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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	I0422.70125US01
First Named Inventor	Jana Barbero Calzado
Title	ZIKA VIRUS VACCINE
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 102]
   Both the claims and abstract must start on a new page.
   (See MPEP § 608.01(a) for information on the preferred arrangement)
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 23]
6. **Inventor's Oath or Declaration** [*Total Pages* 2]
   (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))
   a. [ ] Newly executed (original or copy)
   b. [X] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.*
   See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
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ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [ ] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [X] **Other:** Sequence Listing (text file)

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

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Application Data Sheet

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Correspondence Customer Number::	23628

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Attorney Docket Number::	I0422.70125US01
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Request for Non-Publication?::	No
Total Drawing Sheets::	23
Small Entity?::	No
Petition included?::	No

Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::	No

Representative Information

Representative Customer Number::	23628

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Prior Appl Status::
This Application	Continuation of	16/063007	06/15/18	Pending
16/063007	a 371 of international	PCT/EP2016/082664	12/23/16	

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed::	DAS Access::
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European Patent Office	16176049.1	06/23/16	Yes	
European Patent Office	16176025.1	06/23/16	Yes	
European Patent Office	16161068.8	03/18/16	Yes	
European Patent Office	15202585.4	12/23/15	Yes	

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Signature	/Chelsea E. Witte-Garcia/	Date (YYYY-MM-DD)	2020-03-10
Name	Chelsea E. Witte-Garcia, Ph.D.	Registration Number	75,713

ZIKA VIRUS VACCINE

RELATED APPLICATIONS

This Application is a continuation of U.S. Application Serial No. 16/063,007, filed June 15, 2018, which is a national stage filing under 35 U.S.C. § 371 of International Patent Application Serial No. PCT/EP2016/082664, filed December 23, 2016, the contents of each of which is herein incorporated by reference in its entirety.

FIELD OF INVENTION

The disclosure relates to methods for the purification of Zika viruses for use in vaccines and in particular relates to an improved sucrose gradient process step allowing the separation of impurities such as protamine sulphate. The disclosure also relates to Zika virus vaccines and compositions and methods for producing said vaccines and administering the vaccines to subjects for the generation of an anti-Zika virus immune response.

BACKGROUND OF THE INVENTION

Adverse responses to protamine sulfate have been known for many years. Previous exposure to protamine can induce a humoral immune response and predispose susceptible individuals to the development of untoward reactions from the subsequent use of this drug. Patients exposed to protamine through the use of protamine-containing insulin or during heparin neutralization may experience life-threatening reactions and fatal anaphylaxis upon receiving large doses of protamine intravenously. Severe reactions to intravenous protamine can occur in the absence of local or systemic allergic reactions to subcutaneous injection of protamine-containing insulin. Although there is no clear evidence for hypersensitivity reactions of protamine sulphate linked to vaccination, vaccines containing protamine impurities have a precaution and contraindication warning in their labels stating that a serious allergic reaction after a previous dose of such a protamine containing vaccine (e.g. IXIARO®, see CDC site www.cdc.gov/japaneseencephalitis/vaccine/) is a contraindication to further doses. Thus elimination of said impurity is a medical request for an improved safety profile. On the other hand protamine sulphate is an excellent tool (and often better than other tools such as benzonase) to purify crude harvests of viruses grown on cell substrates.

In 2007, Zika virus was detected for the first time outside of the endemic regions of Asia and Africa since its discovery in a Rhesus monkey in Uganda in 1947. Since then, the virus has caused a large

epidemic in French Polynesia, spreading through islands in the Pacific and into South and Central America by 2015 (WHO "Zika Situation Report" February 5, 2016). Evidence suggests that in addition to being transmitted by *Aedes* species mosquitos, other vectors may exist, and the virus may be transmitted by blood transfusion, transplacentally, and through sexual transmission (WHO Zika Virus Fact Sheet, Feb. 2016). Though the symptoms of Zika virus infection include mild fever, rash, and conjunctivitis, there is a likely correlation between infection and neurological disorders, including Guillain-Barré syndrome and microcephaly in fetuses/neonates subsequent to infection during pregnancy. There is currently no specific treatment or vaccine for Zika virus and the only preventative measures involve control of the mosquito vector. Zika virus presents a substantial public health threat due to the wide circulation of the *Aedes* mosquito, multiple routes of transmission, and potentially severe neurological effects of infection.

A preventative vaccine against Zika virus is a pressing medical need in endemic areas and in geographical areas where the vector is spreading. Furthermore, as Zika infection has dire consequences on embryonic and fetal development, a safe and effective vaccine for women of child-bearing potential or pregnant women is needed. Vaccines administered during pregnancy must be very safe for both the mother and the developing fetus. While live attenuated viral vaccines are highly effective, they are often not considered safe enough for administration to pregnant women. In this regard, inactivated viral vaccines, which lack the ability to propagate in the vaccinated subject, are considered much safer. Development of an inactivated Zika virus vaccine for administration to at-risk patients would fill this need.

SUMMARY OF THE INVENTION

During the course of virus purification, it was observed that addition of protamine sulfate to a virus harvest produced on a cell substrate removed not only contaminating DNA derived from host cells, as expected, but surprisingly also virtually eliminated immature and otherwise non-infectious virus particles from the preparation. This finding provided a streamlined, gentle, reproducible and broadly-applicable process for obtaining highly-purified infectious virus particles for applications such as vaccine preparation. In addition, it was surprisingly found that said protamine sulfate can be very efficiently separated from the virus fraction allowing for a safer vaccine produced at high yields.

Disclosed herein are virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering said Zika virus vaccines for the prevention of Zika virus infection and/or for the

production of an anti-virus immune response in subjects, for example subjects at risk of being exposed to Zika virus. In particular, the invention is directed to a virus vaccine comprising an optimally inactivated Zika virus particle, wherein the Zika virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability. Another advantage of the invention is that related methods of producing said vaccines and compositions are very efficient and provide pure compositions largely devoid of impurities, in particular protamine sulphate, allowing for high volume production of vaccines. Detail experimental examples to the above are provided for Zika virus.

The herein disclosed in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was unexpectedly higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, Nature doi:10.1038/nature18952.). Inactivated viruses are among the safest vaccines and especially preferred for delivery to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing, alignments were performed with the multi alignment package Jalview (Waterhouse et al., 2009, Bioinformatics 25 (9) 1189-1191). In the drawings:

Figure 1: Average distance tree (by % identity, nt), complete genomes.

Figure 2: Neighbor joining tree (by % identity, nt), complete genomes.

Figure 3: Pairwise alignment-Jalview (% identity, nt), complete genomes.

Figure 4: Average distance tree (by % identity, aa), E-protein.

Figure 5: Neighbor joining tree (by % identity. aa), E-protein.

Figure 6: Pairwise alignment-Jalview (% identity, aa), E-protein.

Figures 7A-7C: Alignment (shading: % identity, aa), E-protein.

Figure 8: An example of virus particle maturation in the host cell. As observed in flaviviruses, full maturation of the particles requires proteolytic cleavage of the precursor membrane glycoprotein (prM) by the host protease furin. Not all prM molecules are cleaved, resulting in the release of mature, mosaic or immature-like conformations from the cells. Mosaic and immature forms are generally not infectious—only mature virions are infective and have hemagglutinin (HA) / TCID50 activity. (Figure adapted from Plevka, et al., Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres, EMBO reports (2011) 12, 602–606).

Figure 9: An exemplary downstream virus purification process from the crude harvest to formulation of the drug substance (vaccine), a preferred embodiment of the process of the invention (**A**). A flow-chart of an exemplary virus inactivation process is shown in (**B**).

Figure 10: PS treatment results in selective removal of Zika virus aggregates and Vero HCP and LMW impurities (SEC-HPLC of 30x concentrated Zika Virus harvest day 5).

Figure 11: SEC-HPLC of individual 30x concentrated Zika harvest prior to PS treatment at different time points.

Figure 12: SEC-HPLC of individual 30x concentrated Zika harvest post PS treatment at different time points. The smaller graph indicates the observed cytopathic effect (CPE) over time.

Figure 13: Preparation of the sucrose gradient.

Figure 14: Representative SDS-PAGE from the sucrose gradient harvest of a Zika purification is shown.

Figure 15: Comparison of JEV and ZikaV harvest schedules/yields.

Figure 16: SEC-HPLC elution profile of ZikaV NIV. Data were processed on Dionex Ultimate 3000 / Superose 6 Increase column. Both panels are from the same chromatogram. The upper graph is the complete elution profile; the lower graph is an enlargement of the ZIKAV elution peak.

Figure 17: SEC-MALLS analysis of inactivated ZikaV.

Figure 18: Cumulative particle size distribution of Zika NIV.

Figure 19: Graphical representation of the neutralization of the Zika virus H/PF/2013 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated using the 3-parameter method.

Figure 20: Graphical representation of the neutralization of the Zika virus MR766 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated using the 3-parameter method.

Figure 21: Correlation between JEV antigen content in neutralized inactivated virus (NIV) analysed by ELISA and SEC-HPLC (Dionex Ultimate 3000, Superose 6 column).

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering said virus vaccines for the prevention of virus infection and/or for the production of an anti-virus immune response in subjects, for example subjects at risk of being exposed to virus. In particular, the invention is directed to a virus vaccine comprising an optimally inactivated virus particle, wherein the virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability. Another advantage of the invention is that related methods of producing said vaccines and

compositions are very efficient and provide pure compositions largely devoid of impurities, in particular protamine sulphate, allowing for high volume production of vaccines. Examples to the above are provided for Zika virus.

