degradation during preparation as well as transport prior to administration to a subject, in order to ensure for example, that the correct dosage concentration is delivered to the subject. Some vaccines are sensitive to temperature extremes; either excessive heat or accidental freezing can inactivate the vaccine. Maintaining this "cold chain" throughout distribution is particularly difficult in the developing world. Therefore, there remains a need for improving the stability of both existing and newly developed live, attenuated viruses and live viruses in order to improve manufacturing of vaccines, as well as, transport and delivery of the formulations.

SUMMARY

[0005] In one aspect, the present disclosure provides a lyophilized dengue virus composition comprising: live, attenuated dengue virus; at least one of trehalose and sucrose; urea; a protein agent comprising human serum albumin (HSA); arginine; and at least one salt comprising sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), potassium chloride (KCl) or potassium phosphate (KH_2PO_4); wherein the composition stabilizes the live, attenuated dengue virus.

[0005a] In another aspect, the present disclosure provides a method for stabilizing live, attenuated dengue virus, the method comprising: combining live, attenuated dengue virus with a composition comprising: at least one of trehalose and sucrose; urea; human serum albumin (HSA); arginine; and at least one salt comprising sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), potassium chloride (KCl) or potassium phosphate (KH_2PO_4); and lyophilizing the dengue virus composition, wherein the composition stabilizes the live, attenuated dengue virus.

[0005b] In another aspect, the present disclosure provides a kit for stabilizing live, attenuated dengue virus comprising: a composition as described herein; and at least one container.

[0005c] Examples herein relate to compositions and methods for stabilizing live flaviviruses. In certain examples, compositions and methods disclosed herein concern stabilizing live, attenuated or unattenuated whole flaviviruses. Other examples relate to compositions and methods for reducing degradation of live, attenuated or unattenuated flaviviruses. Yet other examples relate to improved formulations for prolonging stabilization of live attenuated or unattenuated flaviviruses during manufacturing (e.g. vaccines or anti -viral treatments), storage, transport and administration to a subject. Yet other examples relate to uses of compositions or formulations disclosed herein in kits for transportable applications and methods.

[0006] Examples herein relate to formulations for stabilizing live viruses that can include one or more live flaviviruses, one or more carbohydrate agents, and one or more amino acids or salts, esters or amide derivatives thereof. In accordance with these examples, formulations of use herein stabilize live flaviviruses. In other examples, formulations of use herein stabilize live flaviviruses for commercial use.

[0007] In certain examples, formulations disclosed herein can include, but are not limited to, formulations for stabilizing flaviviruses. Flaviviruses can include, but are not limited to, dengue virus, West Nile virus, tick-borne encephalitis virus, yellow fever virus, Japanese encephalitis virus, Kunjin virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, Zika virus, or any related virus thereof.

[0008] In some examples, formulations disclosed herein can further include a buffer. In accordance with these examples, the buffer can include, but is not limited to, phosphate buffered saline (PBS), TRIS buffer, HEPES buffer or the like. In accordance with these examples, the buffer can include at least one salt of sodium chloride (NaCl), monosodium and/or disodium phosphate (Na_2HP0_4), potassium chloride (KC1), and potassium phosphate (KH2PO4). In some examples, the buffer of formulations disclosed

herein can include sodium chloride (NaCl) having a concentration of about 10.0 mM to about 200.0 mM. In other examples, formulations for stabilizing live or live, attenuated flaviviruses do not contain magnesium chloride (MgC^).

[0009] In certain examples, formulations disclosed herein can further include one or more protein agents. In accordance with these examples, the one or more protein agents can include, but is not limited to, albumin, gelatin and/or dextran. For example, the albumin can include, but is not limited to, a recombinant albumin, a native albumin, human serum albumin (HSA), recombinant human serum albumin (rHSA), native human serum albumin (nHSA), or an albumin-like agent. In other examples, the one or more protein agents in the composition can include, but are not limited to, any serum albumin, a human serum albumin (HSA), a bovine serum albumin (BSA), any comparable mammalian serum, gelatin, dextran, a polyol polymer, or combinations thereof. In accordance with these examples, one or more protein agents in a formulation disclosed herein can include albumin, such as human serum albumin or recombinant albumin having a concentration of about 0.01 % to about 2.0% (w/v). In one example, a formulation disclosed herein can include human serum albumin (HSA) having a concentration of about 0.01 % to about 2.0% (w/v).

