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(54) **Title**: VACCINE COMPOSITION COMPRISING AN INACTIVATED CHIKUNGUNYA VIRUS STRAIN

(57) **Abstract**: A vaccine composition for prophylaxis and treatment of Chikungunya virus infections is disclosed which is capable of conferring immunity against any genotypic variants of the Chikungunya virus. More particularly the invention discloses particular nucleotide sequences and their translated proteins thereof, which may be expressed as Virus Like Paricles which for use as a vaccine antigens against Chikungunya virus infections. The compositions disclosed in this invention are also protective against any genotypic variants of the Chikungunya virus which may be propagated by any suitable vector of the disease including Aedis albopictus and Aedis aegypti

VACCINE COMPOSITION COMPRISING AN INACTIVATED CHIKUNGUNYA VIRUS STRAIN

FIELD OF THE INVENTION

The invention relates to stable immunogenic compositions for prophylaxis and treatment against any infections caused by Chikungunya Virus. The present invention particularly relates to compositions of Chikungunya virus (henceforth termed as CHIKV) strains and use of the subunit antigens of the virus thereof, for prophylaxis, therapeutic treatment and diagnosis of Chikungunya infections in humans. More particularly, the invention relates to stable immunogenic vaccine compositions for prophylaxis and treatment against any genotypes or antigenic variants or mutants of Chikungunya virus conferring an antibody titer sufficient for the seroprotection for any genotypic variant or mutant for the Chikungunya virus. The invention also relates to vaccine compositions for immunization against Chikungunya virus in combination with other bacterial and viral infections selected from the following list that include but is not limited to vaccines for Japanese encephalitis virus, dengue vaccines, West Nile virus vaccine and Chandipura virus vaccine and rabies vaccines. Combinations with other viral vaccines are also within the scope of the invention.

BACKGROUND OF THE INVENTION

Chikungunya virus (CHIKV) is an alphavirus of the family *Togaviridae*. It is a positive strand RNA virus that causes a generally non-fatal infection characterized by high fever and sudden onset of polyarthralgia. Hemorrhagic and neurological manifestations including seizures, lymphadenopathy, fulminant hepatitis and conjunctivitis not hitherto associated with CHIKV infections were reported since the re-surgent infection in 2005 (Sourisseau et al., 2007; Kannan et al., 2007). Phylogenetic analyses based on the partial E1 structural glycoprotein sequences have identified three CHIKV lineages, the West African, Asian and the East, Central and South African (ECSA) (Powers et al., 2000). Asian lineage circulated in India and Southeast Asia until it was replaced by the ECSA genotype, which emerged during the 2005–2006 outbreak in the Indian Ocean islands (Yergolkar et al., 2006). Sub-lineages of ECSA strains that had established locally were spread by travellers from endemic areas to Africa, Asia and Europe and caused local outbreaks (Powers and Logue, 2007).

Nearly 1.39 million suspected cases of Chikungunya virus infection occurred in India in 2006. (National Vector Borne Disease Control Programme (NVBDCP), 2007) which was caused by the ECSA strain carrying the E1-226A (Arankalle et al., 2007). The E1-A226V adaptive mutation that increases transmissibility by *Aedes albopictus* is responsible for the wide geographical spread of the virus since then (de Lamballerie et al., 2008). Host immune pressure and resultant site specific mutations in the human leukocyte antigen (HLA) class-1 restricting elements of CHIKV genome are implicated in the explosive Chikungunya virus outbreaks since 2005 (Tong et al., 2010). Prior art known in the field do not include any vaccine candidate derived from the ECSA strain. Bharat Biotech International Limited has earlier developed (disclosed in WO 2008/026225) the 2006 ECSA strain with E1-226A and its use in the development of potential vaccines against Chikungunya virus infections.

Chikungunya virus strains of the urban (epidemic) transmission cycles show a higher evolutionary rate than that of the enzootic (sylvatic) cycle, and the difference in the evolutionary dynamics between the two transmission cycles are influenced by several factors that determine virus-host interactions such as vector diversity and abundance, vector larval habitats and herd immunity in the population (Volk et al., 2010). Arboviruses like Chikungunya interacts with both the arthropod and the vertebrate hosts, and the selection pressure on the envelope glycoproteins are driven by preferences for vector adaptation and by vertebrate host immune defense mechanisms. Viral evolution tends to select for mutations in the antigenic determinants involved in neutralization as well as those residues involved in vector/host adaptation.

The vaccines under development such as that disclosed in WO 2008030220 and in Akahata et al. 2010 make use of the West African genotype and the E1-A226V isolates. Another CHIKV vaccine development is a DNA vaccine (Mallilankaraman et al., 2011) which is different in scope from that disclosed in this invention. An earlier prototype vaccine which is a live attenuated vaccine used the Asian genotype of the virus (Edelman et al., 2001). DNA vaccines have not been successful in human prophylactic vaccination so far, and live attenuated CHIKV vaccine caused side effects in human subjects (Edelman et al., 2001) who received the vaccine. The CHIKV strain used in the earlier vaccine development (WO 2008/026225) was the 2006 ECSA strain with E1-226A. The strains isolated in 2009-2010

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from India as disclosed in this invention belong to a distinct sub-lineage within the ECSA lineage and carry novel mutations in the E2 and E1 envelope glycoproteins. One of the mutations in the E1 glycoprotein in all the isolates reported in the study maps to a region that determines host vector specificity and is under significant positive selection for enhanced adaptation to *Adis. aegypti*, which is the most abundant mosquito vector in the region and indeed in the tropical countries where prevalence of Chikungunya virus infection is now endemic. Other novel mutations hitherto unreported are also disclosed. Thus it is desirable to make a vaccine composition which would confer immunity to the newly developed and distinct sublineages of the ECSA strain of the Chikungunya virus which would also confer immune protection to the other mutated strains of the ECSA strain propagated by the vector *Aedis aegypti.* Inventors in this application after prolonged research disclose such an effective vaccine in this application including other additional advantages over the earlier vaccine (WO 2008/026225) such as new methods of inactivation of the virus and improved formulations with novel adjuvants that enhance the immunogenicity of the inactivated viral vaccine and the recombinant subunit vaccines and virosomes which are also included herein this invention.

DISCLOSURE OF THE INVENTION

The present invention provides a stable vaccine composition that is capable to prevent as well as provide treatment from infections caused by Chikungunya virus. The said vaccine composition is applicable to any genotypic variants of the Chikungunya virus for prophylaxis and treatment thereof.

The invention provides for a stable vaccine composition that is capable to prevent as well as provide treatment from infections caused by Chikungunya virus propagated by any suitable vector which includes prevention and treatment of Chikungunya infections propagated by the vectors *Aedis albopictus* and *Aedis aegypit* which happens to be the most commonly adaptable vectors of the Chikungunya virus.

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The invention provides for a stable vaccine composition which is effective against any genotypic variants of the Chikungunya virus particularly of the ECSA strain and its particular distinct and unique sublineages as applicable thereof.

The invention provides for a stable vaccine composition wherein the antigenic component of the vaccine includes the whole inactivated virion or the subunit antigens of the recombinant CHIKV viral strains that can be expressed as Virus Like particles (henceforth termed as VLPs) in combination of suitable pharmaceutically acceptable carriers, stabilizers, and adjuvants.

The invention provides a method for preparation of a stable vaccine composition that is capable to elicit an immune response sufficient to prevent as well as provide treatment from infections caused by any genotypic mutants or variants of Chikungunya virus including inactivation of the CHIKV virus and mixing with adjuvants in appropriate amounts.

The invention provides antibodies so generated against the Chikungunya virus strains or its subunit antigens useful for diagnosis of Chikungunya virus infections in humans.

The invention relates to provide major antigenic determinants of the Chikungunya virus which are suitable as effective vaccine candidates and nucleotide and protein sequences disclosed thereof.

The invention includes combined vaccine compositions which are effective for prophylaxis and treatment of infections caused by Chikungunya virus and other other bacterial and viral infections selected from the following list that includes but is not limited to vaccines for Japanese encephalitis virus vaccines, dengue vaccines, West Nile virus vaccine and Chandipura virus vaccine and rabies vaccines.

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SUMMARY OF THE INVENTION

According to one aspect of the invention, the invention includes vaccine compositions which specifically contain the whole inactivated virion or the subunit antigens of the CHIKV virus strains. The compositions of the present invention more particularly relate to vaccine capable of eliciting protective antibody and strong T cell responses against Chikungunya virus infection.

Another aspect of the invention is to provide a vaccine composition, comprising one or more Chikungunya virus antigens, wherein the Chikungunya virus antigens are derived from one or more Chikungunya virus isolates selected from TN01610, TN015110, TN06210, TN06310, TN06410, and AP109 comprising a nucleotide sequence as provided in any one of SEQ ID NO. 1 to SEQ ID NO. 6 and SEQ ID NO. 15 to SEQ ID NO. 20.

Another aspect of the invention is to provide inactivated recombinant CHIKV vaccines along with appropriate adjuvants that offer high protective efficacy.

Yet another aspect of the invention of the present invention more particularly relate to vaccine capable of eliciting protective antibody and strong T cell responses against Chikungunya virus infections.

One another aspect of the invention relate to methods of preparing and using Chikungunya virus (CHIKV) antigens of defined sequences expressed as recombinant proteins, virus like particles and as virosomes which are used to elicit protective immune response. The potency of such subunit vaccines are comparable to that elicited by the vaccine consisting of whole inactivated virion of CHIKV that are inactivated with reagents under conditions that confer high immunogenicity to the vaccine.

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Another aspect of the invention relates to methods of inactivation of the virus which comprises heat, gamma irradiation, ultraviolet light or chemically inactivated whole virion of Chikungunya virus isolates in a stable formulation. A combination of two or more inactivating agents has also been used with similar effect. The virus isolates disclosed in the invention are used in vaccine development, and all the methods are applicable to any genotypes or genotypic variants/serotypes/strains/mutants of Chikungunya virus.

One another aspect of the invention is to provide vaccine compositions against Chikungunya virus that elicit strong immunological response when administered parenterally, preferably intradermally, intramuscularly or sub-cutaneously in mammals preferably in humans, and are effective when administered mucosally and by other routes such as oral routes.

Yet another aspect of the invention is to provide antibodies against Chikungunya virus or the subunit antigens thereof to be used for treatment and diagnosis of Chikungunya virus infections in mammals, preferably humans.

Another aspect of the invention is to provide a method of eliciting a protective immune response in a human individual against Chikungunya virus infection comprising administering the vaccine composition as described herein to a human.

Another aspect of the invention is to provide use of the vaccine composition as described herein in the preparation of a vaccine formulation for eliciting a protective immune response in a human individual against Chikungunya virus infection.

Another aspect of the invention is to provide use of one or more Chikungunya virus isolates comprising a structural polyprotein gene sequence as provided in any one of SEQ ID NO. 1 to SEQ ID NO. 6 in the preparation of an immunodiagnostic agent for detection of Chikungunya virus infection in a human.

