virus in Australia and New Guinea, St. Louis encephalitis virus in North and South America, the Usutu, Koutango, and Yaonde viruses in Africa, and Cacipacore virus in South American.

[00010] Live, attenuated viral vaccines have been developed that are safe and protect against flavivirus diseases, such as yellow fever and Japanese encephalitis. The live, attenuated viral vaccine, 17D, has been widely used to prevent yellow fever. The current flavivirus vaccines are lyophilized in the presence of stabilizers. Nonetheless, the vaccines require storage and shipment at 2 - 8° C, a requirement that is difficult to achieve in the developing world and more remote regions of developed nations. Furthermore, upon reconstitution, the vaccines rapidly lose potency even when stored at 2 - 8° C.

[00011] The measles vaccine is another example of a labile attenuated virus that is used worldwide to prevent disease. Measles virus is an enveloped, non-segmented negative strand RNA virus of the Paramyxovirus family. Measles is a highly contagious, seasonal disease that can affect virtually every child before puberty in the absence of vaccination. In developing countries, mortality rates in measles-infected children can by as high as 2 to 15%. Indeed, despite efforts to institute worldwide immunization, measles still causes greater than 7,000 deaths in children per year. The measles vaccine is a live, attenuated virus that is manufactured in primary chicken fibroblast cells. The vaccine is stabilized with gelatin and sorbitol and is then lyophilized. The stabilized, lyophilized vaccine has a shelf life of 2 years or more if stored at 2 to 8° C. However, the lyophilized vaccine still requires a cold chain that is difficult to maintain in the developing world. Furthermore, upon reconstitution, the vaccine loses 50% of its potency within 1 hour at room temperature (20 to 25° C).

[00012] Thus, a need exists in the art for improved vaccine formulations.

SUMMARY

[00013] Examples herein concern methods and compositions to reduce or prevent deterioration or inactivation of a live attenuated virus composition. Certain compositions disclosed can include combinations of components that reduce deterioration of a live attenuated virus. Other examples herein concern combinations of excipients that greatly enhance the stability of live attenuated viruses. Yet other compositions and methods herein are directed to reducing the need for lower temperatures (e.g. refrigerated or frozen storage) while increasing the shelf life of aqueous and/or reconstituted live attenuated virus.

[00013a] In one aspect, the present disclosure refers to a live, attenuated dengue virus composition comprising: one or more live, attenuated dengue viruses; one or more albumins selected from the group consisting of lactalbumins and serum albumins; and trehalose. In another aspect, the present disclosure refers to a method for decreasing inactivation of a live, attenuated dengue virus composition comprising, combining one or more live attenuated dengue viruses with a composition comprising, one or more albumins selected from the group consisting of lactalbumins and serum albumins, and trehalose. In yet another aspect, the present disclosure refers a kit for decreasing the inactivation of a live, attenuated virus composition comprising: at least one container; and a composition comprising a composition as described herein. In yet another aspect, the present disclosure refers to the use of the virus composition as described herein in the manufacture of a medicament for use in reducing the onset of or in preventing Dengue fever.

[00014] In accordance with these examples, certain live attenuated viruses are directed to flaviviruses. Some examples, directed to compositions, can include, but are

not limited to, one or more live, attenuated viruses, such as one or more live, attenuated flaviviruses in combination with one or more high molecular weight surfactants, proteins, and carbohydrates.

[00015] Compositions contemplated herein can increase the stabilization and/or reduce the inactivation and/or degradation of a live attenuated virus including, but not limited to, a live attenuated Flavivirus, Togavirus, Coronavirus, Rhabdovirus, Filovirus, Paramyxovirus, Orthomyxovirus, Bunyavirus, Arenavirus, Retrovirus, Hepadnavirus, Pestivirus, Picornavirus, Calicivirus, Reovirus, Parvovirus, Papovavirus, Adenovirus, Herpes virus, or Poxvirus.

[00016] Other examples concern live, attenuated virus compositions and methods directed to a vaccine compositions capable of reducing or preventing onset of a medical condition caused by one or more of the viruses contemplated herein. In accordance with these examples, medical conditions may include, but are not limited to, West Nile infection, dengue fever, Japanese encephalitis, Kyasanur forest disease, Murray valley encephalitis, Alkhurma hemorrhagic fever, St. Louis encephalitis, tick-borne encephalitis, yellow fever and hepatitis C virus infection.