Disclosed herein are downstream processes for purifying Zika virus particles from a crude preparation. The downstream process can be applied to either a virus which has not adapted for propagation on a particular cell substrate or for a partially / fully cell substrate adapted Zika virus particle.

Aspects of the invention provide processes for the purification of infectious Zika virus particles comprising the steps of (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate; (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, to obtain a virus preparation (b); and further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation to obtain a virus preparation (c) comprising the infectious virus particles.

In some embodiments, the concentration of protamine sulphate in step (b) is about 1 to 10 mg/ml, more preferably about 1 to 5 mg/ml, more preferably about 1 to 2 mg/ml. In one embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/mL. In one embodiment, the concentration of protamine sulphate is 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml. In a preferred embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/ml.

In some embodiments, the residual host cell DNA of the virus preparation (c) is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL. In a preferred embodiment, the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL. In some embodiments, the residual host cell protein of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the residual host cell protein of the virus preparation (c) is less than 100 ng/mL. In some embodiments, the residual non-infectious virus particles of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the content of residual non-infectious virus particles of the virus preparation (c) is less than 100 ng/mL.

In some embodiments, the residual protamine is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL, more preferably is below the detection limit of HPLC, in particular below the detection limit in the final drug substance. In some embodiments, the PS content is tested by HPLC or size exclusion chromatography (SEC). For example, HPLC is validated for PS determination in JEV sucrose gradient pool samples as a routine release assay and is very sensitive (i.e., LOQ 3 µg/mL; LOD 1 µg/mL). In the current invention, PS content in in Zika virus DS samples was <LOD. In one embodiment, the HPLC assessment of PS content can be performed on a Superdex Peptide 10/300GL column (GE: 17-5176-01) using 30% Acetonitrile, 0,1% Trifluoroacetic acid as solvent with a flow rate of 0,6 ml/min at 25°C and detection at 214 nm. A more sensitive method of measurement for residual protamine in a purified virus preparation is mass spectrometry (MS). In some embodiments, the residual PS levels in a Zika virus preparation are tested by MS or other such highly sensitive method, e.g. nuclear magnetic resonance (NMR). With this method, residual PS, as well as fragments and/or break-down products of PS, can be detected at trace amounts, such as levels as low as, for example, 10^6, 10^7 or 10^8 molecules per typical sample load. In some embodiments, the PS levels are tested in the sucrose gradient pool. In some embodiments, the PS levels are tested in the drug product. In some embodiments, the PS levels are tested in the drug substance.

In some embodiments, the crude harvest (a) comprising the virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b). In some embodiments, the one or more pre-purification step(s) comprises digesting host cell genomic DNA in the crude harvest (a) comprising the virus particles and impurities by enzymatic treatment. In some embodiments, the one or more pre-purification step(s) comprises filtration, ultrafiltration, concentration, buffer exchange and/or diafiltration. In some embodiments, the one or more pre-purification steps is filtration using a filter having a pore size equal to or less than 1 µm. In some embodiments, the filter has a pore size equal to or less than 0.2 µm. In a preferred embodiment, the filter has a pore size of 0.2 µm. In some embodiments, the concentration and/or ultra/diafiltration and/or buffer exchange is performed by tangential flow filtration (TFF). In some embodiments, ultra/diafiltration of the crude harvest (a) comprising the virus particles and impurities is performed using a hollow fiber membrane having a cut-off of equal to or less than 300 kDa. In a preferred embodiment, the hollow fiber membrane has a cut-off of about 100 kDa.

The process according to the current invention may also comprise the use of a sucrose gradient, preferably an optimized sucrose gradient. The sucrose gradient is preferably optimized for the removal of protamine sulfate, also for the removal of immature viral particles or other viral particles which are non-infectious or host cell proteins or nucleic acids (DNA, RNA, mRNA, etc) or other host

cell debris. In the current invention the optimized sucrose gradient comprises at least two, at least three, at least four layers of sucrose solutions with different densities. In one embodiment, the virus preparation to be purified is provided in a sucrose solution which has a density of about 8%, about 9%, about 10%, about 11%, about 12% sucrose (w/w), preferably about 10%. In one embodiment, one sucrose solution in the gradient has a density of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55% sucrose (w/w), preferably about 50%. In one embodiment, one sucrose solution in the gradient has a density of about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40% sucrose (w/w), preferably about 35%. In one embodiment, one sucrose solution in the gradient has a density of about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20% sucrose (w/w), preferably about 15% sucrose. In a preferred embodiment, the sucrose gradient comprises three layers of sucrose solutions of about 50%, about 35% and about 15% (w/w) sucrose and the virus composition to be purified is contained in about 10% (w/w) sucrose. Because the invention provided for means to not only test for host cell DNA but also immature viral particles, the skilled person in the art is able to more precisely optimize the sucrose gradient for most efficient purification and include additional tools such as PRNT assay to monitor purification success.

In some embodiments, the virus particle is a live virus, a chimeric virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In a further step, the virus particles of the invention may be optionally inactivated. In some embodiments, the virus particle is an attenuated form of the virus particle. For example, the virus may have reduced infectivity, virulence, and/or replication in a host, as compared to a wild-type virus. In some embodiments, the virus is a mutated or modified virus, for example the nucleic acid of the virus may contain at least one mutation relative to the wild-type virus. In some embodiments, the virus is a recombinant live virus, meaning a virus that is generated recombinantly and may contain nucleic acid from different sources.

In some embodiments, the Zika virus particle is a live virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In preferred embodiments, the Zika virus is a Zika virus from the Asian lineage.

In some embodiments, the relative reduction of impurity of the final virus preparation relative to the liquid medium (a) comprising the virus particles and impurities is in a range from 60 to 95%. In some embodiments, the residual impurity of the final virus preparation is less than 1%.

In some embodiments, the Zika virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line. In some embodiments, said cell line is a duck cell line. In some embodiments, said cell line is a diploid avian cell line. In some embodiments, said cell line is an EB66 cell line. In a preferred embodiment, said cell line is a Vero cell line.

Aspects of the invention provide a use of any of the processes described herein for manufacturing a composition for immunization against a viral infection. In a preferred embodiment, the composition or vaccine is directed against Zika virus such as e.g. a Zika virus of the Asian lineage.

Other aspects provide compositions comprising the Zika virus particles obtainable by any of the processes described herein for treating and/or preventing (i.e. protecting from) a viral infection. In a preferred embodiment, the viral infection is caused by Zika virus such as e.g. a Zika virus of the Asian lineage.

Furthermore, disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering the Zika virus vaccines for the prevention of Zika virus infection and/or for the production of an anti-Zika virus immune response in subjects, for example subjects at risk of being exposed to Zika virus.

Zika virus is a flavivirus closely related to Dengue virus and is similarly transmitted by the *Aedes* species mosquito, although other arthropod vectors for Zika virus are possible. Since it was first isolated from a Rhesus monkey in the Zika forest of Uganda in 1947, there were very few reported incidents of human infection, especially outside of the endemic regions of Africa and Asia until a large outbreak in French Polynesia in 2007 (Haddow *et al. PLoS Neglected Tropical Diseases* (2012) 6(2), Malone *et al. PLoS Neglected Tropical Diseases* (2016) 10(3),). The virus has since spread through islands of the Pacific, including Oceania, and into South and Central America (WHO "Zika Situation Report" February 5, 2016).

In addition to being spread by the bite of an infected mosquito, evidence also suggests transmission may occur between individuals, such as from the blood of an infected individual, *in utero*/transplacental transmission from an infected mother to the fetus, sexual transmission between

sexual partners, and possibly by other local transmission routes. There is a possible association between Zika virus infection during pregnancy and microcephaly in the fetus/neonate. Microcephaly is a rare condition in which a baby's head circumference is significantly less than expected based on the average for their age, sex, and ethnicity. This is a result of the brain failing to undergo proper embryonic development, and in 90% of cases is associated with mental retardation (Rocha *et al.* (2016) *Bull World Health Organ* 8 Feb. 2016).

There is a probable association between individuals having had a prior Zika virus infection and the incidence of Guillain-Barré syndrome, a neurological disorder in which the individual's immune system destroys the myelin sheath surrounding axons of the peripheral nervous system (WHO "Zika Situation Report" February 5, 2016).

No specific treatments or vaccines for Zika virus currently exist, and the only measures at this time to prevent infection are through vector control and avoiding travel to regions experiencing outbreaks.

Described herein are Zika virus vaccines and compositions comprising inactivated Zika virus that provide a safe method for generating an immune response to Zika virus, including virus-neutralizing antibodies, that may help prevent against Zika virus infection.

Any strain of Zika virus may be used in the methods and compositions described herein. In some embodiments, the Zika virus is an isolate from an infected subject during a Zika virus outbreak. In some embodiments, the Zika virus is a strain isolated from Africa or from the African virus lineage. In some embodiments, the Zika virus is a strain isolated from Asia or from the Asian lineage (includes also strains from French Polynesia). In some embodiments, the Zika virus is a strain isolated from the Americas (South America, Central America, or North America), such as a Suriname Zika virus strain. In some embodiments, the Zika virus has an RNA genome corresponding (but not limited) to the DNA sequence provided by GenBank Accession No. AY632535.2, KU321639.1, KU497555.1, KU501215.1, KU509998.1, KU527068.1, KU681081.3, KU681082.3, KU707826.1, KU744693.1, or LC002520.1 or RNA genome disclosed partially or fully herein (SEQ ID NO: 2 to 69).