One another aspect of the invention is to provide a composition for eliciting protective antibody and strong T cell responses either singly or in combination with other vaccines included within the scope of the invention. The other vaccines in combination are but not limited to vaccines for Japanese encephalitis virus vaccines, dengue vaccines, West Nile virus vaccine and Chandipura virus vaccine and rabies vaccines.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1:

Immunogenicity of CHIKV whole virion antigen inactivated by several inactivation methods were tested for potency. The details of inactivation procedures are provided in Example 2. Potency of the 15 μg of the inactivated viral vaccine was tested in three intramuscular injections in 4-6 week old Balb/c mice (8 nos per group) at intervals of 0, 7 and 21 days and bled 7 days after the last dose administration. Only a single dose of the live virus was administered for comparison. The potency of the vaccine preparations were tested by estimating the titer of neutralizing antibodies by $PRNT_{50}$.

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Figure 2:

Immunogenicity of the CHIKV vaccine preparation with and without adjuvants was tested in three intramuscular injections in 4-6 week old Balb/c mice (8 nos per group) at intervals of 0, 7 and 21 days and bled 7 days after the last dose administration. The composition of the adjuvanted vaccine formulations are provided in Example 5. The potency of the vaccine preparations were tested by estimating the titer of neutralizing antibodies by $PRNT_{50.}$

DETAILED DESCRIPTION OF THE INVENTION

No detailed study on evolution of CHIKV serotypes due to sequence diversity has been reported. We report for the first time the adaptive evolution of ECSA strains of CHIKV to *Ae.aegypti* as found in the 2009-2010 virus isolates from India. Incidentally, *Ae.aegypti* is the most prevalent vector in India and indeed in several tropical countries. Despite unique mutations in isolates reported in the current invention, the virus strains cross neutralize the Asian genotypes and various ECSA sub-lineages of CHIKV indicating that they are good candidates for vaccine development. Using virus strains or antigens derived from such strains thereof, that are better adapted to the most prevalent vector in the region is important for vaccine development rather than using strains of West African or Asian genotype which are not so widely prevalent now than the ECSA genotype. Even among the ECSA genotype, using candidates such as LR2006 isolates from Reunion Island that carry E1-A226V mutation which is an adaptive mutation to increase transmissibility in *Ae.albopictus* is less advantageous as *Ae.albopictus* vector in India is prevalent widely only along the West coast of India such as in the states of Kerala and South coastal Karnataka, whereas the mosquito vector that is most abundant in the rest of the country is *Ae.aegypti*. The virus strains isolated and reported in this invention are unique in that they show adaptive evolution to *Ae.aegypti* and at the same time also infect *Ae.albopictus.* Apart from the unique mutations that increase adaptation to *Aedes aegypti*, the advantage of the invention is that the virus isolates cross neutralize the Asian genotypes and various ECSA variant strains and hence are good candidate vaccines. Hence, a subunit vaccine derived from the virus antigens or recombinant antigens of these isolates are good vaccine candidates as well, as the recombinant vaccine antisera also cross neutralizes the different genotypes and genotypic variants.

Hence, using the Indian virus strains that show unique adaptation to *Ae.aegypti* and also infects *Ae.albopictus* is advantageous than using the West African, Asian or ECSA E1-226A and other variant strains as *Ae.aegypti* is the most widely prevalent vector in the India which has the highest incidence of CHIKV infection in the world.

The Chikungunya virus isolates within the scope of the invention are those that belong to the ECSA (East, Central and South African) genotype whose structural polyprotein sequence comprises of the capsid, E3, E2, 6K and E1 (C-E3-E2-6K-E1) proteins. The isolates obtained from the Indian epidemic of 2009-2010 are unique in the sequence reported so far. The structural polyprotein sequence comprising the C-E3-E2-6K-E1 proteins have been deposited in the public sequence repository (GenBank) on 27th April 2010 and have been assigned the accession numbers HM159385 to HM159390. The sequences were published in March 2012 after the date of filing the provisional patent. The unique nucleotide sequences reported in this invention are SEQ ID NO.1 (isolate TN01610), SEQ ID NO.2 (isolate TN15110) SEQ ID NO.3 (isolate TN06210), SEQ ID NO. 4 (TN06310), SEQ ID NO. 5 (TN06410) and SEQ ID NO.6 (AP0109), whose corresponding protein sequences when translated are SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12 and SEQ ID NO.13 respectively. The CHIKV strain CHIKV/03/06 has structural polyprotein of SEQ ID NO.7 and was isolated during the 2006 Indian epidemic, the corresponding protein sequence is SEQ ID NO.14. The names of the virus isolates are provided in the brackets. The full length genomic RNA sequences of the above mentioned virus isolates of the current invention are provided in SEQ ID NO.15 to SEQ ID NO.20.

The sequence of the isolates disclosed in the invention have unique genetic signatures such as the combination of T1766C (E2-V264A) + A3058G (E1-K211E) + 3104C (E1-226A) in the structural polyprotein sequence in addition to other amino acid changes when compared to the S27 African prototype (Gen Bank Acc No. AF369024). The position of nucleotide substitution in the structural polyprotein and the corresponding amino acid change in the individual proteins within the polyprotein is indicated in brackets. Other unique mutations that are being reported are Capsid-A232V in TN06310, E3-D40N in TN15110, E2-K47N in TN06210, E2-G55R in TN01610 and AP0109, E2-K66E in TN064110, E1-P58L in AP0109, and E1-G195R in TN15110 and TN06310. Codon by codon analyses by maximum likelihood estimates of 'ω' (the ratio of non-synonymous to synonymous substitutions) of the ECSA strains show that the amino acid mutation E1-K211E in the isolates reported in the invention (of SEQ ID NO. 8 to SEQ ID NO.13) is under significant positive selection (posterior probability of ≥ 0.97; $p<0.05$) and is suggestive of adaptive mutation to increase infectivity in the *Aedes* mosquito vectors, particularly in *Aedes aegypti*. The amino acid residue E1-211E is conserved in the Asian genotypes of CHIKV which are circulated by *Ae.aegypti*. Additional mutations disclosed in this invention such as the three novel mutations E2-K47N, E2-G55R and E2-K66E also cluster in the same region of the E2 protein that are reported to increase the infectivity of the Sindbis virus in *Aedes aegypti.* The E2 aa 52 – 82 region is exposed at the top of the spike, which is the point of contact with cellular receptors. Codon by codon maximum likelihood estimates of 'ω' by SLAC (Single Likelihood Ancestor Counting), eFEL (Fixed Effects Likelihood), iFEL (internal Fixed Effects Likelihood) and REL (Random Effects Likelihood) identified amino acid sites across the capsid and the structural glycoproteins under significant purifying selection. Among the amino acid sites that were negatively selected, the E2-199Y residue was selected as the genetic loci under most significant purifying selection by all the four likelihood estimates (posterior probability >0.99 by REL, $p<0.01$ by iFEL, $p=0.001$ by SLAC and $p=0.00$ by eFEL). E2-199Y is an important residue in Chikungunya virus determining virus fitness in mosquitoes.

Viral evolution tends to select for mutations in the antigenic determinants involved in neutralization as well as those residues involved in vector/host adaptation. Because of its high immunological specificity, the serum neutralization test is often the gold standard against which the specificity of the other serological techniques is evaluated. The antisera raised against the virus isolates reported in the invention neutralized the virus isolates of Asian and ECSA lineages and several variant strains of ECSA genotype including the E1-A226V ECSA variant strain, indicating that they are good vaccine candidates as they have broad neutralizing activity.

The properties of Chikungunya virus particles as an immunogen, adaptation and propagation of the virus in host cell lines to a high titer, determination of the identity of the virus by RT-PCR, methods of purification and inactivation of the virus, preparation of stable vaccine formulation in a pharmaceutically acceptable carrier suitable for administration in humans, the viral assays and tests for vaccine potency in animal models are also within the scope of the invention. The virus particles obtained from infected patients or isolated from the vectors of the virus where the virus resides, are adapted in cell lines and propagated *in vitro* in cell culture in several passages.

The use of the CHIKV strains in the development of an inactivated whole virion vaccine is one aspect of the invention. The Chikungunya virus strains were infected in mammalian cell lines for production of the virions. The mammalian cells include but are not limited to Vero cells (ATCC CCL-81), MRC-5 or any other cell line suitable for vaccine production for human use.

The whole virions obtained from cell culture were inactivated with different inactivating agents. The optimum time, temperature and use of stabilizers such as sugars like sucrose, lactose, trehalose and other sugars and sugar combinations, and the addition of sugar alcohols such as mannitol or sorbitol either alone or in combination with different sugars, addition of human serum albumin either alone or in combination with sugars, amino acids and sugar alcohols during the inactivation process are within the scope of the invention. The virus was rendered non-infectious by inactivating either by heat, gamma irradiation or ultra violet light or by chemical means with formalin and beta-propiolactone (BPL) among others under conditions that retained high immunogenicity of the vaccine preparation. The conditions of virus inactivation were optimized and are presented in Example 2. Chemical inactivating agents are selected from the following list which includes but is not limited to: formalin, beta-propiolactone, glutaraldehyde, N-acetylethyleneimine, binary ethyleneimine, tertiary ethyleneimine, ascorbic acid, caprylic acid, psolarens, detergents including non-ionic detergents etc. is added to a virus suspension to inactivate the virus. The concentration of the sugars, sugar alcohols, human serum albumin and amino acids either when used alone or in various combinations were in the concentration range of 0.01% to 20%, preferably 0.1% to 10% and most preferably 0.1% to 5%. Time and temperature of inactivation in the presence of the stabilizers were optimized from 2-8^{0}C to 37^{0}C for varying period of time such as 30 min to 20 days. Such vaccine formulations were highly immunogenic and elicited protective neutralizing antibodies.

The structural glycoproteins C-E3-E2-6K-E1 of the Chikungunya virus are the major antigenic determinants. Hence, the structural glycoproteins are excellent vaccine candidates for subunit vaccine for prophylaxis of CHIKV infections. The sequence of the structural proteins as defined in SEQ ID NO.8 to SEQ ID NO. 14. The recombinant non-structural proteins are also immunogenic and are good candidate vaccines. The eukaryotic expression system of choice includes mammalian cells, baculovirus in insect cells, and yeast cells of any species, most preferably *Pichia pastoris* or *Saccharomyces cerevisiae.* Genes encoding the subunit antigens were also expressed in prokaryotic cells such as *E.coli* using any of the suitable prokaryotic expression vectors. *Pichia pastoris* as recombinant expression host is advantageous at industrial scale as it is cost effective for large scale manufacture compared to other eukaryotic expression systems. Recombinant proteins derived from *Pichia pastoris* have been successfully commercialized and have been found safe for human use. The structural proteins such as C-E3-E2-6K-E1 of the sequences disclosed in this application are capable of assembling into 'virus like particles' (VLPs). Alternatively, the VLPs contain only the E3-E2-6K-E1 or E2-6K-E1 or only E2-E1 proteins and are immunogenic and elicited protective immune response when administered in animals. The subunit antigens comprising E3-E2-6K-E1 or E2-6K-E1 are also capable of assembling into virosomes as CHIKV is an enveloped virus. Virosomes comprising E3-E2-6K-E1 or E2-6K-E1 or only E2-E1 are also immunogenic. The liposomes and virosomes can contain different combination of lipid soluble substances which include but are not limited to cholecalciferol, cholesterol, phospholipids etc. and the viral envelope proteins. The methods for virosomes preparation such as solubilization of the virus particles with detergents or with short chain phospholipids and reconstitution of the envelope proteins after removal of the chaotropic agents and the non-envelope proteins and RNA that are applicable to any enveloped virus are also applicable to CHIKV.