[00010] In other examples, the one or more carbohydrate agents of formulations disclosed herein can include, but are not limited to, trehalose (e.g. D-trehalose dehydrate), galactose, fructose, lactose, sucrose, chitosan, mannitol or combinations thereof. In accordance with these examples, the one or more carbohydrate agent concentration can be from about 0.5% to about 15.0% (w/v). In certain examples, the one or more carbohydrate agents in a formulation disclosed herein can include trehalose and/or sucrose. In other examples, the one or more carbohydrate agents in a formulation disclosed herein can include mannitol in combination with at least one of trehalose and sucrose. In other examples, the one or more carbohydrate agents in the composition can include mannitol in combination with both trehalose and sucrose. In certain examples, formulations disclosed herein do not include sorbitol. In other examples, some formulations disclosed herein do not contain poloxamer 407 or other poloxamer.

[00011] In some examples, for example, those according to any of paragraphs [0004] -[00010], the one or more amino acids in a formulation can include, but are not limited to, alanine, arginine, methionine, or combinations thereof. In certain examples, one or more amino acids can include an amino acid derivative or salt thereof. In yet other examples, an amino acid derivative can include monosodium glutamate (MSG) or

potassium glutamate. In certain examples, the one or more amino acids or derivatives thereof are present in a formulation at a concentration of about 0.5 mM to about 150.0 mM.

[00012] In some examples, such as those according to any of paragraphs [0004]- [00011], formulations disclosed herein can include one or more osmolytes. In accordance with these examples, one or more osmolytes can include urea (for example a carbamide) or other substitutable agent, for example, caprolactam, PEG 400 or PEG 600. In accordance with these examples, the one or more osmolyte concentration (e.g. urea) can be about 0.01% to about 0.5% (w/v).

[00013] In certain examples, formulations disclosed herein can include sucrose and/or trehalose in a buffer and urea. In other examples, the formulation can further include one or more amino acids. In certain examples, the one or more amino acids can include at least one of alanine and methionine. In accordance with these examples, these formulations can further include at least one of mannitol and MSG. In certain examples, a formulation can include trehalose and mannitol in a buffer without urea or amino acids. In accordance with these examples, the buffer can include PBS (or TRIS, HEPES or other suitable buffer), NaCl and albumin (e.g., HSA).

[00014] In some examples, such as those according to any of paragraphs [0004]- [00013], a formulation can include a base buffer, sucrose, albumin, mannitol, alanine, methionine, MSG and/or urea in any combination. In accordance with these examples, certain formulations can include recombinant HSA having a concentration of about 0.01% to about 2.0% (w/v), sucrose concentration having a concentration of about 1.0% to about 15.0% (w/v), mannitol concentration having a concentration of about 0.1% to about 15.0% (w/v), alanine having a concentration of about 0.5 mM to about 100 mM, methionine concentration having a concentration of about 1.0 mM to 5.0 mM, MSG concentration having a concentration of about 1.0 mM to about 50.0 mM, and urea concentration having a concentration of about 0.05% to about 1.0% (w/v).

[00015] In some examples, formulations contemplated herein can include recombinant albumin or HSA, trehalose and/or sucrose in a base buffer, mannitol, alanine, methionine, MSG and urea.

[00016] In some examples, such as those according to any of paragraphs [0004]- [00015], recombinant HSA concentration can be about 0.01% to about 2.0% (w/v), trehalose concentration can be about 1.0% to about 10.0% (w/v), sucrose concentration can be about 1.0% to about 15.0% (w/v), mannitol concentration can be about 0.1% to about 15.0% (w/v), alanine can be about 0.5 mM to about 100 mM, methionine concentration can be about 1.0

mM to 5.0 mM, MSG concentration can be about 5.0 mM to about 100.0 mM, and urea concentration can be about 0.05% to about 1.0% (w/v).

[00017] In some examples, such as those according to any of paragraphs [0004]- [00016], formulations can include recombinant HSA, sucrose, alanine and urea. In other examples, recombinant HSA concentration can be about 0.01% to about 0.2%, sucrose can be a concentration can be about 1.0% to about 15.0% (w/v), alanine can be about 0.5 mM to about 100 mM, and urea concentration can be about 0.05% to about 1.0% (w/v). In certain examples regarding this paragraph [00017], sucrose can be replaced with trehalose at a concentration of about 1.0% to about 10% (w/v).

