

Pacific island of Yap, and then disseminated from island to island across the Pacific, leading to an extensive outbreak in 2013-2014 in French Polynesia, spreading then to New Caledonia, the Cook Islands, and ultimately, to Easter Island. An Asian lineage virus was subsequently transferred to the Western Hemisphere by routes that remain undetermined (Faria *et al.* (2016) *Science*. 352(6283):345-349). The virus may be transmitted zoonotically by *Aedes aegypti*, *A. albopictus*, and possibly by *A. hensilli* and *A. polynesiensis* (Weaver *et al.* (2016) *Antiviral Res.* 130:69-80). Additionally, it is thought that other vectors for transmitting the virus may exist, and the virus may be transmitted by blood transfusion, transplacentally, and/or through sexual transmission.

[0005] In late 2015, a significant increase in fetal abnormalities (e.g., microcephaly) and Guillain-Barre syndrome (GBS) in areas of widespread Zika virus infection raised alarm that Zika virus might be much more virulent than originally thought, prompting the World Health Organization (WHO) to declare a Public Health Emergency of International Concern (PHEIC) (Heymann *et al.* (2016) *Lancet* 387(10020): 719-21). Although the WHO has since declared an end to the PHEIC, Zika continues to pose in particular a significant threat for pregnant women and their unborn babies.

[0006] While Zika virus poses a substantial public health threat, no FDA-approved vaccine or treatment currently exists, and the only preventative measures for controlling Zika virus involve managing mosquito populations.

[0007] In recent efforts to characterize a recombinant Zika virus for the development of a potential vaccine, a non-human cell adapted Zika virus was identified that harbors a mutation in the viral Envelope protein at position 330 (Weger-Lucarelli *et al.* (2017) *Journal of Virology* 91(1): 1-10). The authors of this study found that full-length infectious cDNA clones of Zika virus strain PRVABC59 were genetically unstable when amplified during cloning, and opted to split the viral genome to address the observed instability, developing and applying a two plasmid system. However, a two plasmid system for the development of a Zika vaccine is less desirable.

BRIEF SUMMARY

[0008] Thus, there is a need to develop vaccines and immunogenic compositions for treating and/or preventing Zika virus. Accordingly, certain ~~aspect~~example of the present

disclosure relate a vaccine or immunogenic composition comprising a dose of 1µg to 40µg of (one) antigen from a Zika virus, wherein the antigen is an inactivated whole virus.

[0008a] In one aspect, the present disclosure relates to a use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and the second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, and wherein the administration of the medicament induces 14 and/or 28 days after the prime administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or in a population of at least 20 Zika virus seronegative human subjects of greater than 50, or greater than 100, or greater than 200, or greater than 250, as determined by the plaque reduction neutralization test (PRNT).

[0008b] In another aspect, the present disclosure relates to a use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and the second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, and wherein the administration of the medicament induces 14 and/or 28 days after the prime administration a seroconversion rate of at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT).

[0008c] In another aspect, the present disclosure relates to a use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament

is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and the second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, and wherein the administration of the medicament induces 14 and/or 28 days after the prime administration a seropositivity rate of at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% in a population of at least 20 Zika virus seronegative human subjects or in a population of at least 20 Flavivirus naïve human subjects, as determined by the plaque reduction neutralization test (PRNT).

[0008d] In yet another aspect, the present disclosure relates to a use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, and wherein the administration of the medicament until 7 days after the administration induces systemic side effects in less than 50% of a human subject population of at least 20 flavivirus naïve human subjects or in a population of at least 20 Zika virus seronegative human subjects.

[0008e] In a further aspect, the present disclosure relates to a vaccine or immunogenic composition comprising antigen from a Zika virus, wherein the vaccine or immunogenic composition when administered as multi dose administration including at least a first (prime) and a second (boost) administration taking place from about 1 to about 16 weeks apart, induces 14 and/or 28 days after the first (prime) administration a seroconversion rate of at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT), wherein the antigen is a whole virus inactivated with formaldehyde and wherein the vaccine or immunogenic composition has a residual formaldehyde content of less than 50 µg/mL as determined by a method for determining the residual formaldehyde content in a vaccine or immunogenic composition comprising an inactivated virus comprising the steps of: (a)

providing a composition comprising a virus which has been treated with formaldehyde; (b) mixing the composition of (a) with phosphoric acid and 2,4-dinitrophenylhydrazine (DNPH), thereby providing a mixture; (c) incubating the mixture of (b) under suitable conditions; and (d) analyzing the mixture for the presence of residual formaldehyde.

[0009] Accordingly, certain aspeetexamples of the present disclosure relate a vaccine or immunogenic composition comprising a dose of 1µg to 40µg of (one) antigen from a Zika virus, wherein the Zika virus comprises at least one non-human cell adaptation mutation.

[0010] Accordingly, certain aspeetexamples of the present disclosure relate a vaccine or immunogenic composition comprising a dose of 1µg to 40µg of (one) antigen from a Zika virus, the Zika virus having a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1.

[0011] To meet the above and other needs, the present disclosure is directed, at least in part, to a genetically stable non-human cell adapted Zika virus harboring an adaptation in the Non-structural protein 1 (with a wild-type Envelope protein), allowing for the use of a single virus/viral genome system for vaccine production. The present disclosure is also directed, at least in part, to a Zika virus clonal isolate that is genetically homogenous and/or has been purified away from one or more adventitious agents. Accordingly, the present disclosure provides vaccines and immunogenic compositions useful for treating and/or preventing Zika virus infection (in humans) that include one or more Zika virus antigens (e.g., one or more antigens from a whole inactivated Zika virus) from a Zika virus harboring at least one non-human cell adaptation mutation (e.g., a mutation in Zika virus Non-structural protein 1) and/or a Zika virus clonal isolate.

[0012] The present disclosure is based, at least in part, on the surprising finding that both high and low dose vaccines comprising one or more antigens from separately derived clonal virus populations of non-human cell adapted Zika virus were able to induce robust immune responses and provide significant protection from Zika virus infection (See Examples 2 and Example 6 below). Clonal isolation of the Zika virus strains also allowed for: 1) the successful purification of the virus away from contaminating agents (e.g., adventitious agents that may be co-purified with the parental strain), and 2) the production of a genetically homogeneous viral population. Moreover, the present disclosure is based, at least in part, on the finding that clonal isolated Zika viruses harboring an adaptation mutation in protein NS1 grew well and predictably in Vero cells to high titer, and

surprisingly, were genetically stable/genetically homogenous without any detectable

mutations in the viral envelope protein (See Examples 1 and 2 below). While a similar mutation in Zika virus Non-structural protein 1 may have been observed in the genomic sequencing analysis of 1 out of 3 published sequences of Zika virus strain PRVABC59 (Yun et al. Genome Announc. 2016 Aug 18;4(4)), this reference fails to teach or suggest that a mutation in NS1 may improve stability of the virus; fails to teach or suggest that a virus harboring the mutation may be used in the development of an effective vaccine against Zika virus; and fails to teach or suggest that such a vaccine may be effective in inducing a robust immune response and providing significant protection from Zika virus infection when used at both low and high doses. Thus, without wishing to be bound by theory, the inventors of the present disclosure have determined that the adaptation mutation in protein NS1 appeared to enhance genetic stability within the Zika virus, resulting in increased/enhanced replication efficiency. Further, the Zika strain harboring an adaptation mutation in protein NS1 was able to be passaged multiple times without developing further mutations. Such a stable Zika virus strain is advantageous as a master virus seed (MVS), or subsequent seeds derived from the MVS, for vaccine production and manufacturing, as the risk of the master virus seed developing undesirable mutations is reduced. Moreover, without wishing to be bound by theory, the adaptation mutation in protein NS1 of the Zika strain of the present disclosure may also reduce or otherwise inhibit the occurrence of undesirable mutations, such as a mutation within the envelope protein E (Env) of the Zika virus strain.

[0013] Accordingly, certain aspectexamples of the present disclosure relate to a vaccine or immunogenic composition containing one or more antigen from a Zika virus, where the Zika virus contains at least one non-human cell adaptation mutation. In some embodimentexamples, the at least one non-human cell adaptation mutation is in Zika virus Non-structural protein 1 (NS1). In some embodimentexamples, the at least one adaptation mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some embodimentexamples, the at least one adaptation mutation is a Trp98Gly mutation.

