We Claim:

1. An immunogenic composition comprising:
   a) one or more live attenuated dengue (DEN) virus;
   b) sucrose 3 to 6% (w/v); and
   c) glycine 3 to 6% (w/v);

wherein, the composition is lyophilized~~freeze-dried~~ and the reconstituted composition preserves the desired characteristics of a virus, including virus viability, immunogenicity and stability.

2. The immunogenic composition as claimed in claim 1, wherein the one or more live attenuated dengue (DEN) virus comprises a plurality of live attenuated dengue (DEN) viruses of different serotypes selected from group of DEN-1, DEN-2, DEN-3 and DEN-4.

3. The immunogenic composition as claimed in claim 2, wherein the live attenuated dengue (DEN) viruses is tetravalent comprising dengue virus serotypes DEN-1, DEN-2, DEN-3 and DEN-4.

4. The immunogenic composition as claimed in claim 3, wherein the live attenuated dengue (DEN) viruses is recombinant dengue viruses and/or a chimeric dengue viruses comprising a first nucleotide sequence encoding at

least one structural proteins from a first dengue virus and a second nucleotide sequence encoding non-structural proteins from a second dengue virus.

5. The immunogenic composition as claimed in any one of claims 1-4, wherein said live attenuated dengue virus serotypes DEN-1, DEN-2, DEN-3 and DEN-4 carry 30 nucleotide deletion denoted Δ30 mutation and/or carry 31 nucleotide deletion denoted Δ31 mutation in the 3' untranslated region of dengue virus genome.

6. The immunogenic composition as claimed in any one of the claims 1-5, wherein said live attenuated dengue virus serotypes DEN-1, DEN-2, DEN-3 and DEN-4 have a phenotype which is temperature sensitivity in Vero cells or the human liver cell line HuH-7.

~~7. The immunogenic composition as claimed in any one of claims 1-6, wherein the said composition is lyophilized (freeze-dried).~~

7. ~~8.~~ The immunogenic composition as claimed in any one of claims 1-6~~7~~, wherein the lyophilized composition is reconstituted with an aqueous solution, preferably saline or WFI (water for injection), wherein the final pH of the immunogenic composition is 7-8.

8.~~9.~~ The immunogenic composition as claimed in any one of the claims 1-7~~8~~, wherein the dengue virus is present at a dose of not less than 2.5 $\log_{10}$ PFU per 0.5 ml.

9.~~10.~~ The immunogenic composition as claimed in claim 1 comprising:

a) Dengue virus serotype 1 (rDEN 1Δ30),
b) Dengue virus serotype 2 (rDEN 2/4Δ30),
c) Dengue virus serotype 3 (rDEN 3Δ30/31),
d) Dengue virus serotype 4 (rDEN 4Δ30),
e) Sucrose 3 to 6% (w/v), and
f) Glycine 3 to 6% (w/v).

10.~~11.~~ The immunogenic composition as claimed in claim 9~~10~~ comprising:

a) Dengue virus serotype 1 (rDEN 1Δ30),
b) Dengue virus serotype 2 (rDEN 2/4Δ30),
c) Dengue virus serotype 3 (rDEN 3Δ30/31),
d) Dengue virus serotype 4 (rDEN 4Δ0),
e) Sucrose 4 to 5% (w/v), and
f) Glycine 4 to 5% (w/v).

11.~~12.~~ The immunogenic composition as claimed in claim 9~~10~~ comprising:

a) Dengue virus serotype 1 (rDEN 1Δ30), not less than 2.5 $\log_{10}$ PFU per 0.5 ml,

b) Dengue virus serotype 2 (rDEN 2/4Δ30), not less than 2.5~~3.0~~ log_{10} PFU per 0.5 ml,

c) Dengue virus serotype 3 (rDEN 3Δ30/31), not less than 2.5 log_{10} PFU per 0.5 ml,

d) Dengue virus serotype 4 (rDEN 4Δ30), not less than 2.5 log_{10} PFU per 0.5 ml,

e) Sucrose 5% (w/v), and

f) Glycine 5% (w/v).

12.~~13.~~ The immunogenic composition as claimed in claim 9~~10~~ comprising:

a) Dengue virus serotype 1 (rDEN 1Δ30), not less than 2.5 log_{10} PFU per 0.5 ml,

b) Dengue virus serotype 2 (rDEN 2/4Δ30), not less than 2.5~~3.0~~ log_{10} PFU per 0.5 ml,

c) Dengue virus serotype 3 (rDEN 3Δ30/31), not less than 2.5 log_{10} PFU per 0.5 ml,

d) Dengue virus serotype 4 (rDEN 4Δ30), not less than 2.5 log_{10} PFU per 0.5 ml,

e) Sucrose 4.5% (w/v), and

f) Glycine 5% (w/v).

