims 1 to 3, wherein phosphate salts are contained in the dengue vaccine formulation at an amount of less than 0.3 mg/unit dose.

5. The dengue vaccine formulation according to any one of claims 1 to 4, wherein chloride salts are contained in the dengue vaccine formulation at an amount of less than 2.0 mg/unit dose.

6. The dengue vaccine formulation according to any one of claims 1 to 5, wherein the dengue vaccine formulation comprises:
   a. the trehalose at an amount of 20 to 34 mg/unit dose,
   b. the poloxamer at an amount of 0.1 to 3.0 mg/unit dose,
   c. the urea at an amount of 0.1 to 3.0 mg/unit dose,
   d. the arginine hydrochloride at an amount of 0.1 to 2.0 mg/unit dose,
   e. the tromethamine at an amount of 0.05 to 1.0 mg/unit dose, and
   f. the human serum albumin at an amount of 0.05 to 0.5 mg/unit dose.

7. The dengue vaccine formulation according to any one of claims 1 to 6, wherein hydrochloric acid is contained in the dengue vaccine formulation at an amount of 0.01 to 0.5 mg/unit dose.

8. The dengue vaccine formulation according to any one of claims 1 to 7, preferably in dry form, wherein the dengue vaccine formulation comprises based on the dry weight of the vaccine formulation:
   a. 65 to 95 % w/w trehalose,
   b. 0.05 to 5.0 % w/w poloxamer,
   c. 0.1 to 6.0 % w/w urea,
   d. 0.1 to 5.0 % w/w arginine hydrochloride,
   e. 0.05 to 5.0 % w/w tromethamine, and
   f. 0.05 to 3.0 % w/w human serum albumin.

9. The dengue vaccine formulation according to any one of claims 1 to 8, wherein a unit dose of the dengue vaccine formulation in dry form upon reconstitution with a pharmaceutically acceptable aqueous diluent to a volume of 0.5 mL comprises:
   a. the trehalose in a concentration of from 40 to 80 g/L,
   b. the poloxamer in a concentration of 0.1 to 5.0 g/L,
   c. the urea in a concentration of 0.1 to 6.0 g/L,
   d. the arginine hydrochloride in a concentration of 0.5 to 5.0 g/L,
   e. the tromethamine in a concentration of 0.1 to 1.5 g/L, and
   f. the human serum albumin in a concentration of from 0.1 to less than 1 g/L.

10. The dengue vaccine formulation according to any one of claims 1 to 9, wherein a unit dose of the dengue vaccine formulation in dry form upon reconstitution with a pharmaceutically acceptable aqueous diluent to a volume of 0.5 mL comprises chloride salts in a concentration of less than 5 g/L.

11. The dengue vaccine formulation according to any one of claims 1 to 10, wherein a unit dose of the dengue vaccine formulation in dry form upon reconstitution with a pharmaceutically acceptable aqueous diluent to a volume of 0.5 mL has an osmolality of 100 to 700 mOsmol/kg.

12. The dengue vaccine formulation according to any one of claims 1 to 11, wherein a unit dose of the dengue vaccine formulation in dry form upon reconstitution with a pharmaceutically acceptable aqueous diluent to a volume of 0.5 mL comprises:
   (i) the dengue virus serotype 1 in an amount of at least 3.3 log10 pfu/0.5 mL,
   (ii) the dengue virus serotype 2 in an amount of at least 2.7 log10 pfu/0.5 mL,
   (iii) the dengue virus serotype 3 in an amount of at least 4.0 log10 pfu/0.5 mL, and

(iv) the dengue virus serotype 4 in an amount of at least 4.5 log10 pfu/0.5 mL.

13. The dengue vaccine formulation according to any one of claims 1 to 12, wherein the tetravalent dengue virus composition includes at least one non-chimeric dengue virus and at least one chimeric dengue virus.

14. The dengue vaccine formulation according to any one of claims 1 to 13, wherein the dengue virus serotype 1 is a chimeric dengue serotype 2/1 strain, the dengue virus serotype 2 is a non-chimeric dengue serotype 2 strain, the dengue virus serotype 3 is a chimeric dengue serotype 2/3 strain and the dengue virus serotype 4 is a chimeric dengue serotype 2/4 strain, preferably the dengue serotype 1 is TDV-1, the dengue serotype 2 is TDV-2, the dengue serotype 3 is TDV-3 and the dengue serotype 4 is TDV-4.

15. The dengue vaccine formulation according to any one of claims 1 to 14, wherein the tetravalent dengue virus composition is “TAK-003”.

16. The dengue vaccine formulation according to any one of claims 1 to 15, which is a lyophilized dengue vaccine formulation.

17. The dengue vaccine formulation according to any one of claims 1 to 16, wherein the amount of dengue virus before lyophilization and the amount of dengue virus immediately after lyophilization differs by less than 0.46 log10 pfu/unit dose for each of serotypes 1 to 4.