Purification of the virus was achieved by physical or chemical means and preferably by a combination of both. Physical methods utilize the physical properties of the virus such as density, size, mass, sedimentation coefficient etc. and include any of the following techniques but are not limited to: ultracentrifugation, density gradient centrifugation, ultrafiltration etc. Purification through chemical means employs methods such as adsorption/desorption through chemical or physiochemical reactions such as ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, gel filtration chromatography, hydroxyapatite matrix, salting with inorganic salts one such example being ammonium sulphate, and by the use of proprietary Himax™ technology, organic salts and organic compounds such as polyethylene glycol. Purification of the virus or the recombinant virus antigens was achieved by either one or a combination of two or more of the above mentioned methods.

The antigenic compositions of the above mentioned CHIKV candidate vaccines, such as the inactivated whole virion vaccines or the recombinant vaccines were formulated in pharmaceutically acceptable carrier for immunization in mammals, preferably humans. The Chikungunya virus vaccine formulation was adjuvanted and adjuvants were selected from the following list, which includes but is not limited to: alum; calcium phosphate; inulin of any polymorphic form, preferably gamma inulin; adjuvants containing inulin in combination with other organic and inorganic compounds such as aluminum hydroxide, aluminum phosphate, aluminum sulphate phosphate and calcium phosphate; liposomes, chitosan and complex carbobhydrates such as dextran, dextrins, starch, inulin, mannans and glucomannans, galactomannans, beta-glucans, heparin, cellulose, pectins and pectinates, lectins and any other carbohydrates either synthetic or derived from any source, any biodegradable and biocompatible polymers, such as poly lactide and poly(lactide co-glycolides; PLG) or PLGA; any emulsions including but not limited to oil in water emulsions one such example being ASO3, other squalene based adjuvants such as MF59 etc., any water in oil emulsion; liposomes prepared with cholecalciferol as one of the ingredients along with other lipid soluble compounds; liposomes of other compositions; RIBI adjuvant systems, saponins including but not limited to QS-21, QuilA, tomatine, ISCOMs, ISCOMATRIX etc, lipopeptides, glycopeptides, lipopolysaccharides, muramyl dipeptides and any peptide based adjuvants, oligonucleotides, any TLR ligands as adjuvants, any cytokine, vitamins and non-toxic bacterial toxins etc. The most compatible and cost effective adjuvant was selected in the final vaccine formulation after testing for immunogenicity which was enhanced by the addition of adjuvants. In addition to the above, any other organic and inorganic substances that have good immunopotentiating activity can also be used as adjuvants either singly or in combinations to enhance the immunogenicity of Chikungunya virus vaccines. In addition to the inactivated whole virion vaccine, the aforementioned adjuvants or adjuvant combinations are also effective with recombinant Chikungunya virus vaccine using recombinant subunit antigens either when presented as virosome, virus like particles (VLPs) or when expressed, purified and formulated as individual recombinant proteins. The use of suitable adjuvants in the vaccine formulations reduces the amount of antigen required and helps in the manufacture of low-cost vaccines thus conferring economic advantage.

The buffer used in the formulations is phosphate or phosphate-citrate buffer or any other pharmaceutically acceptable buffer. The vaccines optionally contain preservative(s), stabilizer(s) etc. The excipients were selected from a list that includes but is not limited to reducing and non-reducing sugars, sugar alcohols such sorbitol and mannitol, glycerol, amino acids, human serum albumin, inulin, thiomerosol and a choice of adjuvant from the aforementioned list of adjuvants. The excipients are added in the range of 0.01% to 20% for the liquid formulation and upto 60% of the total solids for a lyophilized formulation. The vaccine formulations were also presented as emulsions, either as water in oil emulsion or as oil in water emulsion. Such emulsions of vaccine antigens contain preservatives and stabilizers and other adjuvants. Such a stable formulation of the immunogen either in a liquid or in a lyophilized form and after reconstitution in a pharmaceutically acceptable buffer or water is suitable for administration parenterally in human host and is also formulated for mucosal administration. The vaccine formulations were highly immunogenic and neutralized homologous and heterologous CHIKV strains.

For potency testing of the vaccine, the vaccine formulations were tested in Balb/c mice and rabbits. The resultant serum is assayed by *in vitro* neutralization tests and the antibody titer is determined by ELISA. Seroconversion was observed in the animals immunized with the vaccine formulations described in the present invention. Efficacy of the recombinant vaccine in offering a protective immune response was comparable with the whole virion vaccine and the titers of the neutralizing antibody responses were determined by either serum neutralization test (SNT), plaque reduction neutralization test ($PRNT_{50}$) and ELISA among other methods. Passive immunization of the vaccine antibody offered good protection against virus infection indicating therapeutic use of CHIKV antibodies. The presence of virus in infected patients samples were accurately determined using CHIKV antibodies. Chikungunya virus vaccine obtained by the methods included in the scope of the current invention elicits strong neutralizing antibodies in combination with other vaccines. The vaccines that can be included in the combination are selected from the following list that includes but is not limited to vaccines for Japanese encephalitis virus, Dengue vaccines, West Nile virus vaccine and Chandipura virus vaccine and rabies vaccines. Combinations with other viral vaccines are also within the scope of the invention. As known to those skilled in the art, a bivalent or polyvalent vaccine can be prepared by mixing vaccines produced from two or more CHIKV strains, and is mixed in a suitable ratio based on the antigen content. Such mixing provides a vaccine preparation having a broad antigenic spectrum for protection against the infection.

According to the present invention, the methods and compositions of CHIKV strains of the current invention is applicable to any CHIKV strain. The vaccines of this invention offered good immune protection against plural strains of CHIKV in addition to the virus strains used in production of the vaccine. The CHIKV isolates reported in the study have broad neutralizing activity as they cross neutralize different genotypes /genotypic variants / strains of CHIKV and are ideal vaccine candidates for development of whole inactivated virion vaccine or recombinant vaccines comprising the antigens derived from these virus isolates. The methods disclosed in the invention are applicable to any genotype/genotypic variants/serotype/strain of Chikungunya virus and as demonstrated offer good cross protection against multiple gentotypes/genotypic variants of the virus.

The invention is further described in the following examples. It should be noted that features, integers, characteristics, ranges, compounds, and/or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith and should be considered within the scope of the invention.

Example 1: Isolation of virus strains

The virus strains were isolated from blood samples collected from febrile patients with their informed consent during an epidemic outbreak in India in 2009-2010. The blood samples were collected during the acute phase of Chikungunya virus infection when patients reported high fever, acute polyarthralgia and painful swelling in joints and rashes. The patients' sera samples were transported on dry ice to the laboratory. About 0.05 ml of the serum was used for infection of Vero cells (ATCC No. CCL-81) in 25^2cm flask in medium containing DMEM (Dulbecco's Modified Eagle Medium; Sigma- Aldrich Catalog # D5523) containing 1% fetal bovine serum (FBS). The flasks were incubated at 34^0C to 37^0C. The virus was harvested 48 hours after infection. Scaled up cultures of the virus were made in cell stacks or in cell factories or in bioreactors in liquid culture All the blood samples were negative for Dengue infection by specific IgM ELISA (National Institute of Virology, Pune). The infectious titer of the virus increased more than 10 fold after the virus particles were passaged once in suckling mice brain or after passage in mosquito cell lines such as C6/36 cells, and also after repeated passage of the virus in cell culture *in vitro*.

Example 2: Purification and inactivation of CHIKV virus

The two virus isolates TN01610 and TN15110 were purified from the infected Vero cell monolayers from scaled up cultures by initial ultrafiltration to remove cellular debris, and by filtration and concentration through a 300 kD membrane followed by purification by ion exchange and gel filtration column chromatography. Heat inactivation of the virus was carried out at different temperatures ranging from 45^0C to 60^0C for 30 min to 4 hrs and optimally at 56^0C for 30 min. Inactivation by ultraviolet (UV) light was done at 254 nm for varying period of time from 30 – 120 min on ice, and optimally for 40 min. Chikungunya virus was inactivated effectively by formalin at ratios upto 1:3000 for formalin:virus at 2^0C -8^0C upto 7 days, and with beta propiolactone at 1:1000 to 1:2500 (beta propiolactone:virus) for upto 7 days at 2^0C -8^0C. In both the cases, the time of inactivation was reduced to 24-48 hrs when carried out at ambient temperatures of +20 - 25^0C. Formalin and beta propiolactone were removed by dialysis. During inactivation, use of additives such as glycine, mannitol, sorbitol and sugars and sugar combinations increased the stability of the vaccine preparation. The sugars used may be selected from sucrose, lactose, trehalose, maltose at varying concentrations from 0.5% to 5%. Inactivation of the virus by gamma irradiation was carried out by exposure of the virus samples to a dose of 10 kGy (Kilo Gray) to 25 kGy from a ^{60}Co source (Ms.Gamma Agro-Medical Processings Pvt.Ltd. Hyderabad) and optimally to 20 kGy. Complete inactivation of the virus samples by all of the above methods were confirmed by three serial passages in Vero cells for absence of virus cytopathic effect, and additionally by the absence of growth abnormalities and death when inoculated by intracerebral route in the brain of 2-day old mice. The inactivated virus antigens were tested for potency as candidate vaccines..

Example 3: Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) and Sequencing:

Viral RNA was isolated using Absolutely RNA Miniprep kit (Stratagene, La Jolla, CA) from infected Vero cells (ATCC CCL-81), after a single passage. RT-PCR was carried out using the AccuScript High Fidelity 1st Strand cDNA Synthesis Kit (Stratagene) as per the kit protocols, and the 3,747 bp structural polyprotein gene was amplified with the PfuUltra High-Fidelity DNA polymerase (Stratagene). PCR primers were designed based on the consensus sequence of the S27-African prototype (AF369024) and the Indian 2006 isolate (HM159384), and used to amplify overlapping sequences of the structural polyprotein gene. PCR reaction consisted of initial denaturation at 95^0C for 1 min, followed by 32 thermal cycling steps at 94^0C for 40 sec, annealing at 52–65^0C (depending on the primer sets) for 30 sec and extension at 70^0C for 3 min, followed by final extension at 70^0C for 10min. PCR products were purified by QIAquick gel extraction kit (QIAGEN, Hilden, Germany) after separation on 1% agarose gel and used for DNA sequencing. Nucleotide sequencing of CHIKV structural polyprotein gene gel purified PCR products were sequenced on both strands of DNA by BigDye terminator v3.1 reaction (Applied Biosystems, Foster City, CA) and the sequence data was analyzed using Sequencher v4.7 (GeneCodes, Ann Arbor, MI). The sequences were deposited in GenBank on 27th April 2010 before filing the provisional patent and published by GenBank on 02 March 2012. The unique nucleotide sequences reported in this invention are SEQ ID NO.1 (isolate TN01610), SEQ ID NO.2 (isolate TN15110) SEQ ID NO.3 (isolate TN06210), SEQ ID NO. 4 (TN06310), SEQ ID NO.5 (TN06410) and SEQ ID NO.6 (AP0109), whose corresponding protein sequences when translated are SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12 and SEQ ID NO.13 respectively. The CHIKV strain CHIKV/03/06 has structural polyprotein gene of sequence SEQ ID NO.7 and was isolated during the 2006 Indian epidemic and its corresponding protein sequence is SEQ ID NO.14. The names of the virus isolates are provided in the brackets. For complete genomic RNA sequences, the sequencing reactions were performed using sequencing by synthesis (SBS) technology on the Illumina GAIIx (Genotypic Technology Pvt. Ltd. Bangalore). The complete nucleotide sequences (in the form of cDNA) of the virus genomic RNA of the above mentioned virus strains are provided in SEQ ID NO.15 to SEQ ID NO.20. Mutations identified with reference to strain S27-African prototype (AF369024) were mapped to the individual structural proteins and are presented in Table 1.