In some embodiments, the process of the invention results in an enrichment of infectious Zika virus particles from the crude harvest comprising infectious Zika virus particles and non-infectious virus particles and other virus products such that the enrichment of the infectious virus particles is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, preferably at least 80%,

especially at least 85% relative to the total virus particle content of the crude harvest (a) comprising the virus particles and impurities.

In some embodiments, the residual impurity of the final virus preparation with respect to all impurities in the crude harvest is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, preferably less than 5% as determined by SEC-HPLC (Size Exclusion Chromatography – HPLC).

A unique aspect of the current invention is the realization that know-how related to the vaccine design and purification approach used for the Japanese Encephalitis Vaccine (JEV) IXIARO® (see Srivastava A.K. et al., 2001, Vaccine 19, 4557-4565, WO99/11762) may be employed and improved upon in order to expedite the development of a Zika virus vaccine and provide it to the subjects in need as soon as possible. The industrial process as disclosed for IXIARO®, providing a very effective vaccine against JEV, was complemented by further significant improvements disclosed herein in order to provide a more efficient (higher yield) and safer (less or no protamine sulphate with its allergic potential) Zika vaccine compared to the available JEV vaccine. A particular innovation of the herein disclosed vaccines is their greatly reduced protamine salt (SEQ ID NO: 1) content in the final drug substance facilitated by the development of an improved sucrose gradient. Said sucrose gradient not only allowed the separation of protamine sulphate but also allowed for a very effective inactivation by formaldehyde and resulted in the case of Zika with over 90% yield with the improved process disclosed herein vs about 35% yield with the published JEV process, see experimental part for comparison).

Aspects of the disclosure relate to methods of producing a virus in Vero tissue culture cells. Vero cells are a commonly used tissue culture cell line derived from the kidney of an African green monkey. The Vero cells used in the methods described herein are the Vero (WHO) cell line, obtained from the Health Protection Agency general cell collection under catalogue number 88020401.

Vero cells can be grown to confluent monolayers, for example in tissue culture flasks; in suspension (on microcarriers), for example in roller bottles; or in any other cell culture system for viral production. In some embodiments, the Vero cells are grown in a bioreactor for viral production. For plaque assays or the plaque reduction neutralization test (PRNT), Vero cells are grown in monolayers in tissue culture flasks, dishes, or wells of a plate. To infect the Vero cells with the virus, the culture medium is inoculated with virus and the cells are incubated with the virus for a period of time. The cells may be washed after inoculation to remove any virus that did not adsorb to the cells in a given amount of time.

The methods provided herein involve passaging the virus in Vero cells. As used herein, the terms "passage" or "passaging" refer to infecting a population of Vero cells with virus and subsequently inoculating a second population of Vero cells with virus produced by infection of the first Vero cell population. In some embodiments, a portion of the culture medium from the infected Vero cells (containing virus that was released from the infected cells) is used to inoculate a second population of Vero cells. This is referred to as one passage or one round of passaging. The passaging may be performed serially, for example, a portion of the culture medium from the infected second population of Vero cells is used to inoculate a third population of Vero cells, and so on. In some embodiments, virus obtained from a single plaque is used to inoculate another population of cells.

In some embodiments, the virus is passaged in Vero cells several times, such as at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times. In some embodiments, the virus is passaged in Vero cells at least 4 times or 5 times. In some embodiments, the virus is passaged in Vero cells at least 30 times. It is important that the virus population, i.e. the virus sequences, stays as much as possible constant over said passaging. If adaption of the virus occurs (i.e. appearance of mutated viruses in the original virus population), it is preferred that said passages are not used in the context of manufacturing of said virus, e.g. for Zika it was found that up to passage 3 and culturing to day 7 can be used without major shifts in virus population, i.e. introduction of virus population with mutations. However this observation needs to be done for each virus strain and may be different.

In some embodiments, the Vero cells are incubated for at least 2 days after inoculation with the virus at e.g. a typical 0.01 MOI (multiplicity of infection) to allow for viral production prior to passaging. In some embodiments, the Vero cells are incubated for at least 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 days e.g. at least 7 days after inoculation with the virus prior to passaging. The number of days the Vero cells are incubated after viral inoculation may depend on factors such as the multiplicity of infection used to inoculate the cells and the viral titer desired in the culture medium. Serial passaging of the virus in Vero cells may result in generation of a Vero cell adapted virus strain.

The culture medium from the infected Vero cells may be harvested (collected) to obtain the virus. In some embodiments, the culture medium is harvested from infected Vero cells and is replaced with fresh culture medium, which is then harvested after another period of time. In some embodiments, the culture medium harvested from infected Vero cells is pooled from independent Vero cell cultures and/or from independent days. Harvesting can be repeated up to 4 times by 7 or 9 days post infection,

for example, and result in a high yield of virus per unit cell culture. In order to minimize the adaption of Zika virus strain to Vero cells, it was found that Vero cells could be incubated for at least 7 days, more preferably 5 days, prior to passaging and subsequently supernatants could be harvested at days 2, 3, 5 and 7 or 2, 3, and 5 (see also experimental part). The harvested culture medium can be stored at +4°C prior to purification of the virus from the culture medium up to 2 weeks.

In some embodiments, debris from infected and lysed Vero cells may be removed from the harvested culture medium, referred to as a "clarification" of the culture medium. The harvested culture medium may be clarified by common methods known in the art, such as low-speed centrifugation, for example, at 1500 *g* for 10 min, and/or by filtration through a filter of pore size of 0.45 µm. The harvested culture medium can be stored at +4°C prior to concentration.

To concentrate the titer of the Zika virus in the harvested culture medium, it may be subjected to concentration by any method known in the art. For example, the harvested culture medium may be concentrated by methods including, without limitation, ultrafiltration, ultracentrifugation, centrifugal concentrator, vacuum centrifugation, and lyophilization. In some embodiments, the harvested culture medium is concentrated by ultrafiltration and the retentate containing the Zika virus is collected. In some embodiments, the harvested culture medium is concentrated by precipitation in which polyethylene glycol (PEG) 8000 is dissolved in the culture medium (up to 10%) and the precipitate is dissolved in a buffer, for example phosphate-buffered saline (PBS, pH 7.0).

The harvested culture medium may be precipitated to produce a virus supernatant. In some embodiments, the harvested culture medium is precipitated to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. In some embodiments, the harvested culture medium is concentrated prior to precipitation. In some embodiments, the harvested culture medium is precipitated by adding protamine sulfate (e.g. SEQ ID NO: 1) to the harvested culture medium and incubating the mixture, for example at +4°C or on ice. In some embodiments, the harvested culture medium is treated with benzonase to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. However, it was found that the treatment with protamine sulfate is preferred (see experimental part). In some embodiments, the precipitated culture medium is centrifuged to collect precipitated material and the supernatant containing the virus, referred to as a "virus supernatant", is collected.

The virus supernatant may be further purified after precipitation, for example density gradient ultracentrifugation. In some embodiments, the virus supernatant is further purified by sucrose

gradient. Fractions may be collected from the sucrose gradients and assayed for presence of the virus. Methods for assaying for virus positive fractions include plaque assay, hemagglutination assay, polyacrylamide gel electrophoresis, and antigen assays such as Western blotting and ELISA. The fractions containing virus may be pooled based on titer of the virus and level of other impurities. The level or amount of impurities present in the virus supernatant can be estimated by testing for Vero cell DNA, virus aggregates and/or Vero cell protein (see experimental part). A particular embodiment of the invention is the improved sucrose gradient that allows for an efficient protamine separation as shown in the experimental part. It was surprisingly found that the addition of a virus-containing fraction with 10% (w/w) sucrose to a simple three layer sucrose density gradient (e.g. a gradient comprising a 15% (w/w) sucrose solution, a 35% (w/w) sucrose solution, and a 50% (w/w) sucrose solution) resulted in efficient separation of protamine sulphate without much loss of virus. Thus a particularly preferred embodiment of the invention is the use of a sucrose density gradient that is able to efficiently separate protamine sulphate, wherein said sucrose density gradient is used in the purification of virus such as the viruses described herein, i.e. a Zika virus.

To achieve a safe vaccine or composition for the administration to subjects, the virus supernatant may be inactivated (see experimental part for Zika virus). As used herein, the terms "inactivated" and "optimally inactivated" may be used interchangeably and refer to a process (or its result) by which the virus is rendered unable to infect a host cell (non-infectious), but that does not affect or substantially affect the antigenicity of the virus, for example, the immunogenic antigens exposed on the surface of the virus are able to stimulate an immune response in a subject (*e.g.*, antigen-specific antibodies). By "does not affect or substantially affect the antigenicity of the virus" is meant that the inactivated virus retains at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even essentially 100% of the antigenicity of a virus that is not subjected to inactivation.

