CLAIMS

What is claimed is:

1. A liquid frozen or lyophilized live chikungunya vaccine formulation comprising: a) an effective amount of at least one strain of live chikungunya virus; b) about 1 to 50% (w/v) sugar; c) about 1 mM to about 20 mM phosphate; d) about 1 mM to about 50 mM of at least one carboxylate buffering agent; e) optionally about 1 mM to about 10 mM $MgCl_2$; f) optionally about 0.1 % to about 5 % (w/v) D-sorbitol; g) optionally about 1 mM to 20 mM L-methionine; and h) optionally about 0.001% to about 0.1 % (w/v) human serum albumin.
2. The formulation of claim 1, wherein the human serum albumin is a recombinant human serum albumin (rHSA).
3. The formulation according to any preceding claims wherein said at least one carboxylate buffering agent is selected from the group consisting of succinate, citrate, fumarate, tartarate, maleate and lactate.
4. The formulation according to any preceding claims wherein said sugar is selected from the group consisting of sucrose, mannitol, lactose, sorbitol, dextrose, fucose and trehalose, preferably sucrose or trehalose, most preferably sucrose.
5. The formulation according to any preceding claims wherein the concentration of sugar is between about 1 to about 10%; the concentration of phosphate is between about 1 to about 10 mM; and said at least one carboxylic acid is citrate or succinate at a concentration between about 10 to about 30 mM.
6. The formulation according to any preceding claims further comprising: k) at least one diluent selected from the group consisting of tissue culture medium, saline and water to volume.
7. The formulation according to any preceding claims wherein the pH is between about pH 5.0 to about pH 8.0, preferably between pH 7.0 and pH 7.5, most preferably pH 7.3.
8. The formulation according to any preceding claims wherein said phosphate is selected from the group consisting of monophosphates, polyphosphates and phosphorylated compounds.
9. The formulation according to claim 8 wherein said monophosphate is potassium phosphate.
10. The formulation according to any preceding claims wherein formulation comprises an effective amount of at least one strain of chikungunya virus; b) sucrose at a concentration of about 5% (w/v); c) potassium phosphate at a concentration of about 5 mM to about 10 mM; d) sodium citrate at a concentration of about 25 mM; e) $MgCl_2$ at a concentration of about 10 mM; f) D-sorbitol at a

concentration of about 0.5 % (w/v), g) L-methionine at a concentration of about 10 mM; and h) recombinant human serum albumin at a concentration of about 0.01% (w/v).

11. The formulation according to any preceding claims wherein formulation comprises an effective amount of at least one strain of chikungunya virus; b) about 5% (w/v) sucrose; c) about 5 mM potassium phosphate; d) about 25 mM sodium citrate; e) about 10 mM $MgCl_2$; f) about 0.5 % (w/v) D-sorbitol, g) about 10 mM L-methionine; and h) about 0.01% (w/v) recombinant human serum albumin.

12. The formulation according to any preceding claims wherein said chikungunya virus is selected from an attenuated chikungunya virus of SEQ ID NO: 1; variants with 99% sequence identity to SEQ ID NO: 1 of which have the 60aa deletion as in SEQ ID NO: 1; and/or combinations thereof.

13. The formulation according to any preceding claims wherein said chikungunya virus comprises essentially an attenuated chikungunya virus of SEQ ID NO: 1 and a variant with 99% sequence identity to SEQ ID NO: 1, wherein the variant also has the 60aa deletion in the nsP3 protein as in SEQ ID NO: 1.

14. The formulation according to any preceding claims wherein said at least one strain of chikungunya virus is selected from an attenuated chikungunya virus population that comprises substantially two variants, said two variants both expressing the E1 wild type amino acid sequence as encoded in the relevant part of nucleic acid sequence SEQ ID NO: 1 and wherein one variant expresses the wild type E2 structural protein as defined in SEQ ID NO:2 and wherein the other variant expresses the E168K mutation in the E2 structural protein as defined in SEQ ID NO:3.

15. The lyophilized chikungunya vaccine formulation according to any preceding claims wherein said chikungunya virus is an attenuated chikungunya virus population that comprises substantially two variants, said two variants expressing E2 structural proteins as defined by the amino acid sequences of SEQ ID NO: 2 and SED ID NO: 3 (with E168K), respectively, and wherein said two variants have a combined dose of between about 10^3 and 2 x 10^4 $TCID_{50}$/dose and a target potency of about 5 x 10^3 $TCID_{50}$/dose.

16. The lyophilized chikungunya vaccine formulation according to any preceding claims wherein said chikungunya virus is an attenuated chikungunya virus population that comprises one or more variants and wherein the variant has one or more mutations in E2 which mutations are shown in the group of variants encoding for an E2 amino acid sequence with E168K (SEQ ID NO: 3), G55R (SEQ ID NO: 4), E247K (SEQ ID NO: 5), G82R (SEQ ID NO: 6) and/or H232Y (SEQ ID NO: 7).

17. A method of preparing chikungunya virus vaccine formulations, comprising:

(a) cultivating a chikungunya virus and mixing the chikungunya virus with a concentrated stabilizing solution to form a virus bulk; and, optionally,

(b) dialyzing the virus bulk to form a chikungunya virus vaccine solution; wherein the vaccine solution comprises a) about 1 to 50% (w/v) sugar; b) about 1 mM to about 20 mM phosphate; c) about 1 mM to about 50 mM of at least one carboxylate; d) about 1 mM to about 10 mM $MgCl_2$; e) about 0.1 % to about 5 % (w/v) D-sorbitol; g) about 1 mM to 20 mM L-methionine; and f) about 0.001% to about 0.1 % (w/v) human serum albumin.

18. The method of claim 17, wherein the stabilizing solution comprises a) about 5% (w/v) sugar; b) about 20 mM phosphate; c) about 25 mM citrate; d) about 10 mM $MgCl_2$; e) about 0.5 % (w/v) D-sorbitol; f) about 10 mM L-methionine; and g) about 0.01% (w/v) human serum albumin.

19. The method of claim 17 or 18, wherein said dialyzing step results in a formulation of any of claims 1 to 16.

20. The method of any one of claims 17 to 19 further comprising the step of lyophilizing the vaccine solution.

21. The vaccine formulation of any one of claims 1 to 16 for use in a method of vaccinating a human subject to stimulate a protective immune response against Chikungunya virus in said subject.