TABLE I. Unique mutations in the Chikungunya virus structural genes reported in this study.

Amino acid position		Nucleotide change in polypeptide	strain S27-African prototype	CHIKV/03/06	TN01610	TN151100	TN06210	TN06310	TN06410	AP0109
Poly-peptide	Protein									
232	C-232	c695t	A	.	.	.	.	V	.	.
301	E3-40	g901a	D	.	.	N	.	.	.	.
372	E2-47	a1116t	K	.	.	.	N	.	.	.
380	E2-55	g1138a	G	.	R	.	.	.	.	R
391	E2-66	a1171g	K	.	.	.	.	.	E	.
589	E2-264	t1766c	V	.	A	A	A	A	A	.
867	E1-58	c2600t	P	.	.	.	.	.	.	L
1004	E1-195	g3010c	G	.	.	R	.	R	.	.
1020	E1-211	a3058g	K	.	E	E	E	E	E	E

Unique mutations identified in the capsid, E1, E2 and the E3 structural glycoproteins in the 2009-2010 CHIKV isolates from the States of Tamil Nadu and Andhra Pradesh.

"." Amino acids identical to the reference strain S27-African prototype (AF369024). The GenBank accession numbers of the isolates from Tamil Nadu are HM159385 (TN01610), HM159386 (TN15110), HM159387 (TN06210), HM159388 (TN06310), HM159389 (TN06410), and from Hyderabad, Andhra Pradesh are HM159384 (CHIKV/03/06) and HM159390 (AP0109).

Example 4: Phylogenetic Analyses and Inference of Selection Pressure

The sequences reported in this study and those retrieved from GenBank were screened for recombination by the Genetic Algorithm Recombination Detection (GARD) (Kosakovsky Pond et al. 2006) prior to phylogenetic analysis. Evolutionary analyses were performed in MEGA5 (Tamura et al. 2007) using Kimura-2 parameter model of nucleotide substitution with 1000 bootstrap replicates. Multiple sequence alignment was performed using ClustalW2.0.3. The ECSA structural polyprotein sequences from 2005-2010 retrieved from GenBank and those reported in the study were used in the inference of selection pressure on the ECSA lineage. About 52 unique sequences were short listed by Hyphy (Pond et al. 2005) from 58 sequences retrieved from GenBank for the analyses. Codon-based Maximum Likelihood estimates of ω or the dN/dS (the ratio of non-synonymous to synonymous substitutions) were inferred by Random Effects Likelihood (REL), Fixed Effects Likelihood (eFEL) and selection along the internal branches of phylogeny was tested using Internal Fixed Effects Likelihood (iFEL) method in HyPhy. In the likelihood methods, positive selection was inferred as significant if the p value of the likelihood ratio test (LRT) was less than 0.05 or when the Bayes factor was equal to or larger than 100 for a site. Statistical testing of positive selection operating on the entire protein was inferred by Single Likelihood Ancestor Counting (SLAC) method in HyPhy. Inference of ω by empirical Bayesian method using LRT (Likelihood Ratio Test) with the MEC (Mechanistic Empirical Combination) model for positive selection, and M8a model for purifying and neutral selection was carried out using Selecton v2.2 (Stern et al. 2007). The amino acid sites of CHIKV structural proteins under significant positive and purifying selection is provided in accompanying Table II.

TABLE II. Amino acid sites of CHIKV structural proteins under significant positive and purifying selection

Method	Codon no. in structural polyprotein	Positively selected amino acid	Negatively selected amino acid	p-value	Posterior probability	Bayes factor†
REL	523	E2-198R			0.87	111.55
	524		**E2-199Y**		0.999	505.10
	645	E2-320T			0.86	100.71
	711	E2-386A			0.87	108.86

	1020	**E1-211K**			0.97	532.15
	1035	**E1-226A**			0.98	773.33
	1078	E1-269V			0.86	100.10
	1113	E1-304 P			0.88	120.58
iFEL	28		C-28I	0.034		
	273		E3-12N	0.008		
	326		E2-1S	0.036		
	397		E2-72N	0.008		
	524		**E2-199Y**	**0.003**		
	834		E1-25S	0.036		
	909		E1-100N	0.005		
	916		E1-107H	0.016		
	1020	**E1-211K**		0.040		
	1035	**E1-226A**		0.006		
	1120		E1-311D	0.042		
	1245		E1-436F	0.009		

The amino acids under positive selection in the capsid (C) and in the E1, E2 and E3 glycoproteins in the 2009-2010 Indian CHIKV isolates were inferred by Random Effects Likelihood (REL) and by Internal Fixed Effects Likelihood (iFEL) methods using the HyPhy package. The amino acid sites under significant positive and purifying selection in the E1 and E2 proteins respectively (Bayes factor >500, posterior probability ≥0.97 and $p<0.05$) are indicated in boldface. †Bayes factor is statistical estimation of posterior odds/prior odds for positive selection (dN>dS) at the site.

Example 5: Cloning and Expression of the Structural Polyprotein Sequences

The virus isolates reported in this patent was used as the source for cloning and expression of all viral antigens. The complete open reading frame of the Chikungunya virus structural polyprotein encoded by the SEQ ID NO.1 was amplified by RT-PCR of the viral genomic RNA using the primers CHKVCPFP as the forward primer and CHKVE1RP as the reverse primer to obtain a ~ 3747 bp PCR fragment. The sequence of the PCR primers used for PCR amplification is:

CHKVCPFP:

5' ACAGAATTCATATGGAGTTCATCCCAACCCAAAC 3'

CHKVE1RP:

5' AATTGGATCCGCGGCCGCTTAGTGCCTGCTGAACGACACGC 3'

The PCR fragment was digested with Nde1 and BamH1 and cloned into the Nde1 and BamH1 sites of the prokaryotic expression vector, pET-11B and the recombinant plasmid containing the insert was transformed in *E.coli* DH5a. The recombinant plasmid DNA isolated from DH5a was used to transform the *E.coli* strain BL21(DE3). The PCR gene fragment was digested with EcoR1 and Not 1, gel purified by standard protocols and cloned into EcoR1 and Not1 sites of the yeast expression vector pPIC3.5K (Invitrogen Corporation, Carlsbad, USA) and transformed in *E.coli* DH5a. Recombinant plasmid DNA isolated from *E.coli* clone was linearized with BglII and was transformed into *Pichia Pastoris* GS115 as per the protocol from manufacturers (Invitrogen). The gene has been cloned into the *AOX1* locus and expressed under the *AOX1* promoter by methanol induction. The cloning, screening, isolation of the recombinant *Pichia* strains and induction of the cloned gene with methanol were carried out as per the User's manual "A Manual of Methods for Expression of Recombinant Proteins in *Pichia pastoris*" Version M Jan 2002, of *Pichia* Expression Kit, Catalog # K1710-01, Invitrogen Corporation, Carlsbad, USA).

Example 6: *In vivo* potency testing of the vaccine formulations:

The inactivated virus sample in vaccine formulations was tested with different adjuvants for potency. The adjuvants tested (at concentrations per single human dose) include a) aluminum hydroxide (0.5mg aluminum content) b) aluminum phosphate (0.5 mg aluminum content) c) gamma inulin (10 mg), d) algammulin (a combination of aluminum hydroxide and gamma inulin) at 10 mg, e) cholecalciferol in oil at 0.75 mg per dose, f) an oil in water emulsion OWEM1, containing 4.3% squalene, 0.5% tween-80, and 0.5% Span-85 (Sigma Aldrich product # S7135) in 10 mM phosphate-citrate buffer, f) oil in water emulsion OWEM2 containing 9.5mg squalene, 1 mg tween-80, 1 mg Span-85, 11 mg alpha tocopherol in phosphate-citrate buffer, g) an oil in water emulsion OWEM3 containing at the same concentration of excipients as in OWEM2 except that alpha tocopherol is replaced with 1-10 mg cholecalciferol. The formulated and adjuvanted vaccine preparations were injected intramuscularly in mice and booster doses were administered on day 7 and day 21 after administration of the first dose. Blood was collected at 28 days after the first dose was administered. Pooled sera from each test group were complement inactivated at 56^0C for about 30 min. All the formulations contained 15 µg viral antigen in 40 mM phosphate buffer, pH 6.8 – 7.2 containing 150 mM NaCl. Sera samples were used for estimation of neutralizing antibodies and for the estimation antibody titer by ELISA. Vaccinated animals offered complete protection against viremia with a virus challenge dose of $10^{4.5}$ pfu/ml when monitored over a period of 72 hours after intravenous/intraperitoneal administration of the challenge virus. In another experiment, passive immunization with rabbit antisera with $PRNT_{50}$ titer of 640 when administered intravenously in 4-6 week old Balb/c mice offered complete protection against viremia when challenged with $10^{4.5}$ pfu/ml of the challenge virus. For serotype analyses, antisera against CHIKV/03/06 neutralized heterotypic virus isolates of the Asian genotype (GenBank Acc No. EF027140, isolated in Kolkata in 1963), ECSA, (E1-A226V, E1-211K; GenBank Acc No. FJ000069, isolated in Kerala in 2007) and ECSA (E1-226A, E1-K211E, GenBank Acc No. HM159386, obtained from Tamil Nadu in 2010 with neutralizing antibody titer ≥ 40 indicating heterotypic protection against genotypic variants, and also indicating that no distinct serotypes have evolved.

Example 7: Plaque Reduction Neutralization Assay

One day prior to the assay 6-well plates were seeded with 2.5 x 10^3 Vero cells (ATCC CCL-81) per well and the plates were incubated at 37°C in a 5% CO_2 incubator. To 4-fold dilutions of the sera samples in MEM containing 2% fetal bovine serum, equal volume of the standardized virus (10^5 pfu/ml) was added and incubated at 37°C with 5% CO_2 for 90 min. The cells were washed twice with 1 x PBS pH 7.4 (10 mM phosphate with 150 mM NaCl) and 0.3 ml of each dilution of the serum-virus mixture was added to the corresponding well and incubated for 90 min at 37°C in a 5% CO_2 incubator. Each assay was carried out in triplicates. The cells were overlaid with 2 ml of 0.85% methyl cellulose in MEM containing 10% fetal bovine serum, 1% penicillin-streptomycin and 1% Lglutamine. The plates were incubated at 37°C in a 5% CO_2 incubator for 5 days. At the end of incubation, the plaques were fixed with 10% formalin, washed with 1 x PBS, pH 7.4 and were visualized with 0.1% crystal violet. The highest dilution of serum causing 50% reduction in plaques formed by the control virus sample was estimated as the $PRNT_{50}$ titer. $PRNT_{50}$ assays were carried out to test the potency of the vaccine preparations by various inactivation methods, as well as for adjuvanted CHIKV vaccines and vaccine combination with JEV vaccine..