A variety of methods are known in the art for inactivating viruses. In some embodiments, the virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

In some embodiments, the inactivating is by chemical inactivation and involves contacting the virus with one or more chemical inactivation agents for a period of time under conditions such that the virus is inactivated but the antigenic epitopes are substantially intact. In some embodiments, the virus is inactivated for a period of time that is longer than is required to completely inactivate the virus. In some embodiments, the virus supernatant is inactivated for the number of days required to inactivate the virus plus at least one additional day. Samples of the virus supernatant may be taken at one or

more times throughout the inactivation process and assessed for viral viability (infectivity) by any method known in the art, such as by infecting a monolayer of host cells (*i.e.*, plaque assay). Using such a procedure, the period of time that is required to completely inactivate the virus can be determined, and a longer period of time is selected to ensure complete inactivation.

In some embodiments, the virus is contacted with a chemical inactivation agent for between 1 day and 50 days, between 2 days and 40 days, between 2 days and 30 days, between 2 days and 20 days, between 2 days and 10 days, between 3 days and 9 days, between 4 days and 8 days, between 5 days and 7 days, between 2 days and 5 days, or between 5 and 10 days. In some embodiments, the virus is contacted with one or more chemical inactivation agents for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days, 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, 42 days, 43 days, 44 days, 45 days, 46 days, 47 days, 48 days, 49 days, or at least 50 days.

In some embodiments, the chemical inactivation is performed at about +5°C, +10°C, +15°C, +20°C, +25°C, +30°C, +35°C, +40°C, or about +45°C. In some embodiments, the chemical inactivation is performed at about +4°C. In some embodiments, the chemical inactivation is performed at about +22°C.

Any chemical inactivation agent known in the art may be suitable for inactivating the virus in the methods described herein. It will be appreciated by one of skill in the art that factors such as the chemical inactivation agent and the temperature at which inactivation is performed may affect the length of time (number of days) required to completely inactivate the virus. Examples of chemical inactivation agents include, without limitation, formaldehyde, enzymes, β-propiolactone, ethanol, trifluroacetic acid, acetonitrile, bleach, urea, guanidine hydrochloride, tri-n-butyl phosphate, ethylene-imine or a derivatives thereof, and organic solvents such as Tween, Triton, sodium deoxycholate, and sulphobetaine. A preferred inactivation is inactivation with formaldehyde at 22°C +/-2°C for about 10 days.

In some embodiments, the inactivating agent is neutralized after chemical inactivation of the virus. In some embodiments, the inactivating agent is formaldehyde and is neutralized after chemical inactivation using sodium thiosulphate or sodium metabisulfite.

In some embodiments, the virus is inactivated by thermal inactivation. In some embodiments, the thermal inactivation involves exposing the virus to heat, such as dry heat or vapor heat, for a period of time. In some embodiments, the thermal inactivation involves exposing the virus to temperatures of about +40°C, +45°C, +50°C, +55°C, +60°C, +65°C, +70°C, +75°C, +80°C, +85°C, +90°C,+ 95°C, or about +100°C. In some embodiments, the virus is exposed to heat for at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, about 96 hours, or longer. A preferred thermal inactivation involves exposing the virus to temperatures of about +56°C for 60 minutes.

In some embodiments, the virus is inactivated by exposing the virus to acidic or alkaline conditions for a period of time such that the virus is completely inactivated. The pH of a virus preparation may be adjusted to a desired pH, for example by the addition of an acid, a base, or a buffer with a particular pH to the virus preparation. In some embodiments, the virus is inactivated at an acidic pH of about 2, 2.5, 3, 3.5, 4, 4.5, 5 or about 5.5. In other embodiments, the virus is inactivated at an alkaline pH of about 8, 8.5, 9, 9.5, 10, or about 10.5.

In some embodiments, the virus is inactivated using UV inactivation. UV inactivation involves exposing the virus to energy-rich radiation, such as UV-A, UV-B, or UV-C light for a period of time. It will be appreciated that any two or more methods of inactivation may be combined and performed concurrently or serially.

The inactivated virus may be subsequently dialyzed to remove any undesired material, including the inactivating agent and any neutralizing agent, and/or to replace the buffer with a buffer that is pharmaceutically acceptable for administration to subjects. In some embodiments, the inactivated virus is dialyzed with PBS. In addition or alternatively, the inactivated virus may be filtered, such as sterile filtered, through a 0.22 μm filter.

Any of the methods or uses described herein may be for the prevention of a Zika virus infection in a subject. As used herein, the terms "prevent," "preventing" and "protection from" include the administration of a virus vaccine or composition to a subject to reduce, or delay the onset of the manifestation of clinical or subclinical symptoms, complications, pathologies or biochemical indicia of a disease or infection, or to reduce or inhibit the spread/transmission of the Zika virus. As used herein, antigen(s), such as an inactivated Zika virus, that is administered to a subject prophylactically (e.g., prior to infection) may be referred to as a vaccine.

Zika Vaccine

As described herein Zika virus may cause any of a variety of symptoms upon infection of a subject, and is generally characterized by mild fever; rash (exanthema) on face, neck, trunk, upper arms; headache; sensitivity to light; non-inflammatory joint pain; conjunctivitis; lack of appetite; diarrhea; abdominal pain; and/or dizziness. Zika virus infection during pregnancy is associated with microcephaly in the fetus/neonate. There is also a probable association between the onset of Guillain-Barré syndrome or symptoms thereof. Diagnosis of Zika virus infection in subjects exposed to Zika virus or suspected of being exposed to Zika virus involves detecting the presence of virus-specific antibodies and/or molecular testing, such as PCR or real-time PCR detection of Zika virus.

Provided herein are methods for administering a dose of a therapeutically effective amount of a Zika virus vaccine to a subject in need thereof. In some embodiments, the subject is a mammalian subject, such as a human, non-human primate, rodent, rabbit, sheep, dog, cat, horse, or cow. In some embodiments, the subject is a mouse. In some embodiments, the subject is a human subject, such as a child, an adult, or an elderly adult. In some embodiments, the subject is a female subject. In some embodiments, the subject is pregnant or planning on becoming pregnant. In some embodiments, the subject is at risk of being exposed to Zika virus. In some embodiments, the subject is living in or traveling to an area where Zika virus is present or is thought to be present. In some embodiments, the subject has been previously infected with or vaccinated against Dengue virus; i.e., at risk for antibody-dependent enhancement of disease. In some embodiments, the subject is living in or traveling to an area that is experiencing a Zika virus infection outbreak. In some embodiments, the subject is living in or traveling to an area where an arthropod vector capable of transmitting the Zika virus vector is present or is thought to be present.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject in a therapeutically effective amount or a dose of a therapeutically effective amount. As used herein, a "therapeutically effective amount" of vaccine is any amount that results in a desired response or outcome in a subject, such as those described herein, including but not limited to prevention of infection, an immune response or an enhanced immune response to Zika virus, or prevention or reduction of symptoms associated with Zika disease.

In some embodiments, the therapeutically effective amount of a Zika virus vaccine or composition described herein is an amount sufficient to generate antigen-specific antibodies (*e.g.*, anti-Zika virus

antibodies). In some embodiments, the therapeutically effective amount is sufficient to provide seroprotection in a subject; i.e., to generate sufficient antigen-specific antibodies to prevent/protect from infection. In some embodiments, seroprotection is conferred on at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects. In some embodiments, seroprotection is defined by a reduction in the number of Zika virus plaques by 50% or more in a plaque reduction neutralization test (PRNT) by a 1:10 or higher dilution of sera from a vaccinated subject. In some embodiments, an effective amount of the Zika vaccine is sufficient to seroconvert a subject with at least 70% probability. In some embodiments, the therapeutically effective amount is sufficient to seroconvert a subject with at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98%, or at least 99% probability. Whether a subject has been seroconverted can be assessed by any method known in the art, such as obtaining a serum sample from the subject and performing an assay to detect anti-Zika virus antibodies. In some embodiments, a subject is seroconverted if a serum sample from the subject contains an amount of anti-Zika virus antibodies that surpasses a threshold or predetermined baseline. A subject is generally considered seroconverted if there is at least a 4-fold increase in anti-Zika virus antibodies (*i.e.,* anti-Zika E protein IgG antibodies) in a serum sample from the subject as compared to a serum sample previously taken from the same subject.

In some embodiments, seroconversion of a subject is assessed by performing a plaque reduction neutralization test (PRNT). Briefly, PRNT is used to determine the serum titer required to reduce the number of Zika virus plaques by 50% (PRNT50) as compared to a control serum/antibody. The PRNT50 may be carried out using monolayers of Vero cells or any other cell type/line that can be infected with Zika virus. Sera from subjects are diluted and incubated with live, non-inactivated Zika virus. The serum/virus mixture may be applied to the Vero cells and incubated for a period of time. Plaques formed on the Vero cell monolayers are counted and compared to the number of plaques formed by the Zika virus in the absence of serum or a control antibody. A threshold of neutralizing antibodies of 1:10 dilution of serum in a PRNT50 is generally accepted as evidence of protection (Hombach *et. al.* Vaccine (2005) 23:5205-5211).

In some embodiments, the Zika virus may be formulated for administration in a composition, such as a pharmaceutical composition. The term "pharmaceutical composition" as used herein means a product that results from the mixing or combining of at least one active ingredient, such as an inactivated Zika virus, and one or more inactive ingredients, which may include one or more pharmaceutically acceptable excipient.