13.~~14.~~ The immunogenic composition as claimed in claim 9~~10~~ comprising:

a) Dengue virus serotype 1 (rDEN 1Δ30), not less than 2.5 log_{10} PFU per 0.5 ml,

b) Dengue virus serotype 2 (rDEN 2/4Δ30), not less than 2.5~~3.0~~ log_{10} PFU per 0.5 ml,

c) Dengue virus serotype 3 (rDEN 3Δ30/31), not less than 2.5 log_{10} PFU per 0.5 ml,

d) Dengue virus serotype 4 (rDEN 4Δ30), not less than 2.5 log_{10} PFU per 0.5 ml,

e) Sucrose 6% (w/v), and

f) Glycine 6% (w/v).

14.~~15.~~ A method of manufacturing an immunogenic composition as claimed in claims 1-13~~14~~, said method comprising:

a) Multiple Harvesting of Supernatant comprising at least one serotype of dengue virus in MEM with Hanks salt solution additionally containing Dextrose, L-Glutamine and Sodium Bicarbonate, wherein the multiple harvesting is carried out from a single batch;

b) Filtering the viral harvest by direct flow filtration (DFF) through at least one clarification filter having a pore size of between 6 micrometers to 0.45 micrometers;

c) Treating the viral harvest with a non-specific endonuclease at 34±1°C for 2 hours at least, wherein the non-specific endonuclease is benzonase having concentration in the range of 0.5 units/ml to 5 units/ ml;

d) Concentrating the viral harvest by tangential flow filtration (TFF) using a membrane with a molecular weight cut off (MWCO) of 100kDa;

e) Stabilizing the viral harvest with a stabilizing agent comprising sucrose at a concentration of 3 to 6 % and glycine at a concentration of 3 to 6 % to form a stabilized viral harvest;

f) Sterilizing the stabilized viral harvest by DFF through at least one clarification filter having a pore size of between 0.8 micrometers to 0.2 micrometers to form a sterilized viral harvest of purified virus;

wherein the overall recovery of purified viruses is at least 50%, and

g) ~~Optionally~~ ~~F~~freeze drying Lyophilizing the sterilized viral harvest, comprising the steps of freezing, primary drying and secondary drying, wherein

- the freezing step comprises freezing at -45 °C for 690 minutes to 930 minutes,
- the primary drying step comprises ramping at + 0.5 °C/ minute to 1.0 °C/ minute to achieve shelf temperature of -25 °C, and holding for 1800 minutes to 1980 minutes, and
- the secondary drying step comprises ramping at 0.5 °C/ minute to 1.0 °C/ minute to achieve shelf temperature of + 25 °C, and holding for 420 minutes to 540 minutes.

15.~~16.~~ The method as claimed in claim 14~~15~~, wherein the viral harvest is treated with Benzonase having concentration of 1.25 units/ml.

16.~~17.~~ The method as claimed in claim 14~~15~~, wherein the viral harvest is subjected to tangential flow filtration (TFF) resulting in at least 10X concentration of viral harvest.

17.~~18.~~ The method as claimed in claim 14~~15~~, wherein the stabilizing agent comprises sucrose at a concentration of 4 to 5% (w/v) and glycine at a concentration of 4 to 5% (w/v).

18.~~19.~~ The method as claimed in claim 14~~15~~, wherein the stabilizing agent comprises sucrose at a concentration of 5% (w/v) and glycine at a concentration of 5% (w/v).

19.~~20.~~ The method as claimed in claim 14~~15~~, wherein the stabilizing agent comprises sucrose at a concentration of 4.5% (w/v) and glycine at a concentration of 5% (w/v).

20.~~21.~~ The method as claimed in claim 14~~15~~, wherein the stabilizing agent comprises sucrose at a concentration of 6% (w/v) and glycine at a concentration of 6% (w/v).

21.~~2.~~ The method as claimed in claim 14~~15~~, wherein the freezing step comprises freezing at -45°C for 60 minutes.

22~~3~~. The method as claimed in claim 14~~15~~, wherein the primary drying step comprises ramping at +0.5°C/minute to 1.0°C/minute to achieve shelf temperature of -32°C, and holding for 600 minutes to 1800 minutes.

23~~4~~. The method as claimed in claim 14~~15~~, wherein the secondary drying step comprises ramping at +0.5°C/minute to 1.0°C/minute to achieve shelf temperature of +25°C, holding for 360 minutes to 600 minutes.

24~~5~~. The method as claimed in any one of the claims 14-23~~15-24~~, wherein the lyophilized immunogenic composition is stored at 25°C for 6 months.

256. The method as claimed in any one of the claims 14-2315-24, wherein the lyophilized immunogenic composition is stored at 37°C for 14 days.

267. The method as claimed in any one of the claims 14-2315-24, wherein the lyophilized immunogenic composition is stored at 42°C for 2 days.

27~~8~~. A kit comprising a first container containing an immunogenic composition as claimed in any one of the claims 1-6~~7~~ and ~~10-14~~ 9-13 and a second container containing an aqueous solution, preferably saline or WFI (water for injection) for the reconstitution of the lyophilized ~~(freeze-dried)~~ immunogenic composition.