Example 8: Vaccine combinations

A combination of CHIKV vaccine inactivated by beta-propiolactone was tested in combination with formalin inactivated vaccine for Japanese encephalitis virus (JEV). 15 μg of CHIKV vaccine antigen formulated in alum (0.5mg aluminum/ dose) was tested in combination with inactivated JE (JEV) virus vaccine containing 6 μg of Japanese encephalitis virus whole virion antigen also formulated in alum. The vaccine combination was injected in 8 nos of Balb/c mice with appropriate controls that included either of the antigens alone, and also control animals that received equivalent amount of alum. The animals were boosted at 7 and at 21 days after the first immunization. Blood was collected at 7 days after the last booster injection. Pooled sera from each group were complement inactivated at 56^0C for about 30 min. The sera samples were used for estimation of neutralizing antibody by $PRNT_{50}$ for both CHIKV and JEV. The buffer used in all the formulations was 40 mM phosphate buffer, pH 6.8 – 7.2 containing 150 mM NaCl. All the methods disclosed above are applicable to any genotype/genotypic variants/serotypes and strains of Chikungunya virus.

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We claim:

1. A vaccine composition, comprising one or more Chikungunya virus antigens, wherein the Chikungunya virus antigens are derived from one or more Chikungunya virus isolates selected from TN01610, TN015110, TN06210, TN06310, TN06410, and AP109 comprising a nucleotide sequence as provided in any one of SEQ ID NO. 1 to SEQ ID NO. 6 and SEQ ID NO. 15 to SEQ ID NO. 20.

2. The vaccine composition of claim 1, wherein the one or more Chikungunya virus isolates comprise a structural polyprotein comprising an E1 structural glycoprotein, wherein the structural polyprotein has a non-synonymous mutation K1020E corresponding to E1-K211E in the E1 structural glycoprotein, either singly or in combination with other mutations selected from A232V, D301N, K327N, G380R, K391E, V589A, P867L, G1004R, and A1035V in the structural polyprotein sequence.

3. The vaccine composition of claim 1 or claim 2, comprising one or more structural polyproteins comprising an amino acid sequence as provided in any one of SEQ ID NO. 8 to SEQ ID NO. 13.

4. The vaccine composition of claim 1, wherein the one or more Chikungunya virus antigens comprise a combination of capsid protein and structural glycoproteins of the Chikungunya virus isolates, comprising C-E3-E2-6K-E1, C-E2-E1 and E2-E1 proteins expressed as Virus Like Particles.

5. The vaccine composition of any one of claims 1 to 4, wherein the one or more Chikungunya virus antigens are recombinant polypeptides.

6. The vaccine composition of claim 5, wherein the one or more Chikungunya virus antigens were expressed in *E.coli* or *Pichia pastoris*.

7. The vaccine composition of any one of claims 1 to 6, wherein the Chikungunya virus is inactivated by any of the following methods:

   i) Ultraviolet radiation at 254 nm for 30 min to 120 min; or
   ii) Gamma irradiation by exposing the virus samples to a dose of 10kGy (Kilo Gray) to 25 kGy from a ^{60}Co source.

8. The vaccine composition of any one of claims 1 to 7 further comprising an adjuvant selected from (a) aluminum hydroxide (b) aluminum phosphate (c) gamma inulin, (d) algammulin: a combination of aluminum hydroxide and gamma inulin (e) cholecalciferol in oil (f) an oil in water emulsion OWEM1, containing squalene, tween-80, Span-85 in 10 mM phosphate-citrate buffer, (g) oil in water emulsion OWEM2 containing squalene, tween-80, Span-85, alpha tocopherol in phosphate-citrate buffer, (h) an oil in water emulsion OWEM3 containing squalene, tween-80, Span-85, cholecalciferol in phosphate-citrate buffer.

9. The vaccine composition of any one of claims 1 to 8, wherein the Chikungunya virus antigen is at a dose ranging from 1 μg to 100 μg per human dose in 40 mM phosphate buffer and 150mM NaCl.

10. A combined vaccine composition comprising the vaccine composition of any one of claims 1 to 9 and inactivated Japanese Encephalitis whole virion antigen and an adjuvant selected from (a) aluminum hydroxide (b) aluminum phosphate (c) gamma inulin, (d) algammulin: a combination of aluminum hydroxide and gamma inulin) (e) cholecalciferol in oil (f) an oil in water emulsion OWEM1, containing squalene, tween-80, Span-85 in 10 mM phosphate-citrate buffer, (g) oil in water emulsion OWEM2 containing squalene, tween-80, Span-85, alpha tocopherol in phosphate-citrate buffer, (h) an oil in water emulsion OWEM3 containing squalene, tween-80, Span-85, cholecalciferol in phosphate-citrate buffer for eliciting a protective immune response against Chikungunya virus and Japanese Encephalitis virus infections in a human.

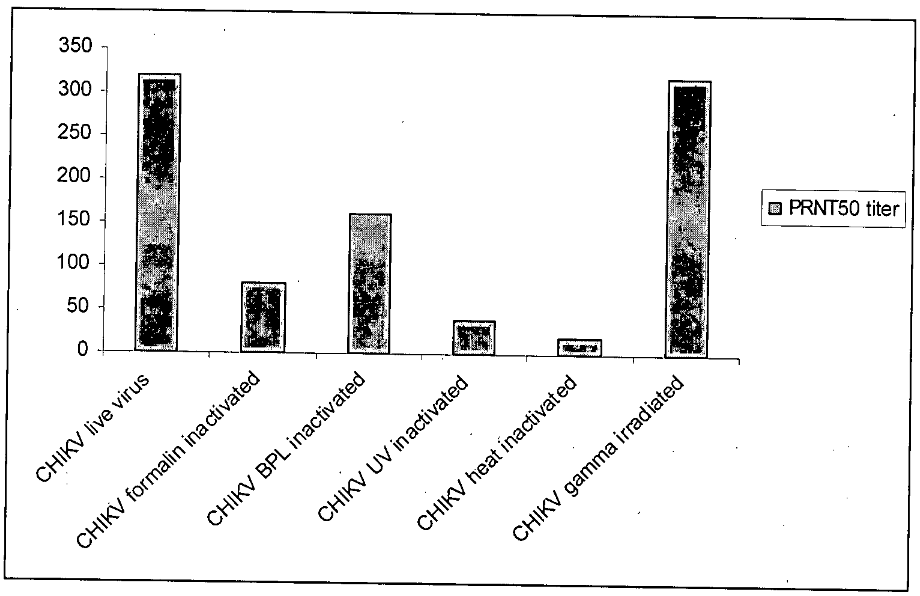
11. A method of eliciting a protective immune response in a human individual against Chikungunya virus infection comprising administering the vaccine composition of any one of claims 1 to 10 to a human.

12. Use of the vaccine composition of any one of claims 1 to 10 in the preparation of a vaccine formulation for eliciting a protective immune response in a human individual against Chikungunya virus infection.

13. The method of claim 11 or the use of claim 12, wherein the vaccine composition is administered through any of the routes selected from intramascular, intradermal, subcutaneous, intravenous, oral or intranasal.

14. Use of one or more Chikungunya virus isolates comprising a structural polyprotein gene sequence as provided in any one of SEQ ID NO. 1 to SEQ ID NO. 6 in the preparation of an immunodiagnostic agent for detection of Chikungunya virus infection in a human.

Figure-1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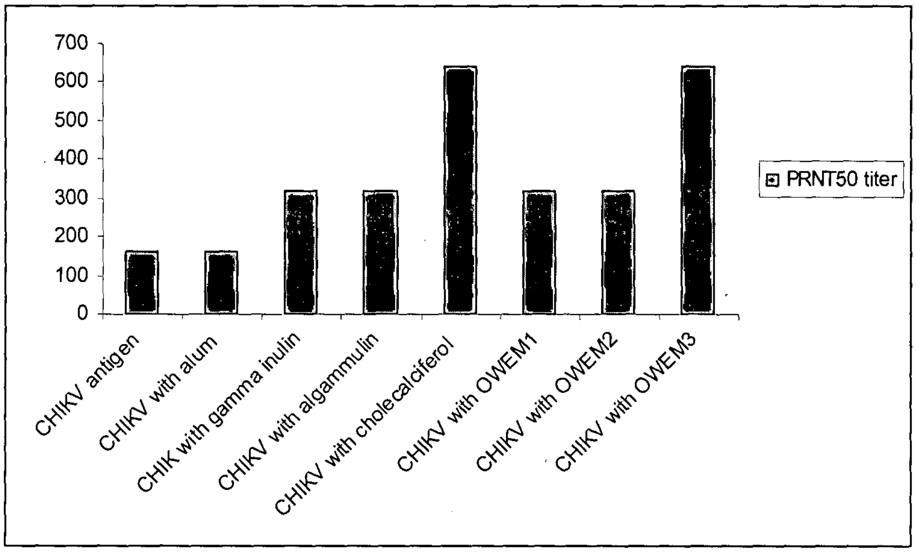
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cagcacacag caccatttgg ctgccaaata gcaacaaacc cggtaagagc ggtgaactgc 3240
gccgtaggga acatgcccat ctccatcgac ataccggaag cggccttcac tagggtcgtc 3300
gacgcgccct ctttaacgga catgtcgtgc gaggtaccag cctgcaccca ttcctcagac 3360
tttgggggcg tcgccattat taaatatgca gccagcaaga aaggcaagtg tgcggtgcat 3420
tcgatgacta acgccgtcac tattcgggaa gctgagatag aagttgaagg gaattctcag 3480
ctgcaaatct ctttctcgac ggccttagcc agcgccgaat tccgcgtaca agtctgttct 3540
acacaagtac actgtgcagc tgagtgccac cccccgaagg accacatagt caactacccg 3600
gcgtcacata ccaccctcgg ggtccaggac atctccgcta cggcgatgtc atgggtgcag 3660
aagatcacgg gaggtgtggg actggttgtt gctgttgccg cactgattct aatcgtggtg 3720
ctatgcgtgt cgttcagcag gcactaa 3747

<210> 8
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 8

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1   5   10   15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
            20   25   30

Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
        35   40   45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
    50   55   60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65   70   75   80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
                85   90   95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
            100   105   110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
        115   120   125

Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
    130   135   140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145   150   155   160

Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
                165   170   175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
            180   185   190

Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
        195   200   205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
    210   215   220

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Val Ala Ile Val Leu Gly Gly Ala Asn Glu Gly Ala Arg Thr Ala Leu
225                 230                 235                 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
                245                 250                 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
            260                 265                 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
        275                 280                 285

Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asp Asn Val Met
    290                 295                 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305                 310                 315                 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
                325                 330                 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
            340                 345                 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
        355                 360                 365

Gly Thr Leu Lys Ile Gln Val Ser Leu Gln Ile Arg Ile Lys Thr Asp
    370                 375                 380

Asp Ser His Asp Trp Thr Lys Leu Arg Tyr Met Asp Asn His Met Pro
385                 390                 395                 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
                405                 410                 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
            420                 425                 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
        435                 440                 445

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
    450                 455                 460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465                 470                 475                 480
```

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
485 490 495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
500 505 510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
515 520 525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
530 535 540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545 550 555 560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg
565 570 575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Ala Thr Cys Arg
580 585 590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val
595 600 605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
610 615 620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
625 630 635 640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
645 650 655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr
660 665 670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
675 680 685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu
690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu

```
            725                     730                     735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
        740                     745                     750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
    755                     760                     765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770                     775                     780

Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785                 790                     795                 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
                805                     810                     815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
            820                     825                     830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
        835                     840                     845

Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
    850                     855                     860

Pro Ser Pro Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865                 870                     875                 880

