CLAIMS

What is claimed is:

1. A lyophilized live chikungunya formulation lyophilized from a composition comprising: a) an effective amount of at least one strain of live chikungunya virus; b) 1 to 20% (w/v) sugar; c) 1 mM to 20 mM phosphate; d) 1 mM to 50 mM of at least one carboxylate buffering agent; e) 1 mM to 10 mM $MgCl_2$; f) 0.1% to 5% (w/v) D-sorbitol; g) 1 mM to 20 mM L-methionine; and h) 0.001% to 0.1 % (w/v) human serum albumin (HSA), wherein said formulation has a pH in the range of 5.0 to 8.0.

2. The formulation of claim 1, wherein:

   - the human serum albumin is a recombinant human serum albumin (rHSA);

-said at least one carboxylate buffering agent is selected from the group consisting of succinate, citrate, fumarate, tartrate, maleate and lactate;

-said sugar is selected from the group consisting of sucrose, mannitol, lactose, sorbitol, dextrose, fucose and trehalose, preferably sucrose or trehalose, most preferably sucrose;

-the concentration of sugar is between 1% to 10%; the concentration of phosphate is between 1 mM to 10 mM; and said at least one carboxylate buffering agent is citrate or succinate at a concentration of between 10 mM to 30 mM;

-said formulation further comprises: k) at least one diluent selected from the group consisting of tissue culture medium, saline and water to volume; and/or

-the pH is between pH 7.0 and pH 7.5, preferably pH 7.3.

3. The formulation according to claim 1 or 2 wherein said phosphate is selected from the group consisting of monophosphates, polyphosphates and phosphorylated compounds.

4. The formulation according to claim 3 wherein said monophosphate is potassium phosphate.

5. The formulation according to any one of claims 1 to 4 wherein said formulation comprises an effective amount of at least one strain of chikungunya virus; b) sucrose at a concentration of 5% (w/v); c) potassium phosphate at a concentration of 5 mM ; d) sodium citrate at a concentration of 25 mM; e) $MgCl_2$ at a concentration of 5 mM; f) D-sorbitol at a concentration of 0.5 % (w/v); g) L-methionine at a concentration of 10 mM; and h) recombinant human serum albumin at a concentration of 0.01% (w/v).

6. The formulation according to any one of claims 1 to 5 wherein said live chikungunya virus is selected from an attenuated chikungunya virus of SEQ ID NO: 1; variants with 99% sequence identity to SEQ ID NO: 1 which have the 60aa deletion as in SEQ ID NO: 1; and/or combinations thereof.

7. The formulation according to any one of claims 1 to 5 wherein said live 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.

8. The formulation according to any one of claims 1 to 5 wherein said at least one strain of live chikungunya virus is selected from an attenuated chikungunya virus population that comprises substantially two variants, said two variants both expressing the wild type E1 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.

9. The formulation according to any one of claims 1 to 5 wherein said live 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 SEQ ID NO: 3 (with E168K), respectively, and wherein said two variants have a combined dose of between 10^3 and 2 x 10^4 $TCID_{50}$/dose and a target potency of 5 x 10^3 $TCID_{50}$/dose.

10. The lyophilized live chikungunya formulation according to any one of claims 1 to 5 wherein said live 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) mutations.

11. A method of preparing a lyophilized live chikungunya virus formulation, comprising:

(i) cultivating a chikungunya virus and mixing the chikungunya virus with a concentrated stabilizing solution to form a virus bulk (i), wherein said virus bulk (i) comprises a) 5% (w/v) sucrose; b) 5 mM phosphate; c) 25 mM citrate; d) 5 mM $MgCl_2$; e) 0.5% (w/v) D-sorbitol; f) 10 mM L-methionine; and g) 0.01% (w/v) human serum albumin; and, optionally,

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

(iii) lyophilizing said virus bulk (i) or composition (ii).

12. The method of claim 11, wherein said dialyzing step (ii) results in the formulation according to any one of claims 1 to 10.

13. The formulation of any one of claims 1 to 10 for use in a method of vaccinating a human subject to stimulate a protective immune response against Chikungunya virus in said subject.