[00017] In certain examples, compositions contemplated herein can be partially or wholly dehydrated or hydrated. In other examples, protein agents contemplated of use in compositions herein can include, but are not limited to, lactalbumin, human serum albumin, a recombinant human serum albumin (rHSA), bovine serum albumin (BSA), other serum albumins or albumin gene family members. Saccharides or polyol agents can include, but are not limited to, monosaccharides, disaccharides, sugar alcohols, trehalose, sucrose, maltose, isomaltose, cellibiose, gentiobiose, laminaribose, xylobiose, mannobiose, lactose, fructose, sorbitol, mannitol, lactitol, xylitol, erythritol, raffinose, amylse, cyclodextrins, chitosan, or cellulose. In certain examples, surfactant agents can include, but are not limited to, a nonionic surfactant such as alkyl poly(ethylene oxide), copolymers of poly(ethylene oxide) and poly(propylene oxide) (EO-PO block copolymers), poly(vinyl pyrroloidone), alkyl polyglucosides (such as sucrose monostearate, lauryl diglucoside, or sorbitan monolaureate, octyl glucoside and decyl maltoside), fatty alcohols (cetyl alcohol or olelyl alcohol), or cocamides (cocamide MEA, cocamide DEA and cocamide TEA).

[00018] In other examples, the surfactants can include, but are not limited to, the Pluronic F127, Pluronic F68, Pluronic P123, or other EO-PO block copolymers of greater than 3,000-4,000 MW.

[00019] In some examples, vaccine compositions can include, but are not limited to, one or more protein agent that is serum albumin; one or more saccharide agent that is trehalose; and one or more surfactant polymer agent that is the EO-PO block copolymer Pluronic F 127.

[00020] Some examples herein concern partially or wholly dehydrated live, attenuated viral compositions. In accordance with these examples, a composition may be 20 % or more; 30% or more ; 40% or more; 50% or more; 60% or more; 70 % or more; 80% or more; or 90% or more dehydrated.

[00021] Other examples concern methods for decreasing inactivation of a live attenuated viruses including, but not limited to, combining one or more live attenuated viruses with a composition capable of reducing inactivation of a live, attenuated virus including, but not limited to, one or more protein agents; one or more saccharides or polyols agents; and one or more high molecular weight surfactants, wherein the composition decreases inactivation of the live attenuated virus. In accordance with these examples, the live attenuated virus may include, but is not limited to, a Flavivirus, Togavirus,

Coronavirus, Rhabdovirus, Filovirus, Paramyxovirus, Orthomyxovirus, Bunyavirus, Arenavirus, Retrovirus, Hepadnavirus, Pestivirus, Picornavirus, Calicivirus, Reovirus, Parvovirus, Papovavirus, Adenovirus, Herpes virus, or a Poxvirus. Additionally, methods and compositions disclosed herein can include freeze drying or other dehydrating methods for the combination. In accordance with these methods and compositions, the methods and compositions decrease inactivation of the freeze dried or partially or wholly dehydrated live attenuated virus. In other methods, compositions for decreasing inactivation of a live attenuated virus may comprise an aqueous composition or may comprise a rehydrated composition after dehydration. Compositions described herein are capable of increasing the shelf life of an aqueous or rehydrated live attenuated virus.

[00022] In certain particular examples, a live attenuated virus for use in a vaccine composition contemplated herein may include, but is not limited to, one or more live, attenuated flavivirus vaccines, including but not limited to, attenuated yellow fever viruses (such as 17D), attenuated Japanese encephalitis viruses, (such as SA 14-14-2), attenuated

dengue viruses (such as DEN-2/PDK-53 or DEN-4Δ30) or recombinant chimeric flaviviruses.

[00023] In certain examples, compositions contemplated herein are capable of decreasing inactivation and/or degradation of a hydrated live attenuated virus for greater than 24 hours at room temperatures (e.g. about 20° to about 25° C) or refrigeration temperatures (e.g. about 0° to about 10° C). In more particular examples, a combination composition is capable of

maintaining about 100 percent of the live attenuated virus for greater than 24 hours. In addition, combination compositions contemplated herein are capable of reducing inactivation of a hydrated live attenuated virus during at least 2 freeze and thaw cycles. Other methods concern combination compositions capable of reducing inactivation of a hydrated live attenuated virus for about 24 hours to about 50 days at refrigeration

temperatures (e.g. about 0° to about 10° C). Compositions contemplated in these methods, can include, but are not limited to, one or more protein agent of serum albumin; one or more saccharide agent of trehalose; and one or more EO-PO block copolymer agent of Pluronic F127. In certain examples, the live, attenuated virus composition remains at about 100% viral titer after 7 days at approximately 21° C and about 100% viral titer after 50 days at refrigeration temperatures around 4° C. Other examples herein may include live, attenuated virus composition remaining at about 90%, or about 80% viral titer after 7 days at approximately 21° C and about 90%, or about 80% viral titer after 50 days at refrigeration temperatures around 4° C. Other examples contemplated include live, attenuated virus compositions remaining at about 3x to about 10x the concentration of viral titer after several hours (e.g. 20 hours) at approximately 37° C compared to other compositions known in the art. (see for example, Figs. 4 and 5). Compositions disclosed herein reduce degradation of the live, attenuated virus when the composition is stored at approximately 37° C.