18. The dengue vaccine formulation according to any one of claims 1 to 17, wherein the amount of dengue virus immediately after lyophilization and the amount of dengue virus after lyophilization and storage at about 25 °C and 65% relative humidity for 12 weeks differs by less than 1.6 log10 pfu/unit dose for each of serotypes 1 to 4.

19. The dengue vaccine formulation according to any one of claims 1 to 18, wherein the amount of dengue virus immediately after lyophilization and the amount of dengue virus after lyophilization and storage at 2-8 °C for 12 months differs by less than 0.44 log10 pfu/unit dose for each of serotypes 1 to 4.

20. A container, preferably a vial, comprising one or more than one unit dose of the dengue vaccine formulation according to any one of claims 1 to 19.

21. A container, preferably a vial comprising five unit doses of the dengue vaccine formulation according to any one of claims 1 to 19.

22. The container, preferably the vial, according to claim 21, wherein
a. the trehalose is contained at an amount of 100 to 170 mg,

b. the poloxamer is contained at an amount of 2.0 to 8.0 mg,
c. the urea is contained at an amount of 2.0 to 8.0 mg,
d. the arginine hydrochloride is contained at an amount of 1.5 to 7.5 mg,
e. the tromethamine is contained at an amount of 0.5 to 4.0 mg, and
f. the human serum albumin is contained at an amount of 0.1 to 2.5 mg.

23. A kit comprising more than one, preferably ten, container(s) according to any one of claims 20 to 22, and optionally one or more than one, preferably ten, container(s) comprising a pharmaceutically acceptable aqueous diluent for reconstitution.

24. A kit for preparing a dengue vaccine formulation in reconstituted form comprising:
a) a container, preferably a vial, comprising the dengue vaccine formulation according to any one of claims 1 to 19 in dry form, and
b) a container, preferably a vial, comprising a pharmaceutically acceptable aqueous diluent for reconstitution.

25. The kit according to claim 23 or 24, wherein the pharmaceutically acceptable aqueous diluent is selected from the group consisting of water for injection and an aqueous sodium chloride solution, preferably the pharmaceutically acceptable aqueous diluent is 37 mM aqueous sodium chloride solution.

26. A unit dose of a dengue vaccine formulation in aqueous form obtainable by reconstituting one to ten, preferably five, unit dose(s) of the dengue vaccine formulation according to claims 1 to 19 in dry form with an equal number of aliquots of a pharmaceutically acceptable aqueous diluent and removing the volume of a unit dose of the reconstituted dengue vaccine formulation.

27. The unit dose according to claim 26, wherein the aliquot has a volume of 0.4 to 0.8 mL/unit dose, preferably of 0.4 to 0.7 mL/unit dose, more preferably of 0.45 to 0.65 mL/unit dose.

28. A method of preparing a dengue vaccine formulation according to any one of claims 1 to 19 in dry form comprising the following steps:
(a) providing a dengue vaccine formulation in aqueous form comprising:
A. a tetravalent dengue virus composition comprising:
(i) a live attenuated dengue virus serotype 1,
(ii) a live attenuated dengue virus serotype 2,
(iii) a live attenuated dengue virus serotype 3, and
(iv) a live attenuated dengue virus serotype 4; and
B. an excipient composition comprising:
a. trehalose,
b. poloxamer,

c. urea,
d. arginine hydrochloride,
e. tromethamine, and
f. human serum albumin,

(b) freezing the dengue vaccine formulation in aqueous form in an apparatus with a shelf temperature of below -35 °C to form a frozen dengue vaccine formulation,

(c) subjecting the frozen dengue vaccine formulation to a primary drying step in an apparatus with a shelf temperature of from -31 to -19 °C, for example of from -31 to -21 °C, and at a pressure below 0.1 mbar to form a primary dried product, and

(d) subjecting the primary dried product to a secondary drying step in an apparatus with a shelf temperature of at least 15 °C, for example at least 20 °C, and at a pressure below 1 mbar to form a secondary dried product.

29. The method according to claim 28, wherein the dengue vaccine formulation in aqueous form is subjected to the freezing step b) in a volume, which is less than the volume used for reconstituting the lyophilized dengue vaccine formulation, preferably the dengue vaccine formulation in aqueous form is subjected to the freezing step b) in containers, such as vials, each comprising one or more than one unit dose of the dengue vaccine formulation in a volume, which is about half of the volume used for reconstituting the one or more than one unit dose of the lyophilized dengue vaccine formulation.

30. Use of the dengue vaccine formulation according to any one of claims 1 to 19, or the container according to any one of claims 20 to 22, or the kit according to any one of claims 23 to 25, or the unit dose according to claim 26 or 27, in the manufacture of a medicament for preventing dengue disease in a subject, comprising that a primary vaccination is to be administered with only two administrations of a unit dose comprising the steps of:

a first unit dose of the dengue vaccine formulation is to be administered to the subject, and

a second unit dose of the dengue vaccine formulation is to be administered to the subject, preferably within 3 months of administration of the first unit dose,

and optionally a booster dose is to be administered at least 12 months after administration of said second unit dose of the dengue vaccine formulation.