[00018] In some examples, stabilizing one or more live fiaviviruses can include reducing loss or reducing degradation of the one or more live fiaviviruses upon freezing, freeze drying, at refrigeration temperatures, at room temperature and at about 25°C, as compared to a composition not containing one or more of these agents. In some examples, stabilizing one or more live fiaviviruses as contemplated herein can include obtaining a 10%, a 20%, a 30%, a 40% or a 50% or more reduction in loss or degradation of the live or live, attenuated fiaviviruses.

[00019] In some examples, methods disclosed herein can include partially or wholly dehydrating the formulation that includes the live flavivirus. In other examples, methods can include partially or wholly rehydrating the formulation that includes the live or live, attenuated fiaviviruses prior to administration to a subject as part of an immunogenic composition (e.g. vaccine composition). In yet other examples, methods can include freezing live or live, attenuated flavivirus compositions disclosed herein.

[00020] Other examples relate to kits for stabilizing live or live, attenuated fiaviviruses. In accordance with these examples, kits can include any composition or formulation disclosed herein and at least one container.

[00021] Certain examples concern live, attenuated flavivirus compositions disclosed herein and methods directed to manufacturing an immunogenic composition (e.g. a vaccine) capable of reducing or preventing onset of a medical condition such as an infection or disease caused by one or more of the fiaviviruses. In certain examples, one or more live, attenuated fiaviviruses can be part of a pharmaceutical composition that can include a pharmaceutically acceptable excipient or carrier. In accordance with these examples, the pharmaceutical composition can be administered to a subject in order to reduce onset of a condition caused by a flavivirus infection when administered to a subject, and/or prepared for

administration to a subject. A subject can be a mammal for example, a domesticated animal a pet, livestock, other animal or a human subject (e.g. an adult, adolescent or child).

[00022] In some examples, compositions can include recombinant HSA or HSA, sucrose, methionine and urea. In accordance with these examples, recombinant HSA or HSA can have a concentration ranging from about 0.01% to about 2.0% (w/v), sucrose can have a concentration ranging from about 0.5% to about 10.0% (w/v), methionine can have a concentration ranging from about 1.0 mM to about 5.0 mM, and urea can have a concentration ranging from about 0.05% to about 0.5% (w/v). In other examples, a formulation having recombinant HSA or HSA, sucrose, methionine and urea can further include one or more of alanine at about 5.0 mM to about 25.0 mM, mannitol at about 1.0% to about 15% (w/v) and monosodium glutamate (MSG) at about 1.0 mM to about 20.0 mM. In certain examples, the compositions of this paragraph [00022] can be in a sucrose base buffer disclosed herein. In yet other examples, sucrose can be replaced with trehalose in these compositions of paragraph [00022].

[00023] In other examples, a composition can include recombinant HSA, sucrose and/or trehalose, arginine and urea. In accordance with these examples, recombinant HSA or HSA can have a concentration ranging from about 0.05% to about 0.5% (w/v), sucrose and/or trehalose can have a concentration ranging from about 0.5% to about 10.0% (w/v), arginine can have a concentration ranging from about 1.0 mM to about 50.0 mM, and urea can have a concentration ranging from about 0.01% to about 0.5% (w/v).

[00024] In some examples, a composition can include recombinant HSA, sucrose and/or trehalose, MSG (monosodium glutamate) and urea. In accordance with these examples, recombinant HSA or HSA can have a concentration ranging from about 0.05% to about 2.0% (w/v), sucrose and/or trehalose can have a concentration ranging from about 0.5% to about 10.0% (w/v), MSG can have a concentration ranging from about 1.0 mM to about 20.0 mM and urea can have a concentration ranging from about 0.01% to about 0.5% (w/v).

[00025] In some examples, compositions and methods disclosed herein can include combining one or more live or live, attenuated flaviviruses with a formulation that includes one or more carbohydrate agents and one or more amino acids or salts, esters or amide derivatives thereof. In accordance with these methods, formulations disclosed herein can stabilize one or more live or live, attenuated flaviviruses from degradation. Formulations disclosed herein are capable of stabilizing for example any serotype or strain of flavivirus. In

certain examples, flaviviruses include enveloped viruses, for example, dengue virus (e.g. serotypes 1-4), yellow fever virus, West Nile virus and Zika virus.

BRIEF DESCRIPTION OF THE DRAWINGS

[00026] The following drawings form part of the instant specification and are included to further demonstrate certain aspects of some embodiments disclosed herein. Certain embodiments may be better understood by reference to one or more of these drawings in combination with the detailed description presented herein.