[0014] Accordingly, certain aspectexamples of the present disclosure relate to a vaccine or immunogenic composition comprising a dose of 1µg to 40µg of (one) antigen from a Zika virus, wherein the Zika virus comprises at least one non-human cell adaptation mutation.

[0015] Accordingly, certain aspectexamples of the present disclosure relate a vaccine or immunogenic composition comprising a dose of 1µg to 40µg of (one) antigen from a Zika

virus, the Zika virus having a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1.

[0016] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the at least one adaptation mutation enhances genetic stability as compared to a Zika virus lacking the at least one adaptation mutation. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the at least one adaptation mutation enhances viral replication as compared to a Zika virus lacking the at least one adaptation mutation. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the Zika virus does not comprise a mutation in Envelope protein E (Env).

[0017] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the non-human cell is a mammalian cell. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the non-human cell is a monkey cell. In some embodimentexamples, the monkey cell is from a Vero cell line. In some embodimentexamples, the Vero cell line is a WHO Vero 10-87 cell line.

[0018] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the Zika virus is an African lineage virus or an Asian lineage virus. In some embodimentexamples, the Zika virus is an Asian lineage virus. In some embodimentexamples, the Zika virus is from strain PRVABC59.

[0019] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the vaccine or immunogenic composition is a purified antigen vaccine or immunogenic composition, a subunit vaccine or immunogenic composition, an inactivated whole virus vaccine or immunogenic composition, or an attenuated virus vaccine or immunogenic composition. In some embodimentexamples, the vaccine or immunogenic composition is an inactivated whole virus vaccine or immunogenic composition. In some embodimentexamples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus. In some embodimentexamples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some embodimentexamples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some embodimentexamples, the vaccine

or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some [embodimentexamples](#), the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2. In some [embodimentexamples](#), the vaccine or immunogenic composition comprises a purified inactivated whole Zika which differs from strain PRVABC59 in a Trp98Gly mutation at position 98 of SEQ ID NO: 1.

[0020] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the virus was chemically inactivated. In some [embodimentexamples](#), the virus was chemically inactivated with one or more of a detergent, formalin, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some [embodimentexamples](#), the virus was chemically inactivated with formalin.

[0021] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition further contains an adjuvant. In some [embodimentexamples](#), the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and/or Incomplete Freund's Adjuvant (IFA). In some [embodimentexamples](#), the adjuvant is an aluminum salt. In some [embodimentexample](#), the adjuvant is selected from the group consisting of alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85. In some [embodimentexamples](#), at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the one or more antigens are adsorbed to the adjuvant.

[0022] In some [embodimentexample](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition is a low or medium dose vaccine or immunogenic composition (e.g., containing from about 1 μ g to about 5 μ g antigen or 2 μ g

antigen or 5µg antigen). In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition is a high dose vaccine or immunogenic composition (e.g., containing about 10µg antigen). In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition contains from about 1 µg to about 25 µg of the one or more antigens, in particular 2 µg, 5 µg or 10 µg, or in particular 10 µg of the one or more antigens. In certain such [embodimentexamples](#) the antigen is a purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein. In some [embodimentexamples](#), the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some [embodimentexamples](#), the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2. In certain such [embodimentexamples](#) the Zika virus is a plaque purified clonal Zika virus isolate. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition contains from about 0.1 µg to about 100 µg Zika virus antigen or Env. In some [embodimentexamples](#), the vaccine or immunogenic composition is unadjuvanted. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the Zika virus is a clonal isolate. In some [embodimentexamples](#), the clonal isolate is substantially free of one or more adventitious agents (e.g., free of one or more adventitious agents that may be co-purified with the parental strain).

[0023] Other [aspectexamples](#) of the present disclosure relate to a vaccine comprising a Zika virus having a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some [embodimentexamples](#), the vaccine comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some [embodimentexamples](#), the vaccine comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the

Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2. In certain such embodimentexamples the Zika virus is a plaque purified clonal Zika virus isolate.

[0024] Other aspectexamples of the present disclosure relate to a vaccine or immunogenic composition containing: a) an aluminum salt adjuvant; and b) a purified inactivated whole Zika virus, where the Zika virus contains a non-human cell adaptation mutation, and where the non-human cell adaptation mutation is a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1.

[0025] Other aspectexample of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject in need thereof, including administering to the human subject a therapeutically effective amount of any of the vaccines or immunogenic compositions described herein.

[0026] Other aspectexample of the present disclosure relate to a method for inducing an immune response in a human subject in need thereof, including administering to the human subject an immunogenic amount of any of the vaccines or immunogenic compositions described herein.

[0027] In one aspectexample the present disclosure relates to a method of treating or preventing, in particular preventing Zika virus infection in a human subject in need thereof, comprising administering to the human subject the vaccine or immunogenic composition.

[0028] In one aspectexample the present disclosure relates to a method for inducing an immune response against a Zika virus antigen in a human subject in need thereof, comprising administering to the human subject the vaccine or immunogenic composition.

[0029] In one aspectexample the present disclosure relates to a method of preventing Zika virus disease in a human subject in need thereof, comprising administering to the human subject the vaccine or immunogenic composition. In this case the disease relates to mild fever, maculopapular rash, conjunctivitis and arthralgia. Furthermore, the Zika virus is a neurotropic flavivirus that can potentially cause disease within the central nervous system and Guillain-Barré Syndrome (GBS).

[0030] In one aspectexample the present disclosure relates to a method of preventing Zika virus disease in a fetus or newborn in need thereof, comprising administering to the pregnant human subject or a human subject that intends to become pregnant or woman of childbearing potential the vaccine or immunogenic composition. The Zika disease in this case relates to serious outcomes for the fetus and newborn. The spectrum of congenital anomalies associated with Zika virus infection, known as Congenital Zika Syndrome (CZS), consists of severe microcephaly with partially collapsed skull, cerebral cortices with subcortical calcifications, macular scarring and focal pigmentary retinal mottling, congenital contractures, and marked early hypertonia with symptoms of extrapyramidal involvement.

[0031] In one aspectexample the present disclosure relates to a vaccine or immunogenic composition of for use in a method of treating or preventing, in particular preventing Zika virus infection in a human subject in need thereof, in a method for inducing an immune response against a Zika virus antigen in a human subject in need thereof, and in a method of preventing Zika virus disease in a human subject, fetus or newborn in need thereof.

[0032] In one aspectexample the present disclosure relates to the use of the vaccine or immunogenic composition in the manufacture of a medicament for a method of treating or preventing, in particular preventing Zika virus infection in a human subject in need thereof, a method for inducing an immune response in a human subject in need thereof, and for a method of preventing Zika virus disease in a human subject fetus or newborn in need thereof.

[0033] Within the meaning of this disclosure, the treatment includes the treatment of a single human subject and the treatment of a human subject population. A human subject population is considered to encompass the treatment of more than one individual, e.g. 2 or more individuals.

[0034] Accordingly, certain aspectexamples of the present disclosure relates to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising an antigen from a Zika virus , wherein the vaccine or immunogenic composition is administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart, and

wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the boost administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or of at least 20 Zika virus seronegative human subjects of greater than 300, or greater than 500, or greater than 1000, or greater than 1500, or greater than 2000, or greater than 3000, as determined by the plaque reduction neutralization test (PRNT).

[0035] Accordingly, certain aspectexamples of the present disclosure relates to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus, wherein the vaccine or immunogenic composition is administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart, and wherein the administration of the vaccine or immunogenic composition induces 28 days after the boost administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or of at least 20 Zika virus seronegative human subjects, which are at least 10 times, or at least 15 times, or at least 20 times, or at least 25 times higher than the geometric mean neutralizing antibody titers induced 28 days after the prime administration, as determined by the plaque reduction neutralization test (PRNT). The boost administration thus provides for very high geometric mean neutralizing antibody titers responsible for a long term protection

[0036] Accordingly, certain aspectexamples of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus, wherein the vaccine or immunogenic composition is administered as a single dose or prime administration, and wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the single dose or prime administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or of at least 20 Zika virus seronegative human subjects of greater than 10, or greater than 50, or greater than 100, or greater than 200, or greater than 250, as determined by the plaque reduction neutralization test (PRNT). The high geometric mean neutralizing antibody titers indicate an early onset of protection,

which is beneficial in an outbreak situation or a traveler visiting an endemic area within a short period of time from the administration of the vaccine or immunogenic composition.

