To date, the majority of the vaccines tested clinically are live, attenuated vaccines. The use of non-replicating vaccine candidates is also being explored. For example, Ivy *et al*. (U.S. Patent 6,432,411) disclose a tetravalent subunit vaccine comprising DEN1-4 80% E (the peptide region of DEN1-4 corresponding to amino acids 1-395 of the DENV-2 envelope polypeptide) proteins. Ivy et al, *supra*, also report compositions comprising DENV 1-4 80% E and ISCOMATRIX® adjuvant. Coller *et al*. (WO 2012/154202) disclose tetravalent formulations comprising DEN1-4 80% E of DEN 1-4. Inactivated viruses may also be used as potential vaccine candidates or as components of an effective vaccine (Putnak et al. *Vaccine* 23: 4442–4452 (2005), US 6190859, US 6254873 and Sterner *et al*. WO 2007/002470). Compositions comprising a live attenuated dengue virus vaccine and a non-replicating dengue vaccine are disclosed in International Patent Application No. PCT/US14/042625 (WO2014/204892).

Whole viruses are one of the commonly used antigens in several vaccine products due to their ability to generate humoral and cellular immune responses. Vaccine products containing whole viruses are challenging to stabilize as these are sensitive to heat, freeze/thaw and other processing stresses leading to significant potency losses. These products are typically stored frozen (below -20°C) or as dried powder. Frozen products are not easy to store and distribute as they need a stringent cold-chain requirement to prevent potency loss. Drying of whole viruses, especially enveloped viruses, often leads to significant loss of potency due to the freezing and drying stresses encountered during the drying process. Therefore, there is a need in the art to generate stable formulations of Dengue virus.

SUMMARY

The current disclosure provides stable formulations of live attenuated dengue vaccine. The addition of a cellulose derivative and a sugar alcohol or glycol improved stability and/or yield after drying. Alternatively, the addition of sugar of at least 150 mg/ml improved stability and/or yield after microwave drying.

The disclosure provides a formulation comprising a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV), a buffer at pH about 6.5 to 8.5, a sugar, a glycol or sugar alcohol, and a cellulose derivative selected from the group consisting of carboxymethyl cellulose, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), 2-hydroxyethyl cellulose (2-HEC), crosscarmellose, and methyl cellulose, or a pharmaceutically acceptable salt thereof; optionally an alkali or alkaline salt, and optionally an amino acid selected from the group consisting of Ala, Asp, His, Leu, Lys, Gln, Pro or Glu, or a combination thereof.

In one aspect, the disclosure provides a formulation that comprises a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus at 100-10,000,000 pfu/ml, a buffer at pH 6.5 to 8.5, 50-

300 mg/ml sugar, 2.5-10.0 mg/ml propylene glycol or glycerol, and 0.3-10 mg/ml sodium carboxymethylcellulose, optionally 10-150 mM NaCl, and optionally 10-100 mM amino acid selected from the group consisting of Ala, Asp, His, Leu, Lys, Gln, Pro and Glu or a combination thereof.

In one example, the buffer is selected from the group consisting of succinate, histidine, phosphate, TRIS, Bis-Tris, MES, MOPS, HEPES, acetate and citrate, or a combination

thereof. In another example, the alkali or alkaline salt is magnesium chloride, calcium chloride, potassium chloride, sodium chloride or a combination thereof. In a further example, the sugar is trehalose or sucrose. In one example, the cellulose derivative is a pharmaceutically acceptable salt of carboxymethyl cellulose. In another example, the glycol is selected from the group consisting of propylene glycol, polypropylene glycol, ethylene glycol, polyethylene glycol, and polyethylene glycol monomethyl ethers. In a further example, the sugar alcohol is glycerol.

In an example, the formulation comprises a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus at about 100-10,000,000 pfu/ml, a buffer at pH about 6.5 to 8.5, about 50-300 mg/ml sugar, about 2.5-10.0 mg/ml propylene glycol (PG) or glycerol, and about 0.3-10 mg/ml sodium carboxymethylcellulose (sodium CMC), optionally about 10-150 mM NaCl, and optionally about 10-100 mM amino acid selected from the group consisting of Ala, Asp, His, Leu, Lys, Gln, Pro or Glu, or a combination thereof; a live attenuated dengue vaccine at about 100-100,000 pfu/ml, about 5-300 mM histidine, TRIS, Bis-Tris or phosphate buffer, or a combination thereof at pH about 7.0 to 8.0, about 50-300 mg/ml sugar, about 3-10 mg/ml propylene glycol or glycerol, and about 3-10 mg/ml sodium carboxymethylcellulose, optionally about 15-75 mM NaCl, and optionally about 10-75 mM amino acid selected from the group consisting of Ala, Asp, His, Leu, Lys, Gln, Pro or Glu, or a combination thereof; a live attenuated dengue vaccine at about 600-20,000 pfu/ml, about 5-300 mM potassium phosphate buffer at pH about 7.0-8.0, about 60-120 mg/ml sucrose or trehalose or a combination thereof, about 3-7 mg/ml propylene glycol or glycerol, and about 3-7 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 30-90 mM NaCl, and optionally about 10-75 mM amino acid Leu, Lys or Glu, or a combination thereof; a live attenuated dengue vaccine at about 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol or glycerol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 75 mM NaCl; a live attenuated dengue vaccine at about 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml

K12. In an example, the formulation further comprises a surfactant selected from poloxamer 188 and poloxamer 407 at about 0.0001 to 5% w/v.