Pharmaceutical compositions of the invention, including vaccines, can be prepared in accordance with methods well known and routinely practiced in the art (see *e.g.*, Remington: The Science and Practice of Pharmacy, Mack Publishing Co. 20th ed. 2000; and Ingredients of Vaccines – Fact Sheet from the Centers for Disease Control and Prevention, e.g., adjuvants and enhancers such as alum to help the vaccine improve its work, preservatives and stabilizers to help the vaccine remain unchanged (e.g., albumin, phenols, glycine)). Pharmaceutical compositions are preferably manufactured under GMP conditions. Typically a therapeutically effective dose of the inactivated Zika virus preparation is employed in the pharmaceutical composition of the invention. The inactivated Zika virus is formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, the prophylactic response).

Dosages of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired pharmaceutical response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors.

A physician, veterinarian or other trained practitioner, can start doses of the inactivated Zika virus vaccine employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect (*e.g.*, production of anti-Zika virus antibodies) is achieved. In general, effective doses of the compositions of the present invention, for the prophylactic treatment of groups of people as described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and the titer of anti-Zika virus antibodies desired. Dosages need to be titrated to optimize safety and efficacy. In some embodiments, the dosing regimen entails subcutaneous or intramuscular administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 7. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 14. In some embodiments, the dosing regimen entails subcutaneous

administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 28. In some embodiments, the inactivated Zika virus is administered to the subject once.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject with, prior to, or after administration of one or more adjuvants. An adjuvant is a molecule that enhances a response in a subject, such as an immune response, to an antigen or other molecule. In some embodiments, an adjuvant may stabilize an antigen or other molecule. Determining whether a Zika virus vaccine or compositions thereof are administered with an adjuvant depends on various factors (e.g., type and extent of response desired) and will be evident to one of skill in the art. In some embodiments, administering any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may enhance the production of virus neutralizing (anti-Zika virus) antibodies. In some embodiments, a subject that is administered any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may only require a single administration of the Zika virus vaccine or composition to be seroconverted (produce a level of anti-Zika virus antibodies). Examples of adjuvants may include, without limitation, aluminium salt (aluminium hydroxide or aluminium phosphate), calcium phosphate hydroxide, paraffin oil, killed bacteria, bacterial toxins, toxoids, subunits of bacteria, squalene, thimerosal, detergents, IL-1, IL-2, IL-12, 2-component adjuvants, such as 2-component adjuvants containing an antibacterial peptide and a TLR9 agonist (*e.g.*, IC31®), and combinations such as Freund's complete adjuvant and Freund's incomplete adjuvant. In some embodiments, the Zika virus vaccines or compositions is administered with aluminium hydroxide. In some embodiments, the inactivated Zika virus vaccine or composition is administered with aluminium phosphate salt. A preferred aluminium salt is the aluminium hydroxide with reduced Cu content, e.g. lower than 1,25ppb based on the weight of the Zika composition, an adjuvant described in detail in WO 2013/083726 or Schlegl et al., Vaccine 33 (2015) 5989-5996.

In some embodiments, the adjuvant is comprised of two components. In some embodiments, the 2-component adjuvant comprises an antibacterial peptide and a TLR9 agonist. In some embodiments, the antibacterial peptide is provided by the amino acid sequence $KLKL_5KLK$ (SEQ ID NO: 71). In some embodiments, the TLR9 agonist is a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN). In some embodiments, the I-ODN comprises the nucleic acid sequence $(dIdC)_{13}$ (SEQ ID NO: 70). In some embodiments, the adjuvant is IC31®. In some embodiments, the adjuvant is in nanoparticle form (See, *e.g.*, US Patent No. 8,765,148 B2, incorporated by reference in its entirety). In some embodiments, the adjuvant is IC31®, i.e.

$KLKL_5KLK$ (SEQ ID NO: 71) and the nucleic acid sequence $(dIdC)_{13}$ (SEQ ID NO: 70), in combination with an aluminium salt such as aluminium hydroxide.

The Zika virus vaccines or compositions described herein may be administered to a subject concomitantly with one or more vaccines to another infectious agent, such as another infectious agent is that present or thought to be present in the same geographic area as Zika virus. In some embodiments, the other infectious agent is one that the subject is also at risk of being in contact with. In some embodiments, the other infectious agent is transmitted by the same arthropod vector as Zika virus. In some embodiments, the other infectious agent is Japanese Encephalitis virus, Yellow Fever virus, Dengue virus and/or Chikungunya virus.

Also within the scope of the present disclosure are kits for use in prophylactically administering to a subject, for example to prevent or reduce the severity of Zika virus infection. Such kits can include one or more containers comprising a composition containing inactivated Zika virus, such as an inactivated Zika virus vaccine. In some embodiments, the kit may further include one or more additional containing comprising a second composition, such as a second vaccine. In some embodiments, the second vaccine is a vaccine for another arbovirus. In some embodiments, the second vaccine is a Dengue virus vaccine and/or a Chikungunya virus vaccine.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the composition containing inactivated Zika virus to prevent, delay the onset, or reduce the severity of Zika virus infection. The kit may further comprise a description of selecting a subject suitable for administration based on identifying whether that subject is at risk for exposure to Zika virus or contracting a Zika virus infection. In still other embodiments, the instructions comprise a description of administering a composition containing inactivated Zika virus to a subject at risk of exposure to Zika virus or contracting Zika virus infection.

The instructions relating to the use of the composition containing inactivated Zika virus generally include information as to the dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine readable instructions are also acceptable.

The kits of the present disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as a syringe or an infusion device. The container may have a sterile access port, for example the container may be a vial having a stopper pierceable by a hypodermic injection needle. At least one active agent in the composition is an inactivated Zika virus, as described herein.

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including", "comprising", or "having,", "containing", "involving", and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, virology, cell or tissue culture, genetics and protein and nucleic chemistry described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated.

The present invention is further illustrated by the following examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove. However, the citation of any reference is not intended to be an admission that the reference is prior art.

Table 1. Overview of process buffers and stock solutions.

Buffer	Composition	Final pH	Final conductivity [mS/cm]
A	0.5 M NaOH		n.a.
B	0.1 M NaOH		n.a.
C	25 mM Tris, 150 mM NaCl	7.4±0.2	16.5
D	1 M Tris	7.4±0.2	n.a.
E	4.5 M NaCl	n.a.	n.a.
F	1 M NaCl	n.a.	n.a.
G	1 % SDS	n.a.	n.a.
H	50 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
I	35 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
J	15 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
K	10 x PBS	7.4±0.2	n.a.
L	50 mg/mL Protamine sulphate	7.4±0.2	n.a.
M	Drug substance formulation buffer (10mM Tris(hydroxymethyl)-aminomethan, 5 % Sucrose, 1% (10 mg/mL) rHSA)	7.4±0.2	1.3

Table 2. Abbreviations.

°Bx	Degrees Brix = sugar content (w/w) of an aqueous solution*
BSA	Bovine serum albumin
CC700	Capto™ Core 700
CPE	Cytopathic effect
EtOH	Ethanol
EU	Endotoxin units
DS	Drug Substance
DP	Drug Product
DSP	Downstream Process
HCP	Host cell protein
hcDNA	Host cell DNA
hpi	Hours post infection
HPLC	High Performance Liquid Chromatography
ID	Inner diameter
JEV	Japanese Encephalitis virus
LAL	Limulus amebocyte lysate
LDS buffer	Lithium dodecyl sulfate sample loading buffer
LOD	Limit of detection
LOQ	Limit of quantitation
MALLS	Multiangle light scattering
mAU	Milli absorbance units
MS	Mass spectroscopy
NIV	Neutralized inactivated virus
PBS	Phosphate buffered saline
PD	Process development
PFU	Plaque forming units
p.i.	Post-infection
PS	Protamine sulphate or protamine sulfate
rcf	Relative centrifugal force
rHSA	Recombinant human serum albumin
Rms radius	Root mean square radius
rMSB	Research master seed bank
RSD	Relative standard deviation
SEC	Size exclusion chromatography
SGC	Sucrose gradient centrifugation

SGP	Sucrose gradient purified
SDS	Sodium dodecyl sulphate
TBS	Tris buffered saline
TFF	Tangential flow filtration
TCID50	Tissue culture infectious dose 50%
UF/DF	Ultrafiltration/diafiltration
WFI	Water for injection
ZikaV	Zika virus

*Degrees Brix (°Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. °Bx corresponds to the sucrose content in percent (w/w), e.g., 45 °Bx equals 45 % (w/w) sucrose.

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Table A. Primers for Zika virus sequencing: lower case letters indicate bases not included in ZIKA but containing restriction sites for later cloning when needed (therefore, two Tms provided).