[0037] Within the meaning of this disclosure PRNT refers to Zika virus Neutralizing antibody titers in human subjects determined by a plaque reduction neutralization test (PRNT) as described previously (See Sun, W. et al. Protection of Rhesus monkeys against dengue virus challenge after tetravalent live attenuated dengue virus vaccination. *J. Infect. Dis.* 193, 1658–1665 (2006). Muthumani K, Griffin BD, Agarwal S, et al. In vivo protection against ZIKV infection and pathogenesis through passive antibody transfer and active immunisation with a prMEnv DNA vaccine. *NPJ Vaccines* 2016; 1: 16021).

[0038] Within the meaning of this disclosure the GMT values in human subjects are in particular to be considered to be measured as the following. In the Zika PRNT assay, human serum was 2-fold serially diluted from 1:5 to 1:10,240 and mixed with an equal volume of diluted Zika virus (ZIKV) (PRVABC59) to obtain a final dilution of 1:10 to 1:20,480. Neutralization was allowed to proceed 20 ± 2 hours at $2-8^{\circ}\text{C}$ after which the serum/virus mixture was used to inoculate Vero E6 cells. Virus adsorption was done at $37 \pm 2^{\circ}\text{C}$ with humidity and CO_2 for 60 ± 10 minutes then a methylcellulose overlay was added. The infected cells were incubated at $37 \pm 2^{\circ}\text{C}$ with humidity and CO_2 for 72 ± 2 hours. Plaques were visualized by using crystal violet staining and were counted using a CTL (Cellular Technology Limited) reader. Determination of the fifty percent neutralizing titer (PRNT_{50}) was based upon the percent reduction in viral plaques in the presence of serum compared to that of the virus control without serum and was calculated by linear regression. The titers represent the reciprocal of the highest dilution resulting in a 50% reduction in the number of plaques. Acceptance was assessed by evaluating the virus control (targeting ~60 pfu/well), cell control, positive control (PRNT_{50} of 173-658) and negative control ($\text{PRNT}_{50} < 10$) tested in parallel with clinical samples. Individual samples and positive control results were accepted if the correlation coefficient of the titration curve generated by linear regression is ≥ 0.85 . Additional acceptance criteria were based on the quality of the crystal violet stain and plaques generated for the plate or run. PRNT_{50} results are reported down to the starting dilution of the assay (1:10). PRNT_{50} results that are above the ULOQ will be repeated at a pre-dilution to generate a result within the quantifiable range of the assay. The result from the pre-diluted sample will be multiplied by the dilution factor to generate a final result.

[0039] Within the meaning of this disclosure seropositivity is defined as titer ≥ 10 as determined by the plaque reduction neutralization test (PRNT); Zika virus seronegative human subjects are subjects with a titer <10 as determined by the plaque reduction neutralization test (PRNT), Seroconversion is defined as: Zika virus seronegative human subjects (titer <10) have titer ≥ 10 post-vaccination as determined by the plaque reduction neutralization test (PRNT); Results < 10 as determined by the plaque reduction neutralization test (PRNT) are assigned a titer of 5; Titers between 10 (limit of detection) and 26 (lower limit of quantification) as determined by the plaque reduction neutralization test (PRNT) are assigned a value of 13.

[0040] Flavivirus naïve human subjects for the present disclosure are defined to be human subjects without detectable serum antibodies against a panel of flaviviruses, as measured by a reactive antibody based assay (Luminex). Flavivirus screening assay is based on a luminex platform to simultaneously detect multiple target antigens in the same sample. This bead based assay is sensitive, specific and reproducible. For the present disclosure, the antigens targeted are Zika, Dengue, Yellow fever, JEV, USUV, SLEV and WNV. Due to cross reactivity among Flaviviruses, the current antigen set would help detect any prior Flavivirus exposure. References for luminex concept are: Dias D, Van Doren J, Schlottmann S, Kelly S, Puchalski D, Ruiz W, Boerckel P, Kessler J, Antonello JM, Green T, Brown M, Smith J, Chirmule N, Barr E, Jansen KU, Esser MT. 2005. Optimization and validation of a multiplexed Luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. Clin. Diagn. Lab. Immunol. 12:959–969 [PMC free article] [PubMed]. Ayoub A et al Development of a Sensitive and Specific Serological Assay Based on Luminex Technology for Detection of Antibodies to Zaire Ebola Virus. J Clin Microbiol. 2017 Dec 28;55(1):165-176. doi: 10.1128/JCM.01979-16.

[0041] Within the meaning of this disclosure endemic is defined as areas with risk of infection as defined by the Centers for Disease Control and Prevention, such as for example as of March 2018, namely: Asia: Bangladesh, Burma (Myanmar), Cambodia, India, Indonesia, Laos, Malaysia, Maldives, Pakistan, Philippines, Singapore, Thailand, Timor-Leste (East Timor), Vietnam. The Pacific Islands: Fiji, Marshall Islands, Papua New Guinea, Samoa, Solomon Islands, Tonga. The Caribbean: Anguilla; Antigua and Barbuda; Aruba; Barbados; Bonaire; British Virgin Islands; Cuba; Curaçao; Dominica; Dominican Republic; Grenada; Haiti; Jamaica; Montserrat; the Commonwealth of Puerto Rico, a US

territory; Saba; Saint Kitts and Nevis; Saint Lucia; Saint Martin; Saint Vincent and the Grenadines; Sint Eustatius; Sint Maarten; Trinidad and Tobago; Turks and Caicos Islands; US Virgin Islands. North America: Mexico Central America: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama South America: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Venezuela Africa: Angola, Benin, Burkina-Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo (Congo-Brazzaville), Côte d'Ivoire, Democratic Republic of the Congo (Congo-Kinshasa), Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Mali, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Sudan, Sudan, Tanzania, Togo, Uganda. These areas may change.

[0042] Accordingly, certain aspectexamples of the present disclosure relate to a method for inducing an immune response in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus , wherein the vaccine or immunogenic composition is administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart, and wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the boost administration geometric mean neutralizing antibody titers in a human subject population of at least 20 flavivirus naïve human subjects and/or of at least 20 Zika virus seronegative human subjects of greater than 300, or greater than 500, or greater than 1000, or greater than 1500, or greater than 2000, or greater than 3000, or greater than 5000, or greater than 10,000, determined by the reporter virus particle neutralization assay (RVP).

[0043] Accordingly, certain aspectexamples of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus, wherein the vaccine or immunogenic composition is administered as a single dose or prime administration, and wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the single dose or prime administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or of at least 20 Zika virus seronegative human subjects of greater than 300, or greater than 500, greater than 1000, or greater than 2000, as

determined by the reporter virus particle neutralization assay (RVP). The high geometric mean neutralizing antibody titers indicate an early onset of protection, which is beneficial in an outbreak situation or a traveler visiting an endemic area within a short period of time from the administration of the vaccine or immunogenic composition.

[0044] Within the meaning of this disclosure Reporter virus particle (RVP) neutralization assay refers to Zika Neutralizing antibody titers which were analyzed by titration of serum samples with a constant amount of Zika RVPs in Vero cells grown in 96-well plates. RVPs contained the prME proteins of Zika (strain SPH2012) and a Dengue-based Renilla luciferase reporter. Briefly, sera were heat inactivated at 56°C for 30 min, diluted, and then incubated at 37°C with RVPs. The serum/RVP mixture was then mixed with Vero cells and incubated for 72 hours at 37°C ± 2°C/ 5% CO₂ before detection with luciferase substrate. Data was analyzed using JMP11 non-linear 4 parameter analysis, normalized to a positive tracking control and effective dose 50% (EC50) was reported.