In one example, the formulation further comprises an aluminum adjuvant. The above formulations can be frozen or lyophilized, or reconstituted in solution. In one example, the reconstitution is performed with about 0.5-1.0 ml saline solution, water or Bacteriostatic Water for Injection (BWFI) and optionally a diluent comprising an aluminum adjuvant. In another example, the formulation is the aqueous solution prior to lyophilization or microwave vacuum drying.

In one example, the live attenuated dengue vaccine comprises tetravalent live attenuated dengue virus or live attenuated chimeric flavivirus. In another example, the LAV or the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated (UTR) region; which reduces the replicative capacity of the virus. In a further example, the live attenuated dengue virus is an LAV that comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated (UTR) region, and is immunogenic against dengue serotype 3, wherein the viral genome of the LAV further contains a deletion of nucleotides upstream from the Δ30 deletion corresponding to the TL-3 structure of the 3'UTR.

In preferred examples of the disclosure, the live attenuated dengue vaccine is a live attenuated tetravalent vaccine comprising a DEN1Δ30 virus, a DEN2/4Δ30 virus (a DEN2 Δ30LACV on a DEN4 backbone), a DEN3Δ30 virus and a DEN4Δ30 virus. In another preferred example, the live attenuated dengue virus is an LAV comprising rDEN1Δ30-1545, rDEN2/4Δ30 (ME)-1495,7163, rDEN3Δ30/31-7164, and rDEN4Δ30-7132,7163,8308.

In another aspect, the disclosure provides a formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, about 11 mM phosphate buffer at pH 6.5-8.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose, about 50 mM NaCl, and about 25 mM Leu.

In another aspect, the disclosure provides a formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, about 11 mM potassium phosphate buffer at pH 6.5-8.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose, about 50 mM NaCl, and about 25 mM Leu.

In another aspect, the disclosure provides a formulation that comprises a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV) at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about

5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, about 50 mM NaCl, and about 25 mM Leu.

In another aspect, the disclosure provides a formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, about 11 mM potassium phosphate buffer at about pH 7.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose, about 50 mM NaCl, and about 25 mM Leu.

In another aspect, the disclosure provides a formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, 11 mM potassium phosphate buffer at pH 7.5, 90 mg/ml sucrose, 5 mg/ml propylene glycol, 5 mg/ml sodium carboxymethylcellulose, 50 mM NaCl, and 25 mM Leu.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1: Effect of Sodium CMC, PG, amino acids on DENV4 lyophilization yield for DEN4 formulations.

FIG. 2: Effect of Sodium CMC, PG, amino acids on DENV4 stability for DEN4 formulations. Formulation 26 (*) was not tested due to cake collapse after storage at 25°C.

FIG. 3: Effect of sugar alcohol on DENV4 lyophilization yield for DEN4 formulations.

FIG. 4: Effect of sugar alcohol on DENV4 stability for DEN4 formulations.

FIG. 5: Effect of pH on DENV4 lyophilization yield for DEN4 formulations.

FIG. 6: Effect of pH on DENV4 stability for DEN4 formulations.

FIG. 7: Effect of buffer on DENV4 lyophilization yield for DEN4 formulations.

FIG. 8: Effect of buffer on DENV4 stability for DEN4 formulations.

FIG. 9: Effect of NaCl concentration on DENV4 lyophilization yield. for DEN4 formulations.

FIG. 10: Effect of NaCl concentration on DENV4 stability for DEN4 formulations.

FIG. 11: Effect of propylene glycol and glyercol on lyophilization yields of Dengue serotypes.