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
1	9320_Zika_PF_1F	SEQ ID NO: 74 ttaggatccGTTGTTGATCTGTGTGAAT	69.9	74.6	707
	9321_Zika_PF_1R	SEQ ID NO: 75 taactcgagCGTACACAACCCAAGTT	69.3	75.6	
2	9322_Zika_PF_2F	SEQ ID NO: 76 ttaggatccTCACTAGACGTGGGAGTG	70	73.9	704
	9323_Zika_PF_2R	SEQ ID NO: 77 taactcgagAAGCCATGTCYGATATTGAT	69.8	73.7	
3	9324_Zika_PF_3F	SEQ ID NO: 78 ttaggatccGCATACAGCATCAGGTG	72.3	74.5	712
	9325_Zika_PF_3R	SEQ ID NO: 79 taactcgagTGTGGAGTTCCGGTGTCT	72	76.4	
4	9326_Zika_PF_4F	SEQ ID NO: 80 ttaggatccGAATAGAGCGAARGTTGAGATA	70.9	74	712
	9327_Zika_PF_4R	SEQ ID NO: 81 taactcgAGTGGTGGGTGATCTTCTTCT	70.5	73.7	
5	9328_Zika_PF_5F	SEQ ID NO: 82 ttaggatcCAGTCACAGTGGAGGTACAGTAC	70.3	75	704
	9329_Zika_PF_5R	SEQ ID NO: 83 taactcgagCRCAGATACCATCTTCCC	71.5	77.3	
6	9330_Zika_PF_6F	SEQ ID NO: 84 ttaggatCCCTTATGTGCTTGGCCTTAG	70.7	72.7	698
	9331_Zika_PF_6R	SEQ ID NO: 85 taactcgagTCTTCAGCCTCCATGTG	70.4	76.9	
7	9332_Zika_PF_7F	SEQ ID NO: 86 ttaggatccAATGCCCACTCAAACATAGA	71.9	75	716
	9333_Zika_PF_7R	SEQ ID NO: 87 taactcgagTCATTCTCTTCTTCAGCCCTT	71	74	
8	9334_Zika_PF_8F	SEQ ID NO: 88 ttaggatccAAGGGTGATCGAGGAAT	70.9	75.2	703
	9335_Zika_PF_8R	SEQ ID NO: 89 taactcgagTTCCCTTCAGAGAGAGGAGC	71.9	73.4	
9	9336_Zika_PF_9F	SEQ ID NO: 90 ttaggatccTCTTTTGCAAACTGCGATC	71.9	75	699
	9337_Zika_PF_9R	SEQ ID NO: 91 taactcgagTCCAGCTGCAAAGGGTAT	71	74.9	
10	9338_Zika_PF_10F	SEQ ID NO: 92 ttaggatccGTGTGGACATGTACATTGA	71.4	75.8	706
	9339_Zika_PF_10R	SEQ ID NO: 93 taactcgagCCCATTGCCATAAAGTC	70.4	75.8	

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
11	9340_Zika_PF_11F	SEQ ID NO: 94 ttaggatccTCATACTGTGGTCCATGGA	71.6	78.1	692
	9341_Zika_PF_11R	SEQ ID NO: 95 taactcgagGCCCATCTCAACCCTTG	74	78	
12	9342_Zika_PF_12F	SEQ ID NO: 96 ttaggatccTAGAGGGCTTCCAGTGC	70.9	74	707
	9343_Zika_PF_12R	SEQ ID NO: 97 taactcgAGATACTCATCTCCAGGTTTGTTG	70.2	72.2	
13	9344_Zika_PF_13F	SEQ ID NO: 98 ttaggatccGAAAACAAAACATCAAGAGTG	70.6	75.4	726
	9345_Zika_PF_13R	SEQ ID NO: 99 taactcgagGAATCTCTCTGTCATGTGTCCT	71.9	75.6	
14	9346_Zika_PF_14F	SEQ ID NO: 100 ttaggatccTTGATGGCACGACCAAC	73.1	75.6	715
	9347_Zika_PF_14R	SEQ ID NO: 101 ttaggatccGTTGTTGATCTGTGTGAAT	70.8	77.9	
15	9348_Zika_PF_15F	SEQ ID NO: 102 taactcgagCAGGTCAATGTCCATTG	71.9	75.4	719
	9349_Zika_PF_15R	SEQ ID NO: 103 ttaggatccTGTTGTGTTCCTATTGCTGGT	73.9	77.2	
16	9350_Zika_PF_16F	SEQ ID NO: 104 taactcgaGTGATCAGRGCCCCAGC	72.3	75.4	703
	9351_Zika_PF_16R	SEQ ID NO: 105 ttaggatccTGCTGCCCAGAAGAGAA	72	76.3	
17	9352_Zika_PF_17F	SEQ ID NO: 106 taactcgaGCACCAACAYGGGTTCTT	73.6	76	705
	9353_Zika_PF_17R	SEQ ID NO: 107 ttaggatcCTCAAGGACGGTGTGGC	72	75.5	
18	9354_Zika_PF_18F	SEQ ID NO: 108 taactcgagCAATGATCTTCATGTTGGG	71.7	75.8	699
	9355_Zika_PF_18R	SEQ ID NO: 109 ttaggatccTATGGGGGAGGACTGGT	71	74.1	
19	9356_Zika_PF_19F	SEQ ID NO: 110 taactcGAGCCCAGAACCTTGGATC	73.3	75.5	711
	9357_Zika_PF_19R	SEQ ID NO: 111 ttaggatcCAGACCCCCAAGAAGGC	71.3	76.9	
20	9358_Zika_PF_20F	SEQ ID NO: 112 taactcgagCCCCTTTGGTCTTGTCT	71.7	75	706
	9359_Zika_PF_20R	SEQ ID NO: 113 ttaggatccAGGAAGGATGTATGCAGATG	71.9	73.9	
21	9360_Zika_PF_21F	SEQ ID NO: 114 taactcgagACATTTGCGCATATGATTTTG	70.4	75.7	709
	9361_Zika_PF_21R	SEQ ID NO: 115 ttaggatccAGGAAGGACACACAAGAGT	71.8	75	
22	9362_Zika_PF_22F	SEQ ID NO: 116 taactcgagACAGGCTGCACAGCTTT	70	79.1	581
	9363_Zika_PF_22R	SEQ ID NO: 117 ttaggatccTCTCTCATAGGGCACAGAC	74.8	81.1	

SEQUENCES

SEQ ID NO: 1
A typical form of protamine
PRRRRSSSRP VRRRRRPRVS RRRRRRGGRR RR

Provided below are examples of nucleic acid sequences of the genomes of Zika viruses that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 2

KU321639.1 Zika virus strain ZikaSPH2015, Brazil, complete genome

GTTGTTACTGTTGCTGACTCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTGG
ATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACG
CGGAGTAGCCCGTGTGAGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGTCATGGGCCCATCAGG
ATGGTCTTGGCAATTCTAGCCTTTTTGAGATTCACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTG
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CAAGGTTCCAGCTCAGATGGCGGTGGACATGCAAACTCTGACCCCAGTTGGGAGGTTGATAACCGCTAACCCCGTAATCA
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SEQ ID NO: 3
KU497555.1 Zika virus isolate Brazil-ZKV2015, Brazil, complete genome
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SEQ ID NO: 4
KU501215.1 Zika virus strain PRVABC59, Puerto Rico, complete genome
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SEQ ID NO: 5
KU509998.1 Zika virus strain Haiti/1225/2014, Haiti, complete genome
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SEQ ID NO: 6
KU527068.1 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, complete genome
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SEQ ID NO: 8
KU681082.3 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, complete genome
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SEQ ID NO: 9

KU707826.1 Zika virus isolate SSABR1, Brazil, complete genome

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SEQ ID NO: 11

LC002520.1 Zika virus genomic RNA, strain: MR766-NIID, Uganda, complete genome

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SEQ ID NO: 12
AY632535.2 NC_012532.1 Zika virus strain MR 766, Uganda, complete genome
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SEQ ID NO: 13
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GATGATAGGTTTGCACATGCCCTCAGGTTCTTGAATGATATGGGAAAAGTTAGGAAGGACACACAAGAGTGGAAACCCT
CAACTGGATGGGACAACTGGGAAGAAGTTCCGTTTTGCTCCCACCACTTCAACAAGCTCCATCTCAAGGACGGGAGGTCC
ATTGTGGTTCCCTGCCGCCACCAAGATGAACTGATTGGCCGGGCCCGCGTCTCTCCAGGGGCGGGATGGAGCATCCGGG
AGACTGCTTGCCTAGCAAAATCATATGCGCAAATGTGGCAGCTCCTTTATTTCCACAGAAGGGACCTCCGACTGATGGCCA
ATGCCATTTGTTCATCTGTGCCAGTTGACTGGGTTCCAACTGGGAGAACTACCTGGTCAATCCATGGAAAGGGAGAATGG
ATGACCACTGAAGACATGCTTGTGGTGTGGAACAGAGTGTGGATTGAGGAGAACGACCACATGGAAGACAAGACCCCA
GTTACGAAATGGACAGACATTCCCTATTTGGGAAAAAGGGAAGACTTGTGGTGTGGATCTCTCATAGGGCACAGACCGC
GCACCACCTGGGCTGAGAACATTAAAAACACAGTCAACATGGTGCGCAGGATCATAGGTGATGAAGAAAAGTACATGGA
CTACCTATCCACCCAAGTTCGCTACTTGGGTGAAGAAGGGTCTACACCTGGAGTGCTGTAAGCACCAATCTTAGTGTTGTC
AGGCCTGCTAGTCAGCCACAGCTTGGGGAAAGCTGTGCAGCCTGTGACCCCCCCAGGAGAAGCTGGGAAACCAAGCCTA
TAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCTGTGAGCCCCTCAGAGGACACTGAGTCAAAAAACC
CCACGCGCTTGGAGGCGCAGGATGGGAAAAGAAGGTGGCGACCTTCCCCACCCTTCAATCTGGGGCCTGAACTGGAGAT
CAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAG

In some embodiments, the Zika virus has a RNA genome corresponding to the DNA sequence provided by the nucleic acid sequence of any one of SEQ ID NOs: 2-13 or 72. In some embodiments, the Zika virus has a variant genome that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%. 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-13 or 72.