Asn Leu Pro Asp Tyr Ser Cys Lys Val Phe Thr Gly Val Tyr Pro Phe
                885                     890                     895

Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Ala Glu Asn Thr Gln Leu
            900                     905                     910

Ser Glu Ala His Val Glu Lys Ser Glu Ser Cys Lys Thr Glu Phe Ala
        915                     920                     925

Ser Ala Tyr Arg Ala His Thr Ala Ser Ala Ser Ala Lys Leu Arg Val
    930                     935                     940

Leu Tyr Gln Gly Asn Asn Ile Thr Val Thr Ala Tyr Ala Asn Gly Asp
945                 950                     955                 960

His Ala Val Thr Val Lys Asp Ala Lys Phe Ile Val Gly Pro Met Ser
                965                     970                     975
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Ser Ala Trp Thr Pro Phe Asp Asn Lys Ile Val Val Tyr Lys Gly Asp
980 985 990

Val Tyr Asn Met Asp Tyr Pro Pro Phe Gly Ala Gly Arg Pro Gly Gln
995 1000 1005

Phe Gly Asp Ile Gln Ser Arg Thr Pro Glu Ser Glu Asp Val Tyr
1010 1015 1020

Ala Asn Thr Gln Leu Val Leu Gln Arg Pro Ala Ala Gly Thr Val
1025 1030 1035

His Val Pro Tyr Ser Gln Ala Pro Ser Gly Phe Lys Tyr Trp Leu
1040 1045 1050

Lys Glu Arg Gly Ala Ser Leu Gln His Thr Ala Pro Phe Gly Cys
1055 1060 1065

Gln Ile Ala Thr Asn Pro Val Arg Ala Val Asn Cys Ala Val Gly
1070 1075 1080

Asn Met Pro Ile Ser Ile Asp Ile Pro Glu Ala Ala Phe Thr Arg
1085 1090 1095

Val Val Asp Ala Pro Ser Leu Thr Asp Met Ser Cys Glu Val Pro
1100 1105 1110

Ala Cys Thr His Ser Ser Asp Phe Gly Gly Val Ala Ile Ile Lys
1115 1120 1125

Tyr Ala Ala Ser Lys Lys Gly Lys Cys Ala Val His Ser Met Thr
1130 1135 1140

Asn Ala Val Thr Ile Arg Glu Ala Glu Ile Glu Val Glu Gly Asn
1145 1150 1155

Ser Gln Leu Gln Ile Ser Phe Ser Thr Ala Leu Ala Ser Ala Glu
1160 1165 1170

Phe Arg Val Gln Val Cys Ser Thr Gln Val His Cys Ala Ala Glu
1175 1180 1185

Cys His Pro Pro Lys Asp His Ile Val Asn Tyr Pro Ala Ser His
1190 1195 1200

Thr Thr Leu Gly Val Gln Asp Ile Ser Ala Thr Ala Met Ser Trp
1205 1210 1215

Val Gln Lys Ile Thr Gly Gly Val Gly Leu Val Val Ala Val Ala
    1220   1225   1230

Ala Leu Ile Leu Ile Val Val Leu Cys Val Ser Phe Ser Arg His
    1235   1240   1245

<210> 9
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 9

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1   5   10   15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
            20   25   30

Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
        35   40   45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
    50   55   60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65   70   75   80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
                85   90   95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
            100   105   110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
        115   120   125

Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
    130   135   140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145   150   155   160

Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
                165   170   175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
            180   185   190

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Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
        195                 200                 205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
    210                 215                 220

Val Ala Ile Val Leu Gly Gly Ala Asn Glu Gly Ala Arg Thr Ala Leu
225                 230                 235                 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
                245                 250                 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
            260                 265                 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
        275                 280                 285

Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asn Asn Val Met
    290                 295                 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305                 310                 315                 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
                325                 330                 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
            340                 345                 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
        355                 360                 365

Gly Thr Leu Lys Ile Gln Val Ser Leu Gln Ile Gly Ile Lys Thr Asp
    370                 375                 380

Asp Ser His Asp Trp Thr Lys Leu Arg Tyr Met Asp Asn His Met Pro
385                 390                 395                 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
                405                 410                 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
            420                 425                 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
        435                 440                 445
```

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
    450   455   460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465   470   475   480

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
                485   490   495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
            500   505   510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
        515   520   525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
    530   535   540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545   550   555   560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg
                565   570   575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Ala Thr Cys Arg
            580   585   590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val
        595   600   605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
    610   615   620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
625   630   635   640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
                645   650   655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr
            660   665   670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
        675   680   685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu

690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu
725 730 735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
740 745 750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
755 760 765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770 775 780

Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785 790 795 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
805 810 815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
820 825 830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
835 840 845

Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
850 855 860

Pro Ser Pro Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865 870 875 880

Asn Leu Pro Asp Tyr Ser Cys Lys Val Phe Thr Gly Val Tyr Pro Phe
885 890 895

Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Ala Glu Asn Thr Gln Leu
900 905 910

Ser Glu Ala His Val Glu Lys Ser Glu Ser Cys Lys Thr Glu Phe Ala
915 920 925

Ser Ala Tyr Arg Ala His Thr Ala Ser Ala Ser Ala Lys Leu Arg Val
930 935 940

```
Leu Tyr Gln Gly Asn Asn Ile Thr Val Thr Ala Tyr Ala Asn Gly Asp
945                 950                 955                 960

His Ala Val Thr Val Lys Asp Ala Lys Phe Ile Val Gly Pro Met Ser
                965                 970                 975

Ser Ala Trp Thr Pro Phe Asp Asn Lys Ile Val Val Tyr Lys Gly Asp
            980                 985                 990

Val Tyr Asn Met Asp Tyr Pro Pro  Phe Gly Ala Arg Arg  Pro Gly Gln
        995                 1000                 1005

Phe Gly  Asp Ile Gln Ser Arg  Thr Pro Glu Ser Glu  Asp Val Tyr
    1010                 1015                 1020

Ala Asn  Thr Gln Leu Val Leu  Gln Arg Pro Ala Ala  Gly Thr Val
    1025                 1030                 1035

His Val  Pro Tyr Ser Gln Ala  Pro Ser Gly Phe Lys  Tyr Trp Leu
    1040                 1045                 1050

Lys Glu  Arg Gly Ala Ser Leu  Gln His Thr Ala Pro  Phe Gly Cys
    1055                 1060                 1065

Gln Ile  Ala Thr Asn Pro Val  Arg Ala Val Asn Cys  Ala Val Gly
    1070                 1075                 1080

Asn Met  Pro Ile Ser Ile Asp  Ile Pro Glu Ala Ala  Phe Thr Arg
    1085                 1090                 1095

Val Val  Asp Ala Pro Ser Leu  Thr Asp Met Ser Cys  Glu Val Pro
    1100                 1105                 1110

Ala Cys  Thr His Ser Ser Asp  Phe Gly Gly Val Ala  Ile Ile Lys
    1115                 1120                 1125

Tyr Ala  Ala Ser Lys Lys Gly  Lys Cys Ala Val His  Ser Met Thr
    1130                 1135                 1140

Asn Ala  Val Thr Ile Arg Glu  Ala Glu Ile Glu Val  Glu Gly Asn
    1145                 1150                 1155

Ser Gln  Leu Gln Ile Ser Phe  Ser Thr Ala Leu Ala  Ser Ala Glu
    1160                 1165                 1170

Phe Arg  Val Gln Val Cys Ser  Thr Gln Val His Cys  Ala Ala Glu
    1175                 1180                 1185
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Cys His Pro Pro Lys Asp His Ile Val Asn Tyr Pro Ala Ser His
1190 1195 1200

Thr Thr Leu Gly Val Gln Asp Ile Ser Ala Thr Ala Met Ser Trp
1205 1210 1215

Val Gln Lys Ile Thr Gly Gly Val Gly Leu Val Val Ala Val Ala
1220 1225 1230

Ala Leu Ile Leu Ile Val Val Leu Cys Val Ser Phe Ser Arg His
1235 1240 1245

<210> 10
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 10

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1 5 10 15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
20 25 30

Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
35 40 45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
50 55 60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65 70 75 80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
85 90 95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
100 105 110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
115 120 125

Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
130 135 140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145 150 155 160

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Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
165 170 175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
180 185 190

Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
195 200 205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
210 215 220

Val Ala Ile Val Leu Gly Gly Ala Asn Glu Gly Ala Arg Thr Ala Leu
225 230 235 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
245 250 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
260 265 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
275 280 285

Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asp Asn Val Met
290 295 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305 310 315 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
325 330 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
340 345 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
355 360 365

Gly Thr Leu Asn Ile Gln Val Ser Leu Gln Ile Gly Ile Lys Thr Asp
370 375 380

Asp Ser His Asp Trp Thr Lys Leu Arg Tyr Met Asp Asn His Met Pro
385 390 395 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
405 410 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
420 425 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
435 440 445

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
450 455 460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465 470 475 480

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
485 490 495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
500 505 510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
515 520 525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
530 535 540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545 550 555 560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg
565 570 575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Ala Thr Cys Arg
580 585 590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val
595 600 605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
610 615 620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
625 630 635 640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
645 650 655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr

660 665 670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
675 680 685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu
690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu
725 730 735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
740 745 750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
755 760 765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770 775 780

Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785 790 795 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
805 810 815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
820 825 830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
835 840 845

Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
850 855 860

Pro Ser Pro Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865 870 875 880

Asn Leu Pro Asp Tyr Ser Cys Lys Val Phe Thr Gly Val Tyr Pro Phe
885 890 895

Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Ala Glu Asn Thr Gln Leu
900 905 910

Ser Glu Ala His Val Glu Lys Ser Glu Ser Cys Lys Thr Glu Phe Ala
        915   920   925

Ser Ala Tyr Arg Ala His Thr Ala Ser Ala Ser Ala Lys Leu Arg Val
    930   935   940

Leu Tyr Gln Gly Asn Asn Ile Thr Val Thr Ala Tyr Ala Asn Gly Asp
945   950   955   960

His Ala Val Thr Val Lys Asp Ala Lys Phe Ile Val Gly Pro Met Ser
                965   970   975

Ser Ala Trp Thr Pro Phe Asp Asn Lys Ile Val Val Tyr Lys Gly Asp
            980   985   990

Val Tyr Asn Met Asp Tyr Pro Pro Phe Gly Ala Gly Arg Pro Gly Gln
        995   1000   1005

Phe Gly Asp Ile Gln Ser Arg Thr Pro Glu Ser Glu Asp Val Tyr
    1010   1015   1020

Ala Asn Thr Gln Leu Val Leu Gln Arg Pro Ala Ala Gly Thr Val
    1025   1030   1035

His Val Pro Tyr Ser Gln Ala Pro Ser Gly Phe Lys Tyr Trp Leu
    1040   1045   1050

Lys Glu Arg Gly Ala Ser Leu Gln His Thr Ala Pro Phe Gly Cys
    1055   1060   1065

Gln Ile Ala Thr Asn Pro Val Arg Ala Val Asn Cys Ala Val Gly
    1070   1075   1080

Asn Met Pro Ile Ser Ile Asp Ile Pro Glu Ala Ala Phe Thr Arg
    1085   1090   1095

Val Val Asp Ala Pro Ser Leu Thr Asp Met Ser Cys Glu Val Pro
    1100   1105   1110

Ala Cys Thr His Ser Ser Asp Phe Gly Gly Val Ala Ile Ile Lys
    1115   1120   1125