WHAT IS CLAIMED IS:

1. A formulation that comprises a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus at 100-10,000,000 pfu/ml, a buffer at pH 6.5 to 8.5, 50-300 mg/ml sugar, 2.5-10.0 mg/ml propylene glycol or glycerol, and 0.3-10 mg/ml sodium carboxymethylcellulose, optionally 10-150 mM NaCl, and optionally 10-100 mM amino acid selected from the group consisting of Ala, Asp, His, Leu, Lys, Gln, Pro and Glu or a combination thereof.
2. The formulation of claim 1 that comprises the live attenuated dengue vaccine at 100-100,000 pfu/ml, 5-300 mM histidine, TRIS, Bis-Tris or phosphate buffer, or a combination thereof at pH 7.0 to 8.0, 50-300 mg/ml sugar, 3-10 mg/ml propylene glycol or glycerol, and 3-10 mg/ml sodium carboxymethylcellulose, optionally 15-75 mM NaCl, and optionally 10-75 mM amino acid selected from the group consisting of Ala, Asp, His, Leu, Lys, Gln, Pro and Glu or a combination thereof.
3. The formulation of claim 1 that comprises the live attenuated dengue vaccine at 600-20,000 pfu/ml, 5-300 mM potassium phosphate buffer at pH 7.0-8.0, 60-120 mg/ml sucrose or trehalose or a combination thereof, 3-7 mg/ml propylene glycol or glycerol, and 3-7 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and 30-90 mM NaCl, and optionally 10-75 mM amino acid Leu, Lys or Glu, or a combination thereof.
4. The formulation of claim 1 that comprises the live attenuated dengue vaccine at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol or glycerol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 75 mM NaCl.
5. The formulation of claim 1 that comprises the live attenuated dengue vaccine at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, about 50 mM NaCl, and about 25 mM Leu.
6. The formulation of claim 1 that comprises the live attenuated dengue vaccine at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH about 7.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, and about 30 mM NaCl.
7. The formulation of any one of claims 2-6, further comprising 90-200 mg/ml trehalose.

8. The formulation of claim 1 that comprises the live attenuated dengue vaccine at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH 7.5-8, about 90 mg/ml sucrose, about 110 mg/ml trehalose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, about 50 mM NaCl, and about 25mM Leu.
9. The formulation of any one of claims 1 to 8 that further comprises an aluminum adjuvant.
10. The formulation of any one of claims 1-8 that is frozen or lyophilized.
11. The formulation of any one of claims 1-8 that is reconstituted in solution.
12. The formulation of any one of claims 1-8 that is the aqueous solution.
13. The formulation of claim 11, wherein the reconstitution is performed with 0.5-1.0 ml saline solution, water or Bacteriostatic Water for Injection (BWFI) and optionally a diluent comprising an aluminum adjuvant.
14. The formulation of any one of claims 1 to 13, wherein the live attenuated dengue vaccine is tetravalent.
15. The formulation of any one of claims 1 to 14, wherein the LAV or the LACV comprise a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated (UTR) region.
16. The formulation of any one of claims 1 to 14, wherein the live attenuated dengue virus (LAV) comprises a viral genome that contains a deletion of about 30 nucleotides corresponding to the TL-2 stem-loop structure of the 3' untranslated (UTR) region, and is immunogenic against dengue serotype 3, wherein the viral genome of the LAV further contains a deletion of nucleotides upstream from the Δ30 deletion corresponding to the TL-3 structure of the 3'UTR.
17. The formulation of any one of claims 1 to 13, wherein the live attenuated dengue virus (LAV) comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30.
18. The formulation of any one of claims 1 to 13, wherein the live attenuated dengue virus (LAV) comprises rDEN1Δ30-1545, rDEN2/4Δ30 (ME)-1495,7163, rDEN3Δ30/31-7164, and rDEN4Δ30-7132,7163,8308.
19. A formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, about 11 mM phosphate buffer at pH 6.5-8.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose, about 50 mM NaCl, and about 25 mM Leu.
20. A formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30,

rDEN3Δ30/31, and rDEN4Δ30, about 11 mM potassium phosphate buffer at pH 6.5-8.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose, about 50 mM NaCl, and about 25 mM Leu.

21. A formulation that comprises a live attenuated dengue vaccine comprising at least one live attenuated dengue virus (LAV) or at least one live attenuated chimeric flavivirus (LACV) at 600-20,000 pfu/ml, about 11 mM potassium phosphate buffer at pH 7.0-8.0, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose with average molecular weight of about 90,000, about 50 mM NaCl, and about 25 mM Leu.

22. The formulation of claim 21, wherein the live attenuated dengue vaccine is tetravalent and comprises live attenuated dengue virus rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30.

23. A formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, about 11 mM potassium phosphate buffer at about pH 7.5, about 90 mg/ml sucrose, about 5 mg/ml propylene glycol, about 5 mg/ml sodium carboxymethylcellulose, about 50 mM NaCl, and about 25 mM Leu.

24. A formulation that comprises a live attenuated dengue vaccine at 100-100,000 pfu/ml, wherein the live attenuated dengue vaccine comprises rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30, 11 mM potassium phosphate buffer at pH 7.5, 90 mg/ml sucrose, 5 mg/ml propylene glycol, 5 mg/ml sodium carboxymethylcellulose, 50 mM NaCl, and 25 mM Leu.