[0045] Accordingly, certain [aspectexample](#) of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising(one) antigen from a Zika virus , wherein the vaccine or immunogenic composition is administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart and wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the boost administration a seroconversion rate of at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% in a population of at least 20 seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT).

[0046] Accordingly, certain [aspectexample](#) of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus, wherein the vaccine or immunogenic composition is administered as single dose or prime administration and wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the single dose or prime administration a seroconversion rate of 25%, 30%, 40%, 40%, 50%, 60%, 70%, 75%, 80%,

85%, or 90% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT). The high seroconversion rate after single dose or prime administration indicates an early onset of protection, which is beneficial in an outbreak situation or a traveler visiting an endemic area within a short period of time from the administration of the vaccine or immunogenic composition.

[0047] Accordingly, certain aspeetexamples of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus, wherein the vaccine or immunogenic composition is administered as single dose or prime administration and wherein the administration of the vaccine or immunogenic composition induces 14 and/or 28 days after the single dose or prime administration a seropositivity rate of 25%, 30%, 40%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, or 90% in a population of at least 20 Flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT). The high seroconversion rate after single dose or prime administration indicates an early onset of protection, which is beneficial in an outbreak situation or a traveler visiting an endemic area within a short period of time from the administration of the vaccine or immunogenic composition.

[0048] Certain other aspeetexamples of the present disclosure relate to a method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising (one) antigen from a Zika virus, wherein the vaccine or immunogenic composition is administered as a single dose or as multiple doses as e.g. in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the administration induces systemic side effects in less than 50% of a human subject population of at least 20 human subjects, in particular in a population of at least 20 flavivirus naïve human subjects or in particular in a population of at least 20 Zika virus seronegative human subjects. The above methods are to be understood to also relate to corresponding uses of a vaccine or immunogenic composition comprising (one) antigen from a Zika virus as

disclosed herein for the manufacture of a medicament for the treating or preventing Zika virus infection.

[0049] Within the meaning of this disclosure “systemic side effects” are in particular fever, headache, fatigue, arthralgia, myalgia and malaise.

[0050] The above methods are to be understood to also relate to a vaccine or immunogenic composition comprising (one) antigen from a Zika virus as disclosed herein for use in treating or preventing Zika virus infection.

[0051] In some embodimentexamples the administration is intramuscular or subcutaneous. In some embodimentexamples the administration includes the administration of two doses of the vaccine or immunogenic composition as described herein (e.g. 10 µg purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein) given about 1 to about 16 weeks apart (first (prime) and a second (boost) administration). In certain such embodimentexamples the Zika virus is a plaque purified clonal Zika virus isolate, in particular as described herein.

[0052] The above aspectexamples of the present disclosure relate to the vaccines or immunogenic compositions as described herein for use in treating or preventing Zika virus infection in a human subject in need thereof, for use in inducing an immune response in a human subject in need thereof and for use in preventing Zika virus disease in a human subject, fetus or newborn in need thereof. In some embodimentexamples the administration is intramuscular or subcutaneous. In some embodimentexamples the administration includes the administration of two doses of the vaccine or immunogenic composition as described herein (e.g. 10 µg purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein) given about 1 to about 16 weeks apart (first (prime) and a second (boost) administration). In certain such embodimentexamples the Zika virus is a plaque purified clonal Zika virus isolate, in particular as described herein.

[0053] The above aspectexample of the present disclosure relate to the use of the vaccines or immunogenic compositions as described herein in the manufacture of a medicament for treating or preventing Zika virus infection in a human subject in need thereof for inducing

an immune response in a human subject in need thereof and in preventing Zika virus disease in a human subject, fetus or newborn in need thereof. In some [embodimentexamples](#) the administration is intramuscular or subcutaneous. In some [embodimentexamples](#) the administration includes the administration of two doses of the vaccine or immunogenic composition as described herein (e.g. 10 µg purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein) given about 1 to about 16 weeks apart (first (prime) and a second (boost) administration). In certain such [embodimentexamples](#) the Zika virus is a plaque purified clonal Zika virus isolate, in particular as described herein.

[0054] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the human subject is pregnant or intends to become pregnant or is a woman of childbearing potential.

[0055] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), administration of the vaccine or immunogenic composition induces a protective immune response in the human subject. In some [embodimentexamples](#), the protective immune response induced in the human subject is greater than a protective immune response induced in a corresponding human subject administered a vaccine or immunogenic composition containing one or more antigens from a Zika virus lacking the at least one non-human cell adaptation mutation. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), administration of the vaccine or immunogenic composition induces the generation of neutralizing antibodies to Zika virus in the human subject. In some [embodimentexamples](#), the concentration of neutralizing antibodies generated in the human subject is higher than a concentration of neutralizing antibodies generated in a corresponding human subject administered a vaccine or immunogenic composition comprising one or more antigens from a Zika virus lacking the at least one non-human cell adaptation mutation.

[0056] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition is administered by a route selected from subcutaneous administration, transcutaneous administration, intradermal administration, subdermal administration, intramuscular administration, peroral administration, intranasal administration, buccal administration, intraperitoneal

administration, intravaginal administration, anal administration and/or intracranial administration. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition is administered one or more times. In some [embodimentexamples](#), the vaccine or immunogenic composition is administered as a first (prime) and a second (boost) administration. In some [embodimentexamples](#), the second (boost) administration is administered at least 28 days after the first (prime) administration. In some [embodimentexamples](#) the administration includes the administration of two doses of the vaccine or immunogenic composition as described herein (e.g. 10 µg purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein) given about 1 to about 16 weeks apart (first (prime) and a second (boost) administration). In certain such [embodimentexamples](#) the Zika virus is a plaque purified clonal Zika virus isolate.

[0057] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the virus preparation is mixed with an adjuvant. In some [embodimentexamples](#), the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and/or Incomplete Freund's Adjuvant (IFA). In some [embodimentexamples](#), the adjuvant is an aluminum salt. In some [embodimentexamples](#), the adjuvant is selected from alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and/or Alhydrogel 85. In some [embodimentexamples](#), at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of one or more antigens in the virus preparation are adsorbed to the adjuvant.

[0058] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the at least one non-human cell adaptation mutation is in Zika virus NS1. In some [embodimentexamples](#), the at least one adaptation mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some [embodimentexamples](#), the at least one adaptation mutation is a Trp98Gly mutation. In some

embodimentexamples that may be combined with any of the preceding embodimentexamples, the at least one adaptation mutation enhances genetic stability as compared to a Zika virus lacking the at least one adaptation mutation. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the at least one adaptation mutation enhances viral replication as compared to a Zika virus lacking the at least one adaptation mutation. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the Zika virus does not comprise a mutation in Envelope protein E (Env).

[0059] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the population of Zika viruses is heterogeneous. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the population of Zika viruses comprises a Zika virus clinical isolate. In some embodimentexamples, the Zika virus clinical isolate is from strain PRVABC59. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the population of Zika viruses comprises a Zika virus that has been previously passaged one or more times in cell culture. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the inoculum comprises human serum. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the inoculum comprises one or more adventitious agents. In some embodimentexamples, the Zika virus clonal isolate is substantially free of the one or more adventitious agents.

[0060] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the methods further include one or more additional plaque purifications of the Zika virus clonal isolate. In some embodimentexamples, the Zika virus clonal isolate is further plaque purified two or more times. In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the methods further include passaging the Zika virus clonal isolate one or more times in cell culture. In some embodimentexamples, the Zika virus clonal isolate is passaged two or more times.

[0061] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the methods further include formulating a vaccine or immunogenic composition comprising one or more antigens from the Zika virus clonal isolate. In some embodimentexamples, the vaccine or immunogenic composition is a purified antigen vaccine or immunogenic composition, a subunit vaccine or immunogenic composition, an inactivated whole virus vaccine or immunogenic composition, or an attenuated virus vaccine or

immunogenic composition. In some [embodimentexamples](#), the vaccine or immunogenic composition is a purified inactivated whole virus vaccine or immunogenic composition. In some [embodimentexamples](#), the Zika virus clonal isolate was chemically inactivated. In some [embodimentexamples](#), the Zika virus clonal isolate was chemically inactivated with one or more of a detergent, formalin, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some [embodimentexamples](#), the Zika virus clonal isolate was chemically inactivated with formalin.