[00024] Other examples concern kits for decreasing the inactivation of a live, attenuated virus composition including, but not limited to, a container; and a composition including, but not limited to, one or more protein agents, one or more saccharide or polyol agents, and one or more EO-PO block copolymer agents, wherein the composition decreases inactivation and/or degradation of a live, attenuated virus. In accordance with these examples, a kit composition may include one or more one protein agent of serum albumin; one or more saccharide agent of trehalose; and one or more EO-PO block copolymer agent. Additionally, a kit contemplated herein may further include one or more

live, attenuated viruses including, but not limited to, a Flavivirus, Togavirus, Coronavirus, Rhabdovirus, Filovirus, Paramyxovirus, Orthomyxovirus, Bunyavirus, Arenavirus, Retrovirus, Hepadnavirus, Pestivirus, Picornavirus, Calicivirus, Reovirus, Parvovirus, Papovavirus, Adenovirus, Herpes virus, or Poxvirus. In certain examples, compositions herein can include trehalose as a saccharide agent. In accordance with these examples, trehalose concentration may be equal to or greater than 5% (w/v). In certain examples,

compositions herein can include polymer F 127 as an EO-PO block copolymer agent. In accordance with these examples, polymer F127 concentration may be about 0.1 to about 4 percent (w/v).

[00025] In other examples, compositions contemplated herein may contain trace amounts or no divalent cations. For example, compositions contemplated herein may have trace amounts or no calcium/magnesium (Ca^{+2}/Mg^{+2}).

BRIEF DESCRIPTION OF THE DRAWINGS

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

[00026] Fig.1 represents an exemplary histogram of experiments using various compositions for testing the stability of an exemplary virus, DEN -2 PDK 53 flavivirus, in the compositions.

[00027] Fig. 2 represents an exemplary graph of a kinetic analysis of an exemplary virus, DEN-2 PDK 53 flavivirus, for viral inactivation at 37° C in various exemplary compositions.

[00028] Fig. 3 represents an exemplary histogram of an analysis of an exemplary virus, DEN-2 PDK 53 virus, stored at 370C for 21 hours. Values are expressed as a percentage of the viral titer remaining after incubation relative to the input titer. Formulation percentages refer to (w/v) of the respective excipient.

[00029] Fig. 4 represents an exemplary histogram of an analysis of an exemplary virus, DEN-2 PDK 53 virus, stored at 37° C for 23 hours in different compositions. Values are

What is claimed is:

1. A live, attenuated dengue virus composition comprising:

one or more live, attenuated dengue viruses;

one or more albumins selected from the group consisting of lactalbumins and serum albumins; and

trehalose.

2. The virus composition of claim 1, wherein the composition further comprises one or more poly(ethylene oxide) and poly(propylene oxide) (EO-PO) block copolymers, the one or more EO-PO block copolymers have a molecular weight of 3000 or greater, such as poloxamer 407(Pluronic® F127).

3. The virus composition of any of the preceding claims, wherein the composition is adapted to be partially or wholly dehydrated.

4. The virus composition of any of the preceding claims, wherein the one or more albumins is at least serum albumin from a vertebrate species or a recombinant albumin.

5. The virus composition of any of the preceding claims, wherein the concentration of trehalose is from 5 to 50% (w/v).

6. The virus composition of any of the preceding claims, wherein the one or more albumin comprises serum albumin and the concentration of the serum albumin is from 0.001 to 3% (w/v).

7. The virus composition of any of the preceding claims, wherein the one or more serum albumins are selected from the group consisting of bovine serum albumin and human serum albumin.

8. A method for decreasing inactivation of a live, attenuated dengue virus composition comprising, combining one or more live attenuated dengue viruses with a composition

comprising, one or more albumins selected from the group consisting of lactalbumins and serum albumins, and trehalose.

9. The method of claim 8, wherein the composition further comprises one or more poly(ethylene oxide) and poly(propylene oxide) (EO-PO) block copolymers, the one or more EO-PO block copolymers have a molecular weight of 3000 or greater such as poloxamer 407 (Pluronic® F127).

10. The method of claim 8 or 9, further comprising partially or wholly dehydrating the combination.

11. A kit for decreasing the inactivation of a live, attenuated virus composition comprising:

at least one container; and

a composition comprising a composition of any one of claims 1-7.

12. Use of the virus composition of any of claims 1 to 7 in the manufacture of a medicament for reducing the onset of or for preventing Dengue fever.