[00027] Fig. 1 represents an exemplary histogram plot illustrating data obtained from experiments performed while analyzing stability of live flaviviruses in various formulations, according to some embodiments disclosed herein.

[00028] Fig. 2 represents an exemplary bar graph illustrating data obtained from experiments performed while analyzing stability of live flaviviruses in various formulations, before lyophilization, after lyophilization, and after lyophilization and storage at room temperature (e.g. 25°C) for about 5 weeks, according to some embodiments disclosed herein.

[00029] Figs. 3A and **3B** are exemplary graphs representing data from experiments using various formulations for testing stability of live flaviviruses after exposure to room temperature (e.g. 25°C) for 5 weeks after lyophilization, log loss (3A) and total log loss (3B) at both a 50% bulk drug substance (BDS dose) (solid triangles) and a current dose (CTM Dose) (solid squares), in one embodiment disclosed herein.

[00030] Figs. 4A and **4B** are exemplary graphs representing data from experiments using various formulations for testing stability of live flaviviruses after exposure to room temperature (e.g. 25°C) for 5 weeks after lyophilization, log loss (4A) and total log loss (4B) at both a 50% BDS (solid triangles) and a CTM (solid squares), in one embodiment disclosed herein.

[00031] Figs. 5A and **5B** are exemplary graphs representing data from experiments using various formulations for testing stability of live flaviviruses after exposure to room temperature (e.g. 25°C) for 5 weeks after lyophilization, log loss (5 A) and total log loss (5B) at both a 50% BDS (solid triangles) and a CTM (solid squares), in one embodiment disclosed herein.

[00032] Figs. 6A and **6B** are exemplary graphs representing data from experiments using various formulations for testing stability of live flaviviruses after exposure to room temperature (e.g. 25°C) for 5 weeks after lyophilization, log loss (6A) and total log loss (6B)

What is claimed is:

1. A lyophilized dengue virus composition comprising:

   live, attenuated dengue virus;

   at least one of trehalose and sucrose;

   urea;

   a protein agent comprising human serum albumin (HSA);

   arginine; and

   at least one salt comprising sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), potassium chloride (KCl) or potassium phosphate (KH_2PO_4);

   wherein the composition stabilizes the live, attenuated dengue virus.

2. The dengue virus composition according to claim 1, wherein the composition is partially or wholly rehydrated prior to administration of the composition to a subject as part of an immunogenic composition.

3. The dengue virus composition according to claim 1 or 2, wherein the composition further comprises a base buffer, the base buffer comprises phosphate buffered saline (PBS), HEPES buffer, histidine buffer or Tris buffer.

4. The dengue virus composition according to any one of claims 1 to 3 wherein the at least one salt comprises NaCl at a concentration of 10 mM to 200 mM.

5. The dengue virus composition according to any one of claims 1 to 4 further comprising, mannitol.

6. The dengue virus composition according to any one of claims 1 to 5 further comprising, one or more amino acids or derivatives thereof or salts, esters or amide derivatives thereof.

7. The dengue virus composition according to claim 6, wherein the one or more amino acids or derivatives thereof or salts, esters or amide derivatives thereof comprises methionine, alanine or a combination thereof.

8. The dengue virus composition according to claim 6, wherein the one or more amino acids or derivatives thereof or salts, esters or amide derivatives thereof comprises monosodium glutamate (MSG).

9. The dengue virus composition according to any one of claims 1 to 8, wherein the protein agent further comprises dextran, polyol polymer or gelatin.

10. The dengue virus composition according to any one of claims 1 to 9, wherein the dengue virus comprises four dengue serotypes in the form of a dengue 1/2 chimera, a modified live attenuated dengue-2 virus, a dengue 3/2 chimera, and a dengue 4/2 chimera, wherein the dengue-2 virus as the backbone of the dengue 1/2 chimera, the dengue 3/2 chimera and the dengue 4/2 chimera is a PDK-53 dengue-2 strain.

11. The dengue virus composition according to any one of claims 1 to 10, wherein the composition does not contain magnesium chloride ($MgCl_2$).

12. The dengue virus composition according to any of claims 1 to 11, wherein the protein agent is present in the composition at a concentration ranging from 0.05% to 1.0% (w/v).

13. The dengue virus composition according to any one of claims 1 to 12, wherein the trehalose and sucrose or combination of trehalose and sucrose is present in the composition at a concentration ranging from 0.5% to 15.0% (w/v).