[0062] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the methods further include admixing the vaccine or immunogenic composition with an adjuvant. In some [embodimentexamples](#), the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and Incomplete Freund's Adjuvant (IFA). In some [embodimentexamples](#), the adjuvant is an aluminum salt. In some [embodimentexamples](#), the adjuvant is selected from alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85. In some [embodimentexamples](#), at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the one or more antigens are adsorbed to the adjuvant. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition comprises 1 µg to about 40 µg of the purified inactivated whole virus, or 1 µg to about 30 µg of the purified inactivated whole virus, or 1 µg to about 20 µg of the purified inactivated whole virus, in particular 2 µg, or 5 µg, or 10 µg, or 15 µg or 20 µg or in particular 10 µg purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein. In some [embodimentexamples](#), the vaccine or immunogenic composition comprises 1 µg to about 30 µg of the purified inactivated whole virus, in particular 2 µg, 5 µg or 10 µg, or in particular 10 µg of a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some [embodimentexamples](#), the vaccine or immunogenic composition

comprises 1 µg to about 30 µg of the purified inactivated whole virus, in particular 2 µg, 5 µg or 10 µg, or in particular 10 µg of a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2.

[0063] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition comprises from about 0.1 µg Env to about 100 µg Env. In certain such [embodimentexamples](#) the Zika virus is a plaque purified clonal Zika virus isolate. In some [embodimentexamples](#), the vaccine or immunogenic composition is unadjuvanted.

[0064] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the Zika virus clonal isolate is a homogenous genetic population. In some [embodimentexamples](#), the Zika virus clonal isolate does not contain a mutation in Envelope protein E (Env). In some [embodimentexamples](#), the Zika virus clonal isolate contains at least one mutation. In some [embodimentexamples](#), the at least one mutation is in Zika virus Non-structural protein 1 (NS1). In some [embodimentexamples](#), the at least one mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some [embodimentexamples](#), the at least one mutation is a Trp98Gly mutation. In some [embodimentexample](#) the at least one mutation is not in Envelope protein E (Env). In some [embodimentexamples](#), the at least one mutation enhances genetic stability as compared to a Zika virus lacking the at least one mutation. In some [embodimentexamples](#), the at least one mutation enhances viral replication as compared to a Zika virus lacking the at least one mutation.

[0065] Other [aspectexamples](#) of the present disclosure relate to a vaccine or immunogenic composition containing one or more antigens from a plaque purified clonal Zika virus isolate. In some [embodimentexamples](#), the plaque purified clonal Zika virus isolate was plaque purified from cells contacted with an inoculum comprising a population of Zika viruses. In some [embodimentexamples](#), the cells are non-human cells. In some [embodimentexamples](#), the cells are insect cells. In some [embodimentexamples](#), the insect cells are mosquito cells. In some [embodimentexamples](#), the cells are mammalian cells. In some [embodimentexamples](#), the mammalian cells are monkey cells. In some [embodimentexamples](#), the monkey cells are from a Vero cell line. In some [embodimentexamples](#), the Vero cell line is a WHO Vero 10-87 cell line.

[0066] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the population of Zika viruses was heterogeneous. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the population of Zika viruses comprised a Zika virus clinical isolate. In some [embodimentexamples](#), the Zika virus clinical isolate is from strain PRVABC59. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the population of Zika viruses comprised a Zika virus that had been previously passaged one or more times in cell culture. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the inoculum comprised human serum. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the inoculum comprised one or more adventitious agents. In some [embodimentexamples](#), the plaque purified clonal Zika virus isolate is substantially free of the one or more adventitious agents.

[0067] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the plaque purified clonal Zika virus isolate is modified as compared to a wild-type Zika virus. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the plaque purified clonal Zika virus isolate is a homogenous genetic population. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the plaque purified clonal Zika virus isolate does not include a mutation in Envelope protein E (Env). In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the plaque purified clonal Zika virus isolate comprises at least one mutation. In some [embodimentexamples](#), the at least one mutation is in Zika virus Non-structural protein 1 (NS1). In some [embodimentexamples](#), the at least one mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some [embodimentexamples](#), the at least one mutation is a Trp98Gly mutation. In some [embodimentexamples](#), the at least one mutation is not in Zika virus Envelope protein E (Env). In some [embodimentexamples](#), the at least one mutation enhances genetic stability as compared to a Zika virus lacking the at least one mutation. In some [embodimentexamples](#), the at least one mutation enhances viral replication as compared to a Zika virus lacking the at least one mutation. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the plaque purified clonal Zika virus isolate is an African lineage virus or an Asian lineage virus. In some [embodimentexamples](#), the plaque purified clonal Zika virus isolate is an Asian lineage virus.

[0068] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition is a purified antigen vaccine or immunogenic composition, a subunit vaccine or immunogenic composition, an inactivated whole virus vaccine or immunogenic composition, or an attenuated virus vaccine or immunogenic composition. In some [embodimentexamples](#), the vaccine or immunogenic composition is an inactivated whole virus vaccine or immunogenic composition. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the plaque purified clonal Zika virus isolate was chemically inactivated. In some [embodimentexamples](#), the plaque purified Zika virus was chemically inactivated with one or more of a detergent, formalin, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some [embodimentexamples](#), the plaque purified clonal Zika virus isolate was chemically inactivated with formalin.

[0069] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition further comprises an adjuvant. In some [embodimentexamples](#), the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and Incomplete Freund's Adjuvant (IFA). In some [embodimentexamples](#), the adjuvant is an aluminum salt. In some [embodimentexamples](#), the adjuvant is selected from alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85. In some [embodimentexamples](#), at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the one or more antigens are adsorbed to the adjuvant. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the vaccine or immunogenic composition contains from 0.1 µg to about 25 µg of the purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 as described herein, in particular 2 µg, 5 µg or 10 µg, or in particular 10 µg purified inactivated whole virus or about 0.1 µg Env to about 100 µg Env. In certain such [embodimentexample](#) the Zika virus is a plaque purified clonal Zika virus isolate. In some [embodimentexamples](#), the vaccine or immunogenic composition is unadjuvanted.

[0070] It is to be understood that one, some, or all of the properties of the various embodimentexamples described above and herein may be combined to form other embodimentexamples of the present disclosure. These and other aspectexamples of the present disclosure will become apparent to one of skill in the art. These and other embodimentexamples of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] FIG. 1 shows bright field microscopy images of Vero cell monolayers mock infected (top) or infected with ZIKAV strain PRVABC59 (bottom).

[0072] FIG. 2 shows growth kinetics of ZIKAV PRVABC59 P1 on Vero cell monolayers, as determined by TCID50.

[0073] FIG. 3 shows potency assay testing (TCID50) of Zika virus PRVABC59 P5 clones a-f.

[0074] FIG. 4 shows bright-field microscopy images depicting the cytopathic effect (CPE) of growth of Zika virus PRVABC59 P6 clones a-f on Vero cell monolayers.

[0075] FIG. 5 shows potency assay testing (TCID50) of Zika virus PRVABC59 P6 clones a-f

[0076] FIG. 6 shows an amino acid sequence alignment comparing the envelope glycoprotein sequence of Zika virus near residue 330 from Zika virus strains PRVABC59 P6e (SEQ ID NO: 8) and PRVABC59 (SEQ ID NO: 9) with several other flaviviruses (WNV (SEQ ID NO: 10); JEV (SEQ ID NO: 11); SLEV (SEQ ID NO: 12); YFV (SEQ ID NO: 13); DENV 1 16007 (SEQ ID NO: 14); DENV 2 16681 (SEQ ID NO: 15); DENV 3 16562 (SEQ IDNO: 16); and DENV 4 1036 (SEQ ID NO: 17)).