Tyr Ala Ala Ser Lys Lys Gly Lys Cys Ala Val His Ser Met Thr
    1130   1135   1140

Asn Ala Val Thr Ile Arg Glu Ala Glu Ile Glu Val Glu Gly Asn
    1145   1150   1155

Ser Gln Leu Gln Ile Ser Phe Ser Thr Ala Leu Ala Ser Ala Glu
1160 1165 1170

Phe Arg Val Gln Val Cys Ser Thr Gln Val His Cys Ala Ala Glu
1175 1180 1185

Cys His Pro Pro Lys Asp His Ile Val Asn Tyr Pro Ala Ser His
1190 1195 1200

Thr Thr Leu Gly Val Gln Asp Ile Ser Ala Thr Ala Met Ser Trp
1205 1210 1215

Val Gln Lys Ile Thr Gly Gly Val Gly Leu Val Val Ala Val Ala
1220 1225 1230

Ala Leu Ile Leu Ile Val Val Leu Cys Val Ser Phe Ser Arg His
1235 1240 1245

<210> 11
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 11

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1 5 10 15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
20 25 30

Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
35 40 45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
50 55 60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65 70 75 80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
85 90 95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
100 105 110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
115 120 125

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Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
    130                 135                 140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145                 150                 155                 160

Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
                165                 170                 175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
            180                 185                 190

Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
        195                 200                 205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
    210                 215                 220

Val Ala Ile Val Leu Gly Gly Val Asn Glu Gly Ala Arg Thr Ala Leu
225                 230                 235                 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
                245                 250                 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
            260                 265                 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
        275                 280                 285

Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asp Asn Val Met
    290                 295                 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305                 310                 315                 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
                325                 330                 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
            340                 345                 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
        355                 360                 365

Gly Thr Leu Lys Ile Gln Val Ser Leu Gln Ile Gly Ile Lys Thr Asp
    370                 375                 380
```

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Asp Ser His Asp Trp Thr Lys Leu Arg Tyr Met Asp Asn His Met Pro
385                 390                 395                 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
                405                 410                 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
            420                 425                 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
        435                 440                 445

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
    450                 455                 460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465                 470                 475                 480

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
                485                 490                 495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
            500                 505                 510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
        515                 520                 525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
    530                 535                 540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545                 550                 555                 560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg
                565                 570                 575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Ala Thr Cys Arg
            580                 585                 590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val
        595                 600                 605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
    610                 615                 620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
```

625 630 635 640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
645 650 655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr
660 665 670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
675 680 685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu
690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu
725 730 735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
740 745 750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
755 760 765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770 775 780

Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785 790 795 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
805 810 815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
820 825 830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
835 840 845

Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
850 855 860

Pro Ser Pro Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865 870 875 880

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Asn Leu Pro Asp Tyr Ser Cys Lys Val Phe Thr Gly Val Tyr Pro Phe
                885                     890                 895

Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Ala Glu Asn Thr Gln Leu
            900                     905                 910

Ser Glu Ala His Val Glu Lys Ser Glu Ser Cys Lys Thr Glu Phe Ala
        915                     920                 925

Ser Ala Tyr Arg Ala His Thr Ala Ser Ala Ser Ala Lys Leu Arg Val
    930                     935                 940

Leu Tyr Gln Gly Asn Asn Ile Thr Val Thr Ala Tyr Ala Asn Gly Asp
945                 950                     955                 960

His Ala Val Thr Val Lys Asp Ala Lys Phe Ile Val Gly Pro Met Ser
                965                     970                 975

Ser Ala Trp Thr Pro Phe Asp Asn Lys Ile Val Val Tyr Lys Gly Asp
            980                     985                 990

Val Tyr Asn Met Asp Tyr Pro Pro  Phe Gly Ala Arg Arg  Pro Gly Gln
        995                     1000                 1005

Phe Gly  Asp Ile Gln Ser Arg  Thr Pro Glu Ser Glu  Asp Val Tyr
    1010                 1015                 1020

Ala Asn  Thr Gln Leu Val Leu  Gln Arg Pro Ala Ala  Gly Thr Val
    1025                 1030                 1035

His Val  Pro Tyr Ser Gln Ala  Pro Ser Gly Phe Lys  Tyr Trp Leu
    1040                 1045                 1050

Lys Glu  Arg Gly Ala Ser Leu  Gln His Thr Ala Pro  Phe Gly Cys
    1055                 1060                 1065

Gln Ile  Ala Thr Asn Pro Val  Arg Ala Val Asn Cys  Ala Val Gly
    1070                 1075                 1080

Asn Met  Pro Ile Ser Ile Asp  Ile Pro Glu Ala Ala  Phe Thr Arg
    1085                 1090                 1095

Val Val  Asp Ala Pro Ser Leu  Thr Asp Met Ser Cys  Glu Val Pro
    1100                 1105                 1110