14. The dengue virus composition according to any one of claims 6 to 13, wherein the one or more amino acids or derivatives thereof or salts, esters or amide derivatives is present in the composition at a concentration ranging from 1.0 mM to 25.0 mM.

15. The dengue virus composition according to any one of claims 1 to 14, wherein the urea concentration in the composition is from 0.01% to 1.0% (w/v).

16. The dengue virus composition according to any one of claims 1 to 15, wherein the trehalose, sucrose or combination of trehalose and sucrose is present in the composition at a concentration ranging from 0.5% to 15.0% (w/v), the urea

concentration in the composition is from 0.01% to 1.0% (w/v), and the protein agent concentration in the composition is from 0.05% to 1.0% (w/v).

17. The dengue virus composition according to any one of claims 1 to 16, wherein the HSA concentration is from 0.05% to 0.5% (w/v); wherein the sucrose, trehalose or combination of sucrose and trehalose concentration is from 0.5% to 15.0% (w/v); wherein the arginine concentration is from 1.0 mM to 20.0 mM; and wherein the urea concentration is from 0.1% to 0.3% (w/v).

18. The dengue virus composition according to claim 17, wherein the sucrose, trehalose or combination of sucrose and trehalose concentration is from 5.0% to 15.0% (w/v).

19. The dengue virus composition according to any one of claims 1 to 18, wherein the trehalose and sucrose or combination of trehalose and sucrose is present in the composition at a concentration of less than 10.0% (w/v).

20. A method for stabilizing live, attenuated dengue virus, the method comprising:
    combining live, attenuated dengue virus with a composition comprising:
    at least one of trehalose and sucrose;
    urea;
    human serum albumin (HSA);
    arginine; and
    at least one salt comprising sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), potassium chloride (KCl) or potassium phosphate (KH_2PO_4); and
    lyophilizing the dengue virus composition,
    wherein the composition stabilizes the live, attenuated dengue virus.

21. The method according to claim 20, wherein the dengue virus comprises four dengue virus serotypes in the form of a dengue 1/2 chimera, a modified live, attenuated denuge-2 virus, a dengue 3/2 chimera, and a dengue 4/2 chimera, wherein the dengue-2 virus as the backbone of the dengue 1/2 chimera, the dengue 3/2 chimera and the dengue 4/2 chimera is a PDK-53 dengue-2 strain.

22. The method according to claim 20 or 21, wherein the lyophilized dengue virus composition is stored at 25°C for extended periods.

23. The method according to any one of claims 20 to 22, further comprising partially or wholly rehydrating the composition prior to administration of the composition to a subject as part of an immunogenic composition.

24. The method according to any one of claims 20 to 23, wherein the composition further comprises a buffer.

25. The method according to any one of claims 20 to 24, wherein the composition further comprises one or more protein agents selected from the group consisting of gelatin, dextran, a polyol polymer, or combinations thereof.

26. The method according to any one of claims 20 to 25, wherein the composition further comprises, one or more amino acids or derivatives thereof or salts, esters or amide derivatives thereof.

27. The method according to claim 26, wherein the one or more amino acids or derivatives thereof or salts, esters or amide derivatives thereof are selected from the group consisting of alanine, methionine, MSG or combinations thereof.

28. The method according to any one of claims 20 to 27, wherein the composition comprises 0.5% to 15.0% (w/v) of at least one of trehalose and sucrose, 0.01% to 1.0% (w/v) urea, and 0.05% to 1.0% (w/v) human serum albumin (HSA).

29. The method according to any one of claims 20 to 28, wherein the urea is present in the composition at a concentration ranging from 0.01% to 0.5% (w/v).

30. The method according to any one of claims 20 to 29, wherein the HSA concentration in the composition is from 0.05% to 0.5% (w/v); wherein the sucrose, trehalose or combination of sucrose and trehalose concentration in the composition is from 0.5% to 15.0% (w/v); wherein the arginine concentration in the composition is from 1.0 mM to 20.0 mM; and wherein the urea concentration in the composition is from 0.1% to 0.3% (w/v).

31. The method according to claim 30, wherein the sucrose, trehalose or combination of sucrose and trehalose concentration in the composition is from 5.0% to 15.0% (w/v).

32. A kit for stabilizing live, attenuated dengue virus comprising:

a composition according to any one of claims 1 to 19; and

at least one container.