[0077] FIG. 7 shows an amino acid sequence alignment comparing the NS1 protein sequence of Zika virus near residue 98 from Zika virus strains PRVABC59 P6e (SEQ ID NO: 18) and PRVABC59 (SEQ ID NO: 19) with several other flaviviruses (WNV (SEQ ID NO: 20); JEV (SEQ ID NO: 21); SLEV (SEQ ID NO: 22); YFV (SEQ ID NO: 23); DENV 1 16007 (SEQ ID NO: 24); DENV 2 16681 (SEQ ID NO: 25); DENV 3 16562 (SEQ IDNO: 26); and DENV 4 1036 (SEQ ID NO: 27)).

CLAIMS

What is claimed is:

1. ~~Method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population~~ Use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and the second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus ~~the vaccine or immunogenic composition is administered as a single dose or prime administration,~~ and wherein the administration of the medicament ~~vaccine or immunogenic composition~~ induces 14 and/or 28 days after the ~~single dose or~~ prime administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or in a population of at least 20 Zika virus seronegative human subjects of ~~greater than 10, or~~ greater than 50, or greater than 100, or greater than 200, or greater than 250, as determined by the plaque reduction neutralization test (PRNT).
2. ~~Method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population~~ Use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and the second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, ~~wherein the vaccine or immunogenic composition is administered as single dose or prime administration~~ and wherein the administration of the medicament ~~vaccine or immunogenic composition~~ induces 14 and/or 28 days after the ~~single dose or~~ prime administration a seroconversion rate of ~~at least 25%, at least 30%, at least 40%, at least 50%,~~ at least 60%, at least 70%, at least 75%, at

least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT).

3. ~~Method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population~~ Use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and the second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, ~~wherein the vaccine or immunogenic composition is administered as single dose or prime administration~~ and wherein the administration of the medicament vaccine or immunogenic composition induces 14 and/or 28 days after the ~~single dose or prime administration~~ a seropositivity rate of ~~at least 25%, at least 30%, at least 40%, at least 50%,~~ at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% in a population of at least 20 Zika virus seronegative human subjects or in a population of at least 20 Flavivirus naïve human subjects, as determined by the plaque reduction neutralization test (PRNT)..
4. ~~Method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population~~ Use of a vaccine or immunogenic composition comprising antigen from a Zika virus in the manufacture of a medicament for treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, wherein the use comprises that the medicament is to be administered to individual human subjects of said human subject population as multi dose administration including at least a first (prime) and a second (boost) administration, wherein the first and second administration take place from about 1 to about 16 weeks apart, wherein the antigen is an inactivated whole virus, ~~wherein the vaccine or immunogenic composition is administered as a single dose administration or multi dose administration including at least a first (prime) and a second (boost) administration~~ and wherein the administration of the

~~medicament vaccine or immunogenic composition~~ until 7 days after the administration induces systemic side effects in less than 50% of a human subject population of at least 20 flavivirus naïve human subjects or in a population of at least 20 Zika virus seronegative human subjects.

5. ~~A method of treating or preventing, in particular preventing Zika virus infection in a human subject population in need thereof, comprising administering to individual human subjects of said human subject population a vaccine or immunogenic composition comprising antigen from a Zika virus obtainable by a method for inactivating a Zika virus preparation comprising:~~

~~(a) isolating the Zika virus preparation from one or more cells cultured in vitro, wherein the cells are used to produce the Zika virus preparation, wherein isolating the Zika virus preparation comprises one or more steps selected from: (i) depth filtration, (ii) buffer exchange and/or dilution; (iii) ion exchange chromatography; and~~

~~(b) treating the Zika virus preparation with formaldehyde, optionally with formaldehyde, wherein the numerical result of the multiplication of the formaldehyde concentration as measured in % (w/v) with the period of incubation with formaldehyde as measured in days is 0.025 to 0.5.~~

6.5. ~~_____~~ The ~~method-use~~ of any one of claims 1 to ~~54~~, wherein the ~~vaccine or immunogenic use comprises that the medicament composition~~ is to be administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart, and wherein the administration of the ~~medicament vaccine or immunogenic composition~~ induces 14 and/or 28 days after the boost administration geometric mean neutralizing antibody titers in a population of at least 20 flavivirus naïve human subjects and/or in a population of at least 20 Zika virus seronegative human subjects of greater than 300, or greater than 500, or greater than 1000, or greater than 1500, or greater than 2000, or greater than 3000, as determined by the plaque reduction neutralization test (PRNT).

7.6. ~~_____~~ The ~~method-use~~ of any one of claims 1 to ~~65~~, wherein the ~~vaccine or immunogenic use comprises that the medicament composition~~ is to be administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart and wherein the administration of the ~~medicament vaccine or immunogenic composition~~ induces 14 and/or 28 days after the boost administration

a seroconversion rate of at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT),

and/or

a seropositivity rate of at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% in a population of at least 20 Zika virus seronegative human subjects or in a population of at least 20 Flavivirus naïve human subjects, as determined by the plaque reduction neutralization test (PRNT).

~~8. The method of any one of claims 1 to 7, wherein the administration of the vaccine or immunogenic composition until 7 days after the administration induces headache symptoms in less than 29% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

9.7. The method-use of any one of claims 1 to ~~88~~, wherein the administration of the vaccine or immunogenic composition until 7 days after the administration induces fever in 4% or less, and/or fatigue in 33% or less, and/or arthralgia in 10% or less, and/or myalgia in 17% or less, and/or malaise in 15% or less, headache symptoms in less than 29% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.

~~10.8.~~ The method-use of any one of claims 1 to ~~97~~, wherein the use comprises that the medicament vaccine or immunogenic composition is to be administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the ~~medicament vaccine or immunogenic composition~~ until 7 days after the boost administration induces systemic side effects in less than 40% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.

~~11.9.~~ The method-use of any one of claims 1 to ~~108~~, wherein the use comprises that the medicament vaccine or immunogenic composition is to be administered as multiple doses in a first (prime) and a second (boost) administration and wherein the

administration of the vaccine or immunogenic composition until 7 days after the boost administration induces

- at least 40% less, or at least 45% less fatigue, and/or
- no more fever, and/or
- no more, or at least 10% less, or at least 20% less, or at least 25% less myalgia, and/or
- no more, or at least 10% less, or at least 20% less malaise

compared to 7 days after the prime administration in a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.

12.10. The ~~method-use~~ of any one of claims 1 to ~~119~~, wherein the use comprises ~~that the medicament vaccine or immunogenic composition is to be~~ administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces

~~- fever in less than 4% or in 0%, and/or of a human subject population of at least 20 flavivirus naïve human subjects or at least 20 Zika virus seronegative human subjects.~~

~~13. The method of any one of claims 1 to 12, wherein the vaccine or immunogenic composition is administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fatigue in less than 19%, and/or of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~14. The method of any one of claims 1 to 13, wherein the vaccine or immunogenic composition is administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces myalgia in less than 12% or less than 8%, and/or of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

- ~~15. The method of any one of claims 1 to 14, wherein the vaccine or immunogenic composition is administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces~~ malaise in less than 13% or in 10% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.
- ~~16.11. The method-use~~ of any one of claims 1 to ~~1510~~, wherein the use comprises that the medicament vaccine or immunogenic composition is to be administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the ~~medicament vaccine or immunogenic composition~~ until 7 days after the boost administration induces headache symptoms in 20% or less, and arthralgia in 8% or less, and fever in less than 4%, and fatigue in less than 19%, and myalgia in less than 12%, and malaise in less than 13% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.
- ~~17.12. Method-The use~~ of any one of claims 1 to ~~1611~~, wherein the vaccine or immunogenic composition comprises a dose of 1µg to 40µg of the antigen, preferably wherein the vaccine or immunogenic composition comprises a dose of from about 5 µg to about 15 µg or from about 10 µg to about 40 µg or from about 10 µg to about 30 µg or from about 10 µg to about 20 µg or from about 10 µg to about 15 µg of the antigen or a dose of about 2 µg or about 5 µg or about 10 µg of the antigen~~the antigen is an inactivated whole virus.~~
13. The use according to any one of claims 1 to 12, wherein the vaccine or immunogenic composition further comprises an aluminum salt adjuvant, preferably wherein the aluminum salt adjuvant is aluminum hydroxide.
14. The use according to claim 13, wherein the vaccine or immunogenic composition comprises from about 100 µg to about 600 µg, or from about 100 µg to about 450 µg, or from about 100 µg to about 300 µg, or from about 150 µg to about 250 µg, or from about 100 µg to about 225 µg of the adjuvant.
15. The use according to claim 13 or claim 14, wherein at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the antigen are adsorbed to the adjuvant.