Ala Cys  Thr His Ser Ser Asp  Phe Gly Gly Val Ala  Ile Ile Lys
    1115                 1120                 1125
```

Tyr Ala Ala Ser Lys Lys Gly Lys Cys Ala Val His Ser Met Thr
1130 1135 1140

Asn Ala Val Thr Ile Arg Glu Ala Glu Ile Glu Val Glu Gly Asn
1145 1150 1155

Ser Gln Leu Gln Ile Ser Phe Ser Thr Ala Leu Ala Ser Ala Glu
1160 1165 1170

Phe Arg Val Gln Val Cys Ser Thr Gln Val His Cys Ala Ala Glu
1175 1180 1185

Cys His Pro Pro Lys Asp His Ile Val Asn Tyr Pro Ala Ser His
1190 1195 1200

Thr Thr Leu Gly Val Gln Asp Ile Ser Ala Thr Ala Met Ser Trp
1205 1210 1215

Val Gln Lys Ile Thr Gly Gly Val Gly Leu Val Val Ala Val Ala
1220 1225 1230

Ala Leu Ile Leu Ile Val Val Leu Cys Val Ser Phe Ser Arg His
1235 1240 1245

<210> 12
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 12

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1 5 10 15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
20 25 30

Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
35 40 45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
50 55 60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65 70 75 80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
85 90 95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
100 105 110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
115 120 125

Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
130 135 140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145 150 155 160

Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
165 170 175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
180 185 190

Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
195 200 205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
210 215 220

Val Ala Ile Val Leu Gly Gly Ala Asn Glu Gly Ala Arg Thr Ala Leu
225 230 235 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
245 250 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
260 265 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
275 280 285

Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asp Asn Val Met
290 295 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305 310 315 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
325 330 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
340 345 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
355 360 365

Gly Thr Leu Lys Ile Gln Val Ser Leu Gln Ile Gly Ile Lys Thr Asp
370 375 380

Asp Ser His Asp Trp Thr Glu Leu Arg Tyr Met Asp Asn His Met Pro
385 390 395 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
405 410 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
420 425 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
435 440 445

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
450 455 460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465 470 475 480

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
485 490 495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
500 505 510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
515 520 525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
530 535 540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545 550 555 560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg
565 570 575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Ala Thr Cys Arg
580 585 590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val

595 600 605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
610 615 620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
625 630 635 640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
645 650 655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr
660 665 670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
675 680 685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu
690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu
725 730 735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
740 745 750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
755 760 765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770 775 780

Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785 790 795 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
805 810 815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
820 825 830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
835 840 845

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Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
850 855 860

Pro Ser Pro Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865 870 875 880

Asn Leu Pro Asp Tyr Ser Cys Lys Val Phe Thr Gly Val Tyr Pro Phe
885 890 895

Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Ala Glu Asn Thr Gln Leu
900 905 910

Ser Glu Ala His Val Glu Lys Ser Glu Ser Cys Lys Thr Glu Phe Ala
915 920 925

Ser Ala Tyr Arg Ala His Thr Ala Ser Ala Ser Ala Lys Leu Arg Val
930 935 940

Leu Tyr Gln Gly Asn Asn Ile Thr Val Thr Ala Tyr Ala Asn Gly Asp
945 950 955 960

His Ala Val Thr Val Lys Asp Ala Lys Phe Ile Val Gly Pro Met Ser
965 970 975

Ser Ala Trp Thr Pro Phe Asp Asn Lys Ile Val Val Tyr Lys Gly Asp
980 985 990

Val Tyr Asn Met Asp Tyr Pro Pro Phe Gly Ala Gly Arg Pro Gly Gln
995 1000 1005

Phe Gly Asp Ile Gln Ser Arg Thr Pro Glu Ser Glu Asp Val Tyr
1010 1015 1020

Ala Asn Thr Gln Leu Val Leu Gln Arg Pro Ala Ala Gly Thr Val
1025 1030 1035

His Val Pro Tyr Ser Gln Ala Pro Ser Gly Phe Lys Tyr Trp Leu
1040 1045 1050

Lys Glu Arg Gly Ala Ser Leu Gln His Thr Ala Pro Phe Gly Cys
1055 1060 1065

Gln Ile Ala Thr Asn Pro Val Arg Ala Val Asn Cys Ala Val Gly
1070 1075 1080

Asn Met Pro Ile Ser Ile Asp Ile Pro Glu Ala Ala Phe Thr Arg
1085 1090 1095

Val Val Asp Ala Pro Ser Leu Thr Asp Met Ser Cys Glu Val Pro
1100 1105 1110

Ala Cys Thr His Ser Ser Asp Phe Gly Gly Val Ala Ile Ile Lys
1115 1120 1125

Tyr Ala Ala Ser Lys Lys Gly Lys Cys Ala Val His Ser Met Thr
1130 1135 1140

Asn Ala Val Thr Ile Arg Glu Ala Glu Ile Glu Val Glu Gly Asn
1145 1150 1155

Ser Gln Leu Gln Ile Ser Phe Ser Thr Ala Leu Ala Ser Ala Glu
1160 1165 1170

Phe Arg Val Gln Val Cys Ser Thr Gln Val His Cys Ala Ala Glu
1175 1180 1185

Cys His Pro Pro Lys Asp His Ile Val Asn Tyr Pro Ala Ser His
1190 1195 1200

Thr Thr Leu Gly Val Gln Asp Ile Ser Ala Thr Ala Met Ser Trp
1205 1210 1215

Val Gln Lys Ile Thr Gly Gly Val Gly Leu Val Val Ala Val Ala
1220 1225 1230

Ala Leu Ile Leu Ile Val Val Leu Cys Val Ser Phe Ser Arg His
1235 1240 1245

<210> 13
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 13

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1 5 10 15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
20 25 30

Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
35 40 45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
50 55 60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65 70 75 80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
85 90 95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
100 105 110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
115 120 125

Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
130 135 140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145 150 155 160

Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
165 170 175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
180 185 190

Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
195 200 205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
210 215 220

Val Ala Ile Val Leu Gly Gly Ala Asn Glu Gly Ala Arg Thr Ala Leu
225 230 235 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
245 250 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
260 265 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
275 280 285

Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asp Asn Val Met
290 295 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305 310 315 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
325 330 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
340 345 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
355 360 365

Gly Thr Leu Lys Ile Gln Val Ser Leu Gln Ile Arg Ile Lys Thr Asp
370 375 380

Asp Ser His Asp Trp Thr Lys Leu Arg Tyr Met Asp Asn His Met Pro
385 390 395 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
405 410 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
420 425 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
435 440 445

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
450 455 460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465 470 475 480

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
485 490 495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
500 505 510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
515 520 525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
530 535 540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545 550 555 560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg

565 570 575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Val Thr Cys Arg
580 585 590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val
595 600 605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
610 615 620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
625 630 635 640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
645 650 655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr
660 665 670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
675 680 685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu
690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu
725 730 735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
740 745 750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
755 760 765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770 775 780

Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785 790 795 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
805 810 815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
            820   825   830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
        835   840   845

Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
    850   855   860

Pro Ser Leu Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865   870   875   880

Asn Leu Pro Asp Tyr Ser Cys Lys Val Phe Thr Gly Val Tyr Pro Phe
                885   890   895

Met Trp Gly Gly Ala Tyr Cys Phe Cys Asp Ala Glu Asn Thr Gln Leu
            900   905   910

Ser Glu Ala His Val Glu Lys Ser Glu Ser Cys Lys Thr Glu Phe Ala
        915   920   925

Ser Ala Tyr Arg Ala His Thr Ala Ser Ala Ser Ala Lys Leu Arg Val
    930   935   940

Leu Tyr Gln Gly Asn Asn Ile Thr Val Thr Ala Tyr Ala Asn Gly Asp
945   950   955   960

His Ala Val Thr Val Lys Asp Ala Lys Phe Ile Val Gly Pro Met Ser
                965   970   975

Ser Ala Trp Thr Pro Phe Asp Asn Lys Ile Val Val Tyr Lys Gly Asp
            980   985   990

Val Tyr Asn Met Asp Tyr Pro Pro Phe Gly Ala Gly Arg Pro Gly Gln
        995   1000   1005

Phe Gly Asp Ile Gln Ser Arg Thr Pro Glu Ser Glu Asp Val Tyr
    1010   1015   1020

Ala Asn Thr Gln Leu Val Leu Gln Arg Pro Ala Ala Gly Thr Val
    1025   1030   1035

His Val Pro Tyr Ser Gln Ala Pro Ser Gly Phe Lys Tyr Trp Leu
    1040   1045   1050

Lys Glu Arg Gly Ala Ser Leu Gln His Thr Ala Pro Phe Gly Cys
    1055   1060   1065

Gln Ile Ala Thr Asn Pro Val Arg Ala Val Asn Cys Ala Val Gly
1070 1075 1080

Asn Met Pro Ile Ser Ile Asp Ile Pro Glu Ala Ala Phe Thr Arg
1085 1090 1095

Val Val Asp Ala Pro Ser Leu Thr Asp Met Ser Cys Glu Val Pro
1100 1105 1110

Ala Cys Thr His Ser Ser Asp Phe Gly Gly Val Ala Ile Ile Lys
1115 1120 1125

Tyr Ala Ala Ser Lys Lys Gly Lys Cys Ala Val His Ser Met Thr
1130 1135 1140

Asn Ala Val Thr Ile Arg Glu Ala Glu Ile Glu Val Glu Gly Asn
1145 1150 1155

Ser Gln Leu Gln Ile Ser Phe Ser Thr Ala Leu Ala Ser Ala Glu
1160 1165 1170

Phe Arg Val Gln Val Cys Ser Thr Gln Val His Cys Ala Ala Glu
1175 1180 1185

Cys His Pro Pro Lys Asp His Ile Val Asn Tyr Pro Ala Ser His
1190 1195 1200

Thr Thr Leu Gly Val Gln Asp Ile Ser Ala Thr Ala Met Ser Trp
1205 1210 1215

Val Gln Lys Ile Thr Gly Gly Val Gly Leu Val Val Ala Val Ala
1220 1225 1230

Ala Leu Ile Leu Ile Val Val Leu Cys Val Ser Phe Ser Arg His
1235 1240 1245

<210> 14
<211> 1248
<212> PRT
<213> Chikungunya virus

<400> 14

Met Glu Phe Ile Pro Thr Gln Thr Phe Tyr Asn Arg Arg Tyr Gln Pro
1 5 10 15

Arg Pro Trp Thr Pro Arg Ser Thr Ile Gln Ile Ile Arg Pro Arg Pro
20 25 30

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Arg Pro Gln Arg Gln Ala Gly Gln Leu Ala Gln Leu Ile Ser Ala Val
        35                  40                  45

Asn Lys Leu Thr Met Arg Ala Val Pro Gln Gln Lys Pro Arg Arg Asn
    50                  55                  60

Arg Lys Asn Lys Lys Gln Lys Gln Lys Gln Gln Ala Pro Gln Asn Asn
65                  70                  75                  80

Thr Asn Gln Lys Lys Gln Pro Pro Lys Lys Lys Pro Ala Gln Lys Lys
                85                  90                  95

Lys Lys Pro Gly Arg Arg Glu Arg Met Cys Met Lys Ile Glu Asn Asp
            100                 105                 110

Cys Ile Phe Glu Val Lys His Glu Gly Lys Val Thr Gly Tyr Ala Cys
        115                 120                 125

Leu Val Gly Asp Lys Val Met Lys Pro Ala His Val Lys Gly Thr Ile
    130                 135                 140

Asp Asn Ala Asp Leu Ala Lys Leu Ala Phe Lys Arg Ser Ser Lys Tyr
145                 150                 155                 160

Asp Leu Glu Cys Ala Gln Ile Pro Val His Met Lys Ser Asp Ala Ser
                165                 170                 175

Lys Phe Thr His Glu Lys Pro Glu Gly Tyr Tyr Asn Trp His His Gly
            180                 185                 190

Ala Val Gln Tyr Ser Gly Gly Arg Phe Thr Ile Pro Thr Gly Ala Gly
        195                 200                 205

Lys Pro Gly Asp Ser Gly Arg Pro Ile Phe Asp Asn Lys Gly Arg Val
    210                 215                 220

Val Ala Ile Val Leu Gly Gly Ala Asn Glu Gly Ala Arg Thr Ala Leu
225                 230                 235                 240

Ser Val Val Thr Trp Asn Lys Asp Ile Val Thr Lys Ile Thr Pro Glu
                245                 250                 255

Gly Ala Glu Glu Trp Ser Leu Ala Ile Pro Val Met Cys Leu Leu Ala
            260                 265                 270

Asn Thr Thr Phe Pro Cys Ser Gln Pro Pro Cys Thr Pro Cys Cys Tyr
        275                 280                 285
```

```
Glu Lys Glu Pro Glu Glu Thr Leu Arg Met Leu Glu Asp Asn Val Met
    290                 295                 300

Arg Pro Gly Tyr Tyr Gln Leu Leu Gln Ala Ser Leu Thr Cys Ser Pro
305                 310                 315                 320

His Arg Gln Arg Arg Ser Thr Lys Asp Asn Phe Asn Val Tyr Lys Ala
                325                 330                 335

Thr Arg Pro Tyr Leu Ala His Cys Pro Asp Cys Gly Glu Gly His Ser
            340                 345                 350

Cys His Ser Pro Val Ala Leu Glu Arg Ile Arg Asn Glu Ala Thr Asp
        355                 360                 365

Gly Thr Leu Lys Ile Gln Val Ser Leu Gln Ile Gly Ile Lys Thr Asp
    370                 375                 380

Asp Ser His Asp Trp Thr Lys Leu Arg Tyr Met Asp Asn His Met Pro
385                 390                 395                 400

Ala Asp Ala Glu Arg Ala Gly Leu Phe Val Arg Thr Ser Ala Pro Cys
                405                 410                 415

Thr Ile Thr Gly Thr Met Gly His Phe Ile Leu Ala Arg Cys Pro Lys
            420                 425                 430

Gly Glu Thr Leu Thr Val Gly Phe Thr Asp Ser Arg Lys Ile Ser His
        435                 440                 445

Ser Cys Thr His Pro Phe His His Asp Pro Pro Val Ile Gly Arg Glu
    450                 455                 460

Lys Phe His Ser Arg Pro Gln His Gly Lys Glu Leu Pro Cys Ser Thr
465                 470                 475                 480

Tyr Val Gln Ser Thr Ala Ala Thr Thr Glu Glu Ile Glu Val His Met
                485                 490                 495

Pro Pro Asp Thr Pro Asp Arg Thr Leu Met Ser Gln Gln Ser Gly Asn
            500                 505                 510

Val Lys Ile Thr Val Asn Gly Gln Thr Val Arg Tyr Lys Cys Asn Cys
        515                 520                 525

Gly Gly Ser Asn Glu Gly Leu Thr Thr Thr Asp Lys Val Ile Asn Asn
```

530 535 540

Cys Lys Val Asp Gln Cys His Ala Ala Val Thr Asn His Lys Lys Trp
545 550 555 560

Gln Tyr Asn Ser Pro Leu Val Pro Arg Asn Ala Glu Leu Gly Asp Arg
565 570 575

Lys Gly Lys Ile His Ile Pro Phe Pro Leu Ala Asn Val Thr Cys Arg
580 585 590

Val Pro Lys Ala Arg Asn Pro Thr Val Thr Tyr Gly Lys Asn Gln Val
595 600 605

Ile Met Leu Leu Tyr Pro Asp His Pro Thr Leu Leu Ser Tyr Arg Asn
610 615 620

Met Gly Glu Glu Pro Asn Tyr Gln Glu Glu Trp Val Met His Lys Lys
625 630 635 640

Glu Val Val Leu Thr Val Pro Thr Glu Gly Leu Glu Val Thr Trp Gly
645 650 655

Asn Asn Glu Pro Tyr Lys Tyr Trp Pro Gln Leu Ser Thr Asn Gly Thr
660 665 670

Ala His Gly His Pro His Glu Ile Ile Leu Tyr Tyr Tyr Glu Leu Tyr
675 680 685

Pro Thr Met Thr Val Val Val Val Ser Val Ala Thr Phe Ile Leu Leu
690 695 700

Ser Met Val Gly Met Ala Ala Gly Met Cys Met Cys Ala Arg Arg Arg
705 710 715 720

Cys Ile Thr Pro Tyr Glu Leu Thr Pro Gly Ala Thr Val Pro Phe Leu
725 730 735

Leu Ser Leu Ile Cys Cys Ile Arg Thr Ala Lys Ala Ala Thr Tyr Gln
740 745 750

Glu Ala Ala Ile Tyr Leu Trp Asn Glu Gln Gln Pro Leu Phe Trp Leu
755 760 765

Gln Ala Leu Ile Pro Leu Ala Ala Leu Ile Val Leu Cys Asn Cys Leu
770 775 780

```
Arg Leu Leu Pro Cys Cys Cys Lys Thr Leu Ala Phe Leu Ala Val Met
785                 790                 795                 800

Ser Val Gly Ala His Thr Val Ser Ala Tyr Glu His Val Thr Val Ile
                805                 810                 815

Pro Asn Thr Val Gly Val Pro Tyr Lys Thr Leu Val Asn Arg Pro Gly
            820                 825                 830

Tyr Ser Pro Met Val Leu Glu Met Glu Leu Leu Ser Val Thr Leu Glu
        835                 840                 845

Pro Thr Leu Ser Leu Asp Tyr Ile Thr Cys Glu Tyr Lys Thr Val Ile
    850                 855                 860

Pro Ser Pro Tyr Val Lys Cys Cys Gly Thr Ala Glu Cys Lys Asp Lys
865                 870                 875                 880

Asn Leu Pro Asp Tyr Ser Cys Arg Val Phe Thr Gly Val Tyr Pro Phe
                885                 890                 895

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