CLAIMS

1. A process for preparing an attenuated tetravalent dengue vaccine comprising:

(i) amplifying Vero cells in culture to produce Master and Working banks of Vero cells, wherein the Vero cells are adapted for growth in serum-free medium, are grown in serum-free medium, and are sub-cultured with trypsin of non-animal origin of this cell in 225 cm^2 Tissue Culture (TC)-flasks and later in a Cell Factory System™ (CFS);

(ii) infecting Vero cells from the Master or Working bank with attenuated dengue virus serotypes 1, 2, 3 and 4 from a Seed or Working bank of each virus, wherein the Vero cells are independently infected with attenuated dengue virus serotypes 1, 2, 3, and 4 in separate cultures with serum free medium;

(iii) incubating the 225 cm^2 TC-flasks or Cell Factory System™ (CFS) containing the Vero cells infected with each dengue virus at 36.5°C (± 1°C) for 10 to 20 days;

(iv) harvesting the supernatants of each culture;

(v) filtering each dengue virus suspension from step (iv) through a membrane with 0.2µm of porosity and storing the filtered dengue virus at -80°C (± 5°C);

(vi) preparing dengue virus bulks of the serotypes 1, 2, 3 and 4;

(vii) formulating monovalent vaccines;

(viii) formulating tetravalent vaccine by mixing the monovalent vaccines;

(ix) filling vials with the tetravalent vaccine; (x) lyophilizing the tetravalent vaccine in the vials;

(xi) sealing the lyophilized tetravalent vaccine in

the vials; and

(xii) storing the lyophilized and sealed product at 2-8°C, thereby preparing an attenuated tetravalent dengue vaccine.

2. The process according to claim 1, wherein the supernatant is harvested in step (iv), an equal volume of serum free medium is added to the culture, and steps (iii) and (iv) are repeated.

3. The process according to claim 2, wherein steps (iii) and (iv) are repeated one to about seven times.

4. The process according to claim 1, wherein the dengue virus strains used are rDEN1Δ30-1545 (SEQ ID NO:1); rDEN2/4Δ30(ME)-1495,7163 (SEQ ID NO:2) ; rDEN3Δ30/31-7164 (SEQ ID NO:3), and rDEN4Δ30-7132,7163,8308 (SEQ ID NO:4).

5. A process for preparing an attenuated tetravalent dengue vaccine comprising:

(i) amplifying Vero cells in culture to produce Master and Working banks of Vero cells, wherein the Vero cells are adapted for growth in serum-free medium, are grown in serum-free medium, and are sub-cultured with trypsin of non-animal origin ;

(ii) infecting Vero cells from the Master or Working bank with attenuated dengue virus serotypes 1, 2, 3 and 4 from a Seed or Working bank of each virus, wherein the Vero cells are independently infected with attenuated dengue virus serotypes 1, 2, 3, and 4 in separate cultures with serum free medium;

(iii) incubating the Vero cells infected with each dengue virus at 36.5°C (± 1°C) for 10 to 20 days in a tissue culture flask or Cell Factory System™;

(iv) harvesting the supernatants of each culture;

(v) filtering each dengue virus suspension from step

(iv) through a membrane with 0.2µm of porosity and storing the filtered dengue virus at -80°C (± 5°C);

(vi) preparing dengue virus bulks of the serotypes 1, 2, 3 and 4;

(vii) formulating monovalent vaccines; and

(viii) formulating tetravalent vaccine by mixing the monovalent vaccines.

6. The process according to claim 5 wherein the Vero cell line used is ATCC CCL-81.4.

7. The process according to claim 5, wherein the supernatant is harvested in step (iv), an equal volume of serum free medium is added to the culture, and steps (iii) and (iv) are repeated.

8. The process according to claim 7, wherein steps (iii) and (iv) are repeated one to about seven times.

9. The process according to claim 5, wherein the dengue virus strains used are rDEN1Δ30-1545 (SEQ ID NO:1) ; rDEN2/4Δ30(ME)-1495,7163 (SEQ ID NO:2) ; rDEN3Δ30/31-7164 (SEQ ID NO:3) , and rDEN4Δ30-7132,7163,8308 (SEQ ID NO:4).

10. The process according to claim 9, wherein the multiplicity-of-infection (MOI) of dengue virus strains for each dengue serotype is: 0.01 to 0.03 for DENV 1 and 4, 0.02 to 0.04 for DENV 2, and 0.05 to 0.08 for DENV3.

11. The process according to claim 5, wherein the monovalent vaccines are mixed in the same ratio of volume to obtain the tetravalent dengue vaccine 1,2,3,4 (attenuated).

12. The process according to any one of claims 5 to 11, that further comprises the steps of filling vials with the tetravalent vaccine; lyophilizing the tetravalent vaccine in the vials; sealing the lyophilized tetravalent vaccine in the vials;
and storing the lyophilized and sealed product at 2-8°C.

13. The process according to claim 12, wherein the parameters used in the lyophilizing process are: freezing (-30 to -50°C), vacuum (20 to 100µbar), primary drying from -30 to -50°C (36 to 42h), and -5 to -10°C (18 to 24h) secondary drying 25 to 29°C (8 to 15h).

14. A process for preparing a tetravalent dengue vaccine for administration to a subject that comprises the step of reconstituting the sealed and lyophilized tetravalent dengue vaccine from any one of claims 1 to 4 or 12 to 13 in a composition comprising 0.2M sodium phosphate monobasic dihydrate, 0.2M sodium phosphate dibasic heptahydrate, and water.

15. The process according to claim 14, wherein the sealed and lyophilized vaccine is reconstituted in 5 milliliters of the composition.

16. A tetravalent dengue vaccine kit that comprises an attenuated tetravalent dengue vaccine that is produced by the process as described in any one of claims 1 to 15, a reconstitution composition comprising 0.2M sodium phosphate monobasic dihydrate, 0.2M sodium phosphate dibasic heptahydrate and water.