~~18. Method of claim 17, the Zika virus having a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1.~~

~~19. 16. The useMethod~~ of any one of claims 1 to ~~18~~15, wherein the antigen is purified, and wherein the main peak of the purified antigen in the size exclusion chromatography is more than 85% of the total area under the curve in the size exclusion chromatography.

17. The use of any one of claims 1 to 16, wherein the use comprises that the medicament is to be administered as a multi dose administration comprising at least a first (prime) and second (boost) administration and wherein the first and second administration take place about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, or about 16 weeks apart.

18. The use of any one of claims 1 to 16, wherein the use comprises that the medicament is to be administered as a multi dose administration comprising at least a first (prime) and second (boost) administration and wherein the first and second administration take place from about 1 to about 6 weeks or from about 1 to about 4 weeks apart, preferably wherein the first and second administration take place from about 25 to about 30 days, such as 28 days, apart.

~~20. Method of any one of claims 1 to 19, wherein the vaccine or immunogenic composition is administered to human subjects from a Zika endemic region, optionally subject to an outbreak.~~

~~21. Method of any one of claims 1 to 20, wherein the vaccine or immunogenic composition is administered to human subjects from a Zika non-endemic region travelling to an endemic region.~~

~~22. 19. The useMethod~~ of any one of claims 1 to ~~21~~18, wherein the use comprises that the medicament vaccine or immunogenic composition is to be administered to human subjects 18 to 29 years of age, ~~in particular women of childbearing potential.~~

~~23. Method of any one of claims 1 to 21, wherein the vaccine or immunogenic composition is administered to human subjects~~or 30 to 49 years of age, in particular women of childbearing potential, optionally from a Zika endemic region or a Zika non-endemic region travelling to an endemic region.

20. The use of any one of claims 1 to 19, wherein the antigen is an inactivated whole virus, preferably wherein the antigen is a whole virus inactivated with formaldehyde, and wherein the vaccine or immunogenic composition comprises less than 1.0 TCID₅₀ of residual replicating virus as determined by a method for determining the completeness of inactivation of a Zika virus preparation, the method for determining the completeness of inactivation comprising the steps of:

- (i) inoculating cultured insect cells, such as C6/36 cells, with a Zika virus preparation which was subjected to an inactivation step and incubating the insect cells for a first period of time, such as for 3 to 7 days, thereby producing an insect cell supernatant;
- (ii) inoculating cultured mammalian cells, such as Vero cells, with the insect cell supernatant produced in (i) and incubating the mammalian cells for a second period of time, such as for 3 to 14 days; and
- (iii) determining whether the Zika virus preparation contains a residual replicating virus that produces a cytopathic effect on the mammalian cells.

21. The use of any one of claims 1 to 20, wherein the antigen is a whole virus inactivated with formaldehyde and wherein the vaccine or immunogenic composition has a residual formaldehyde content of less than 50 µg/ml as determined by a method for determining the residual formaldehyde content in a vaccine or immunogenic composition comprising an inactivated virus, the method for determining the residual formaldehyde content comprising the steps of:

- (a) providing a composition comprising a virus which has been treated with formaldehyde;
- (b) mixing the composition of (a) with phosphoric acid and 2,4-dinitrophenylhydrazine (DNPH), thereby providing a mixture;
- © incubating the mixture of (b) under suitable conditions; and
- (d) analyzing the mixture for the presence of residual formaldehyde.

22. The use of any one of claims 1 to 21, wherein the use comprises that the medicament is to be administered intramuscularly or subcutaneously and/or wherein the individuals of the human subject population are flavivirus naïve or Zika virus seronegative.

23. A vaccine or immunogenic composition comprising antigen from a Zika virus, wherein the vaccine or immunogenic composition when administered as multi dose administration including at least a first (prime) and a second (boost) administration

taking place from about 1 to about 16 weeks apart, induces 14 and/or 28 days after the first (prime) administration a seroconversion rate of at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT), wherein the antigen is a whole virus inactivated with formaldehyde and wherein the vaccine or immunogenic composition has a residual formaldehyde content of less than 50 µg/mL as determined by a method for determining the residual formaldehyde content in a vaccine or immunogenic composition comprising an inactivated virus comprising the steps of:

(a) providing a composition comprising a virus which has been treated with formaldehyde;

(b) mixing the composition of (a) with phosphoric acid and 2,4-dinitrophenylhydrazine (DNPH), thereby providing a mixture;

(c) incubating the mixture of (b) under suitable conditions; and

(d) analyzing the mixture for the presence of residual formaldehyde.

24. The vaccine or immunogenic composition of claim 23, wherein the vaccine or immunogenic composition when administered as a first (prime) and a second (boost) administration about 1 to about 16 weeks apart induces 14 and/or 28 days after the boost administration a seroconversion rate of at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% in a population of at least 20 Zika virus seronegative human subjects, as determined by the plaque reduction neutralization test (PRNT).

25. The vaccine or immunogenic composition of claim 23 or 24, wherein the vaccine or immunogenic composition comprises less than 1.0 TCID₅₀ of residual replicating virus as determined by a method for determining the completeness of inactivation of a Zika virus preparation comprising the steps of:

(i) inoculating cultured insect cells, such as C6/36 cells, with the Zika virus preparation which was subjected to an inactivation step and incubating the insect cells for a first period of time, such as for 3 to 7 days, thereby producing an insect cell supernatant;

(ii) inoculating cultured mammalian cells, such as Vero cells, with the insect cell supernatant produced in (i) and incubating the mammalian cells for a second period of time, such as for 3 to 14 days; and

(iii) determining whether the Zika virus preparation contains a residual replicating virus that produces a cytopathic effect on the mammalian cells.

~~24. The method of any one of claims 1 to 23, wherein the vaccine or immunogenic composition is administered as a single dose administration or multi-dose administration including at least a first (prime) and a second (boost) administration wherein the vaccine or immunogenic composition comprises a dose of about 5µg of purified inactivated whole virus.~~

~~25. The method of claim 24 wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces systemic side effects in less than 50%, or in less than 45%, or in less than 40% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces systemic side effects in less than 40%, or in less than 35%, or in less than 30%, or in less than 25%, or in less than 20%, or in less than 15% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~26. The method of claim 24 or 25, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces fever in less than 3%, or 0%, of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fever in less than 4%, or in less than 3%, or 0% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~27. The method of any one of claims 24 to 26, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces headache in less than 29% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces headache in less than 20%, or in less than 15%, or in less than 10%, or in less than 5% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~28. The method of any one of claims 24 to 27, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces fatigue in less than 30%, or in less than 25%, or in less than 20% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fatigue in less than 20%, or in less than 15%, or in less than 10% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~29. The method of any one of claims 24 to 28, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces arthralgia in less than 4% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces arthralgia in less than 5%, or in less than 2%, or in 0% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~30. The method of any one of claims 24 to 29, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces myalgia in 17% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces myalgia in less than 12%, or in less than 10%, or in less than 5% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~31. The method of any one of claims 24 to 30, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces malaise in less than 10% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces malaise in less than 14%, or in less than 10%, or in less than 5%, or 0% of a human subject population~~

~~of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~32. The method of any one of claims 24 to 31, wherein the vaccine or immunogenic composition is administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces~~

- ~~— at least 70% less, or at least 60% less, or at least 50% less, or at least 40% less, or at least 35% less, or at least 30% less systemic side effects, and/or~~
- ~~— no increase in fever, and/or~~
- ~~— at least 80% less, or at least 70% less, or at least 60% less, or at least 50% less, or at least 45% less headache, and/or~~
- ~~— at least 60% less, or at least 55% less, or at least 50% less, or at least 45% less, or at least 40% less fatigue, and/or~~
- ~~— no increase in arthralgia, or at least 80% less, or at least 60% less, or at least 40% less, or at least 20% less, or at least 10% less arthralgia, and/or~~
- ~~— no increase in myalgia, or at least 70% less, or at least 60% less, or at least 40% less, or at least 20% less, or at least 10% less myalgia, and/or~~
- ~~— no increase in malaise, or at least 80% less, or at least 60% less, or at least 40% less, or at least 20% less, or at least 10% less malaise~~

