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 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).

8. The immunogenic composition as claimed in claim 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.

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

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).

11. The immunogenic composition as claimed in claim 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).

12. The immunogenic composition as claimed in claim 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 3.0 log_{10} PFU per 0.5 ml,

c) Dengue virus serotype3 (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).

13. The immunogenic composition as claimed in claim 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 3.0 log_{10} PFU per 0.5 ml,

c) Dengue virus serotype3 (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).

14. The immunogenic composition as claimed in claim 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 3.0 $\log_{10}$ PFU per 0.5 ml,

c) Dengue virus serotype3 (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).

15. A method of manufacturing an immunogenic composition as claimed in claims 1-14, 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 freeze drying 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.

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

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

18. The method as claimed in claim 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).

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

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

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

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

23. The method as claimed in claim 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.

24. The method as claimed in claim 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.

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

26. The method as claimed in any one of the claims 15-24, wherein the lyophilized immunogenic composition is stored at 37°C for14 days.

27. The method as claimed in any one of the claims 15-24, wherein the lyophilized immunogenic composition is stored at 42°C for 2 days.

28. A kit comprising a first container containing an immunogenic composition as claimed in any one of the claims 1-7 and 10-14 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.