~~compared to 7 days after the prime administration in a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~33. The method of any one of claims 1 to 23, wherein the vaccine or immunogenic composition is administered as a single dose administration or multi-dose administration including at least a first (prime) and a second (boost) administration wherein the vaccine or immunogenic composition comprises a dose of about 10µg of purified inactivated whole virus.~~

~~34. The method of claim 33, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces systemic side effects in less than 50% of a human subject population of at~~

~~least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces systemic side effects in less than 40%, or in less than 35%, or in less than 30% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~35. The method of claim 33 or 34, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces fever in less than 4% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fever in less than 4%, or in less than 3%, or 0% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~36. The method of any one of claims 33 to 35, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces headache in less than 29%, or in less than 25%, or in less than 20%, or in less than 15% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces headache in 20% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~37. The method of any one of claims 33 to 36, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces fatigue in 33% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fatigue in less than 20% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~38. The method of any one of claims 33 to 37, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces arthralgia in 10% or less of a human subject population of at~~

~~least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces arthralgia in less than 5% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~39. The method of any one of claims 33 to 38, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces myalgia in 17% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces myalgia in less than 12%, or in less than 10% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~40. The method of any one of claims 33 to 39, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces malaise in 15% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces malaise in less than 13%, or in 10% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~41. The method of any one of claims 33 to 40, wherein the vaccine or immunogenic composition is administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces~~

- ~~— at least 40% less, or at least 35% less, or at least 30% less, or at least 25% less systemic side effects, and/or~~
- ~~— no increase in fever, or at least 80% less, or at least 60% less, or at least 40% less, or at least 20% less, or at least 10% less fever, and/or~~
- ~~— at least 45% less, or at least 40% less fatigue, and/or~~
- ~~— no increase in arthralgia, or at least 65% less, or at least 60% less, or at least 40% less, or at least 20% less, or at least 10% less arthralgia, and/or~~

~~—no increase in myalgia, or at least 45% less, or at least 40% less, or at least 20% less, or at least 10% less myalgia, and/or~~

~~—no increase in malaise, or at least 20% less, or at least 10% less malaise~~

~~compared to 7 days after the prime administration in a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~42. The method of any one of claims 1 to 23, wherein the vaccine or immunogenic composition is administered as a single dose administration or multi-dose administration including at least a first (prime) and a second (boost) administration wherein the vaccine or immunogenic composition comprises a dose of about 2µg of purified inactivated whole virus.~~

~~43. The method of claim 42, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces systemic side effects in less than 50%, or in less than 45%, or in less than 40%, or in less than 35% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces systemic side effects in less than 40%, or in less than 35% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~44. The method of claims 42 or 43, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces fever in less than 3%, or 0%, of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fever in less than 4%, or in less than 3%, or 0% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~45. The method of any one of claims 42 to 44, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces headache in less than 29%, or in less than 25%, or in less than 20% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the~~

~~administration of the vaccine or immunogenic composition until 7 days after the boost administration induces headache in less than 20%, or in less than 15% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~46. The method of any one of claims 42 to 45, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces fatigue in less than 30%, or in less than 25% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces fatigue in less than 20%, or in less than 15% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~47. The method of any one of claims 42 to 46, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces arthralgia in less than 4% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces arthralgia in less than 8% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~48. The method of any one of claims 42 to 47, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces myalgia in 17% or less of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces myalgia in less than 12%, or in less than 10% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~49. The method of any one of claims 42 to 48, wherein the administration of the vaccine or immunogenic composition until 7 days after the single dose or prime administration induces malaise in less than 10% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects and/or wherein the administration of the vaccine or immunogenic~~

~~composition until 7 days after the boost administration induces malaise in less than 13%, or in less than 10%, or in less than 5%, or 0% of a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~50. The method of any one of claims 42 to 49, wherein the vaccine or immunogenic composition is administered as multiple doses in a first (prime) and a second (boost) administration and wherein the administration of the vaccine or immunogenic composition until 7 days after the boost administration induces~~

~~——no increase in fever, and/or~~

~~——at least 50% less, or at least 45% less, or at least 40% less fatigue, and/or~~

~~——no increase in myalgia, or at least 20% less, or at least 10% less myalgia, and/or~~

~~——no increase in malaise, or at least 80% less, or at least 60% less, or at least 40% less, or at least 20% less, or at least 10% less malaise~~

~~compared to 7 days after the prime administration in a human subject population of at least 20 flavivirus naïve human subjects or of at least 20 Zika virus seronegative human subjects.~~

~~51. The method of any one of claims 5 to 50, wherein the cells are non-human cells.~~

~~52. The method of claim 51, wherein the cells are Vero cells.~~

~~53. The method of any one of claims 5 to 52, wherein the Zika virus preparation is treated with formaldehyde at a concentration of 0.005 % (w/v) to 0.02 % (w/v).~~

~~54. The method of any one of claims 5 to 53, wherein the Zika virus preparation is treated for eight to twelve days.~~

~~55. The method of claim 54, wherein the Zika virus preparation is treated for ten days.~~

~~56. The method of any one of claims 5 to 55, wherein the Zika virus preparation is treated at a temperature of 15°C to 30°C.~~

~~57. The method of claim 56, wherein the Zika virus preparation is treated at a temperature of 22°C.~~

~~58. The method of any one of claims 5 to 57, further comprising a step (c) of determining the completeness of inactivation.~~

~~59. The method of claim 58, wherein step (c) comprises:~~

~~(i) inoculating cultured insect cells with a Zika virus preparation treated according to step (b) and incubating the insect cells for a first period of time, thereby producing an insect cell supernatant;~~

~~—(ii) inoculating cultured mammalian cells with the insect cell supernatant produced in (i) and incubating the mammalian cells for a second period of time; and~~

~~(iii) determining whether the Zika virus preparation contains a residual replicating virus that produces a cytopathic effect on the mammalian cells.~~

~~60. The method of claim 59, wherein the insect cells are selected from CCL-125 cells, Aag-2 cells, RML-12 cells, C6/36 cells, C7-10 cells, AP-61 cells, A.t. GRIP-1 cells, A.t. GRIP-2 cells, A.t. GRIP-3 cells, UM-AVE1 cells, Mos.55 cells, Sua1B cells, 4a-3B cells, Mos.42 cells, MSQ43 cells, LSB-AA695BB cells, NIID-CTR cells and TRA-171 cells, such as C6/36 cells.~~

~~61. The method of claim 59 or 60, wherein the first period of time is 3 to 7 days.~~

~~62. The method of any one of claims 59 to 61, wherein the mammalian cells are selected from VERO cells, LLC-MK2 cells, MDBK cells, MDCK cells, ATCC CCL34 MDCK (NBL2) cells, MDCK 33016 (deposit number DSM ACC 2219 as described in WO97/37001) cells, BHK21-F cells, HKCC cells, and Chinese hamster ovary cells (CHO cells), such as VERO cells.~~

~~63. The method of any one of claims 59 to 62, wherein the second period of time is 3 to 14 days.~~

~~64. The method of any one of claims 5 to 63, further comprising a step (d) of neutralizing the formaldehyde-treated Zika virus preparation with sodium metabisulfite.~~

~~65. The method of claim 64, wherein the formaldehyde-treated Zika virus preparation is neutralized at least five, at least seven, at least nine, at least 11, or at least 14 days after formaldehyde treatment.~~

~~66. The method of any one of claims 1 to 65, wherein the vaccine or immunogenic composition has a residual formaldehyde content of less than 50 µg/ml.~~

~~67. A vaccine or immunogenic composition for use in a method of any one of claims 1 to 66.~~

~~68. Use of the vaccine or immunogenic composition in the manufacture of a medicament for the method of any one of claims 1 to 67.~~