Provided below are amino acid sequences of the E-proteins of Zika strains that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 14
isol-ARB15076.AHF49784.1.Central_African_Republic/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
TDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 15
isol-IbH_30656.AEN75265.1.Nigeria/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHSGADTETPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
RDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSIIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 16
ArB1362.AHL43500.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXXXXXXXNR

AEVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 17
ArD128000.AHL43502.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMXXXXXGHETDEN
RAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHRLVRKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWLKKGSSIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 18
ArD158095.AHL43505.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 19
ArD158084.AHL43504.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 20
isol-ARB13565.AHF49783.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 21
isol-ARB7701.AHF49785.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 22
isol-ArD_41519.AEN75266.1.Senegal/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVE
FKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVT
VEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKA
FEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 23
MR766-NIID.BAP47441.1.Uganda/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 24
LC002520.1/326-829 Zika virus genomic RNA, strain: MR766-NIID, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 25
isol-MR_766.AEN75263.1.Uganda/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 26
ArD7117.AHL43501.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAVCTAAKVPAETLHGTVT
VEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKA
FEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 27
AY632535.2/326-825 NC_012532.1 Zika virus strain MR 766, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE

VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 28
MR_766.AAV34151.1.Uganda/291-790 Flavivirus envelope glycoprotein E. |Q32ZE1|Q32ZE1_9FL
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 29
MR_766.YP_009227198.1.Uganda/1-500 envelope protein E [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 30
KU681081.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHTGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVLNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 31
isol-Zika_virus%H.sapiens-tc%THA%2014%SV0127-_14.AMD61710.1.Thailand/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHTGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVLNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 32
CK-ISL_2014.AIC06934.1.Cook_Islands/1-504 Flavivirus envelope glycoprotein E. (Fragment) OS=Zika virus GN=E PE=4 SV=1
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV

EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 33
Natal_RGN.AMB18850.1.Brazil:_Rio_Grande_do_Norte,_Natal/291-794 Flavivirus envelope glycoprotein E.]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 34
isol-Si323.AMC37200.1.Colombia/1-504 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 35
KU707826.1/317-820 Zika virus isolate SSABR1, Brazil, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 36
KU509998.1/326-829 Zika virus strain Haiti/1225/2014, Haiti, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 37
isol-GDZ16001.AML82110.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 38

BeH819015.AMA12085.1.Brazil/291-794 Flavivirus envelope glycoprotein E.]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 39
MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 40
KU501215.1/308-811 Zika virus strain PRVABC59, Puerto Rico, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 41
Haiti%1225%2014.AMB37295.1.Haiti/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 42
KU527068.1/308-811 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 43
isol-Z1106027.ALX35662.1.Suriname/5-508 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF

KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 44
isol-FLR.AMM39804.1.Colombia:_Barranquilla/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 45
PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.Canada/254-757 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 46
BeH818995.AMA12084.1.Brazil/291-794 Flavivirus envelope glycoprotein E. [Zika virus].
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 47
H/PF/2013.AHZ13508.1.French_Polynesia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 48
PRVABC59.AMC13911.1.Puerto_Rico/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 49
KU321639.1/326-829 Zika virus strain ZikaSPH2015, Brazil, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 50
ZikaSPH2015.ALU33341.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 51
103344.AMC13912.1.Guatemala/291-794 polyprotein [Zika virus]. 103344.AMC13912.1.Guatemala Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEIRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 52
isol-Brazil-ZKV2015.AMD16557.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 53
KU497555.1/308-811 Zika virus isolate Brazil-ZKV2015, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 54
isol-ZJ03.AMM39806.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN

RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGARRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 55
isol-FSS13025.AFD30972.1.Cambodia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 56
isol-Z1106032.ALX35660.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 57
isol-Z1106033.ALX35659.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 58
isol-BeH828305.AMK49165.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDTQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFE
ATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGV
LIFLSTAVSA

SEQ ID NO: 59
isol-GD01.AMK79468.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNGTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 60
isol-Z1106031.ALX35661.1.Suriname/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVLAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 61
ACD75819.1.Micronesia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 62
KU681082.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 63
isol-Zika_virus%H.sapiens-tc%PHL%2012%CPC-0740.AMD61711.1.Philippines/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 64
isol-BeH823339.AMK49164.2.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVSTTVSNMAEVRSYCYEATISDIASDSRCPTQGEAYLD
KQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENR
AKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFK
DAHAKRQTAVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVE
VQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFE
ATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGV
LIFLSTAVSA

SEQ ID NO: 65
isol-P6-740.AEN75264.1.Malaysia/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWXRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 66
KU744693.1/326-829 Zika virus isolate VE_Ganxian, China, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMLVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETVDGTVTV
EGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSG

SEQ ID NO: 67
isol-VE_Ganxian.AMK79469.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMLVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETVDGTVTV
EGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSG

SEQ ID NO: 68
ArD157995.AHL43503.1.-/291-794 Flavivirus envelope glycoprotein E.
ISCIGVSNRDLVEGMSGGTWVDVVLEHGGCVTEMAQDKPTVDIELVTMTVSNMAEVRSYCYEASLSDMASASRCPTQGEPSL
DKQSDTQSVCKRTLGDRGWGNGCGIFGKGSLVTCSKFTCCKKMPGKSIQPENLEYRIMLPVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQSAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 69
MR_766.ABI54475.1.Uganda/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
TDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 70
5'-$(dIdC)_{13}$-3'
dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC

SEQ ID NO: 71
KLK peptide
KLKLLLLLKLK

SEQ ID NO: 72
ZIKV Sequence H/PF/2013 as sequenced

CAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTTGGATTTGGAAACGAGAGTT
TCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACGCGGAGTAGCCCGTGTG
AGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGTCATGGGCCCATCAGGATGGTCTTGGCGATTCT
AGCCTTTTTGAGATTCACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTGGGGAAAAAAGAGGCTA
TGGAAATAATAAAGAAGTTCAAGAAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGAAGGAGAAGAAGAGAC
GAGGCGCAGATACTAGTGTCGGAATTGTTGGCCTCCTGCTGACCACAGCTATGGCAGCGGAGGTCACTAGACGTGGGAG
TGCATACTATATGTACTTGGACAGAAACGACGCTGGGGAGGCCATATCTTTTCCAACCACATTGGGGATGAATAAGTGTT
ATATACAGATCATGGATCTTGGACACATGTGTGATGCCACCATGAGCTATGAATGCCCTATGCTGGATGAGGGGGTGGAA
CCAGATGACGTCGATTGTTGGTGCAACACGACGTCAACTTGGGTTGTGTACGGAACCTGCCATCACAAAAAAGGTGAAGC
ACGGAGATCTAGAAGAGCTGTGACGCTCCCCTCCCATTCCACTAGGAAGCTGCAAACGCGGTCGCAAACCTGGTTGGAAT
CAAGAGAATACACAAAGCACTTGATTAGAGTCGAAAATTGGATATTCAGGAACCCTGGCTTCGCGTTAGCAGCAGCTGCC
ATCGCTTGGCTTTTGGGAAGCTCAACGAGCCAAAAAGTCATATACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGC
ATCAGGTGCATAGGAGTCAGCAATAGGGACTTTGTGGAAGGTATGTCAGGTGGGACTTGGGTTGATGTTGTCTTGGAAC
ATGGAGGTTGTGTCACCGTAATGGCACAGGACAAACCGACTGTCGACATAGAGCTGGTTACAACAACAGTCAGCAACAT
GGCGGAGGTAAGATCCTACTGCTATGAGGCATCAATATCGGACATGGCTTCGGACAGCCGCTGCCCAACACAAGGTGAA
GCCTACCTTGACAAGCAATCAGACACTCAATATGTCTGCAAAAGAACGTTAGTGGACAGAGGCTGGGGAAATGGATGTG
GACTTTTTGGCAAAGGGAGCCTGGTGACATGCGCTAAGTTTGCATGCTCCAAGAAAATGACCGGGAAGAGCATCCAGCC
AGAGAATCTGGAGTACCGGATAATGCTGTCAGTTCATGGCTCCCAGCACAGTGGGATGATCGTTAATGACACAGGACATG
AAACTGATGAGAATAGAGCGAAGGTTGAGATAACGCCCAATTCACCAAGAGCCGAAGCCACCCTGGGGGGTTTTGGAAG
CCTAGGACTTGATTGTGAACCGAGGACAGGCCTTGACTTTTCAGATTTGTATTACTTGACTATGAATAACAAGCACTGGTT
GGTTCACAAGGAGTGGTTCCACGACATTCCATTACCTTGGCACGCTGGGGCAGACACCGGAACTCCACACTGGAACAACA
AAGAAGCACTGGTAGAGTTCAAGGACGCACATGCCAAAAGGCAAACTGTCGTGGTTCTAGGGAGTCAAGAAGGAGCAG
TTCACACGGCCCTTGCTGGAGCTCTGGAGGCTGAGATGGATGGTGCAAAGGGAAGGCTGTCCTCTGGCCACTTGAAATG
TCGCCTGAAAATGGATAAACTTAGATTGAAGGGCGTGTCATACTCCTTGTGTACCGCAGCGTTCACATTCACCAAGATCCC
GGCTGAAACACTGCACGGGACAGTCACAGTGGAGGTACAGTACGCAGGGACAGATGGACCTTGCAAGGTTCCAGCTCAG
ATGGCGGTGGACATGCAAACTCTGACCCCAGTTGGGAGGTTGATAACCGCTAACCCCGTAATCACTGAAAGCACTGAGAA
CTCTAAGATGATGCTGGAACTTGATCCACCATTTGGGGACTCTTACATTGTCATAGGAGTCGGGGAGAAGAAGATCACCC
ACCACTGGCACAGGAGTGGCAGCACCATTGGAAAAGCATTTGAAGCCACTGTGAGAGGTGCCAAGAGAATGGCAGTCTT
GGGAGACACAGCCTGGGACTTTGGATCAGTTGGAGGCGCTCTCAACTCATTGGGCAAGGGCATCCATCAAATTTTTGGAG
CAGCTTTCAAATCATTGTTTGGAGGAATGTCCTGGTTCTCACAAATTCTCATTGGAACGTTGCTGATGTGGTTGGGTCTGA
ACACAAAGAATGGATCTATTTCCCTTATGTGCTTGGCCTTAGGGGGAGTGTTGATCTTCTTATCCACAGCTGTCTCTGCTGA
TGTGGGGTGCTCGGTGGACTTCTCAAAGAAGGAGACGAGATGCGGTACAGGGGTGTTCGTCTATAACGACGTTGAAGCC
TGGAGGGACAGGTACAAGTACCATCCTGACTCCCCCCGTAGATTGGCAGCAGCAGTCAAGCAAGCCTGGGAAGATGGTA
TCTGTGGGATCTCCTCTGTTTCAAGAATGGAAAACATCATGTGGAGATCAGTAGAAGGGGAGCTCAACGCAATCCTGGAA
GAGAATGGAGTTCAACTGACGGTCGTTGTGGGATCTGTAAAAAACCCCATGTGGAGAGGTCCACAGAGATTGCCCGTGC
CTGTGAACGAGCTGCCCCACGGCTGGAAGGCTTGGGGGAAATCGTACTTCGTCAGAGCAGCAAAGACAAATAACAGCTT
TGTCGTGGATGGTGACACACTGAAGGAATGCCCACTCAAACATAGAGCATGGAACAGCTTTCTTGTGGAGGATCATGGGT
TCGGGGTATTTCACACTAGTGTCTGGCTCAAGGTTAGAGAAGATTATTCATTAGAGTGTGATCCAGCCGTTATTGGAACAG
CTGTTAAGGGAAAGGAGGCTGTACACAGTGATCTAGGCTACTGGATTGAGAGTGAGAAGAATGACACATGGAGGCTGA
AGAGGGCCCATCTGATCGAGATGAAAACATGTGAATGGCCAAAGTCCCACACATTGTGGACAGATGGAATAGAAGAGAG
TGATCTGATCATACCCAAGTCTTTAGCTGGGCCACTCAGCCATCACAATACCAGAGAGGGCTACAGGACCCAAATGAAAG
GGCCATGGCACAGTGAAGAGCTTGAAATTCGGTTTGAGGAATGCCCAGGCACTAAGGTCCACGTGGAGGAAACATGTGG
AACAAGAGGACCATCTCTGAGATCAACCACTGCAAGCGGAAGGGTGATCGAGGAATGGTGCTGCAGGGAGTGCACAAT
GCCCCCACTGTCGTTCCGGGCTAAAGATGGCTGTTGGTATGGAATGGAGATAAGGCCCAGGAAAGAACCAGAAAGTAAC
TTAGTAAGGTCAATGGTGACTGCAGGATCAACTGATCACATGGATCACTTCTCCCTTGGAGTGCTTGTGATTCTGCTCATG
GTGCAGGAAGGGCTGAAGAAGAGAATGACCACAAAGATCATCATAAGCACATCGATGGCAGTGCTGGTAGCTATGATCC
TGGGAGGATTTTCAATGAGTGACCTGGCTAAGCTTGCAATTTTGATGGGTGCCACCTTCGCGGAAATGAACACTGGAGGA
GATGTAGCTCATCTGGCGCTGATAGCGGCATTCAAAGTCAGACCAGCGTTGCTGGTATCTTTCATCTTCAGAGCTAATTGG
ACACCCCGTGAAAGCATGCTGCTGGCCTTGGCCTCGTGTCTTTTGCAAACTGCGATCTCCGCCTTGGAAGGCGACCTGATG
GTTCTCATCAATGGTTTTGCTTTGGCCTGGTTGGCAATACGAGCGATGGTTGTTCCACGCACTGATAACATCACCTTGGCA
ATCCTGGCTGCTCTGACACCACTGGCCCGGGGCACACTGCTTGTGGCGTGGAGAGCAGGCCTTGCTACTTGCGGGGGGTT
TATGCTCCTCTCTCTGAAGGGAAAAGGCAGTGTGAAGAAGAACTTACCATTTGTCATGGCCCTGGGACTAACCGCTGTGA

GGCTGGTCGACCCCATCAACGTGGTGGGACTGCTGTTGCTCACAAGGAGTGGGAAGCGGAGCTGGCCCCCTAGCGAAGT
ACTCACAGCTGTTGGCCTGATATGCGCATTGGCTGGAGGGTTCGCCAAGGCAGATATAGAGATGGCTGGGCCCATGGCC
GCGGTCGGTCTGCTAATTGTCAGTTACGTGGTCTCAGGAAAGAGTGTGGACATGTACATTGAAAGAGCAGGTGACATCAC
ATGGGAAAAAGATGCGGAAGTCACTGGAAACAGTCCCCGGCTCGATGTGGCGCTAGATGAGAGTGGTGATTTCTCCCTG
GTGGAGGATGACGGTCCCCCCATGAGAGAGATCATACTCAAGGTGGTCCTGATGACCATCTGTGGCATGAACCCAATAGC
CATACCCTTTGCAGCTGGAGCGTGGTACGTATACGTGAAGACTGGAAAAAGGAGTGGTGCTCTATGGGATGTGCCTGCTC
CCAAGGAAGTAAAAAAGGGGGAGACCACAGATGGAGTGTACAGAGTAATGACTCGTAGACTGCTAGGTTCAACACAAGT
TGGAGTGGGAGTTATGCAAGAGGGGGTCTTTCACACTATGTGGCACGTCACAAAAGGATCCGCGCTGAGAAGCGGTGAA
GGGAGACTTGATCCATACTGGGGAGATGTCAAGCAGGATCTGGTGTCATACTGTGGTCCATGGAAGCTAGATGCCGCCT
GGGACGGGCACAGCGAGGTGCAGCTCTTGGCCGTGCCCCCCGGAGAGAGAGCGAGGAACATCCAGACTCTGCCCGGAA
TATTTAAGACAAAGGATGGGGACATTGGAGCGGTTGCGCTGGATTACCCAGCAGGAACTTCAGGATCTCCAATCCTAGAC
AAGTGTGGGAGAGTGATAGGACTTTATGGCAATGGGGTCGTGATCAAAAATGGGAGTTATGTTAGTGCCATCACCCAAG
GGAGGAGGGAGGAAGAGACTCCTGTTGAGTGCTTCGAGCCTTCGATGCTGAAGAAGAAGCAGCTAACTGTCTTAGACTT
GCATCCTGGAGCTGGGAAAACCAGGAGAGTTCTTCCTGAAATAGTCCGTGAAGCCATAAAAACAAGACTCCGTACTGTGA
TCTTAGCTCCAACCAGGGTTGTCGCTGCTGAAATGGAGGAAGCCCTTAGAGGGCTTCCAGTGCGTTATATGACAACAGCA
GTCAATGTCACCCACTCTGGAACAGAAATCGTCGACTTAATGTGCCATGCCACCTTCACTTCACGTCTACTACAGCCAATCA
GAGTCCCCAACTATAATCTGTATATTATGGATGAGGCCCACTTCACAGATCCCTCAAGTATAGCAGCAAGAGGATACATTT
CAACAAGGGTTGAGATGGGCGAGGCGGCTGCCATCTTCATGACCGCCACGCCACCAGGAACCCGTGACGCATTTCCGGA
CTCCAACTCACCAATTATGGACACCGAAGTGGAAGTCCCAGAGAGAGCCTGGAGCTCAGGCTTTGATTGGGTGACGGATC
ATTCTGGAAAAACAGTTTGGTTTGTTCCAAGCGTGAGGAACGGCAATGAGATCGCAGCTTGTCTGACAAAGGCTGGAAA
ACGGGTCATACAGCTCAGCAGAAAGACTTTTGAGACAGAGTTCCAGAAAACAAAACATCAAGAGTGGGACTTTGTCGTG
ACAACTGACATTTCAGAGATGGGCGCCAACTTTAAAGCTGACCGTGTCATAGATTCCAGGAGATGCCTAAAGCCGGTCAT
ACTTGATGGCGAGAGAGTCATTCTGGCTGGACCCATGCCTGTCACACATGCCAGCGCTGCCCAGAGGAGGGGGCGCATA
GGCAGGAATCCCAACAAACCTGGAGATGAGTATCTGTATGGAGGTGGGTGCGCAGAGACTGACGAAGACCATGCACACT
GGCTTGAAGCAAGAATGCTCCTTGACAATATTTACCTCCAAGATGGCCTCATAGCCTCGCTCTATCGACCTGAGGCCGACA
AAGTAGCAGCCATTGAGGGAGAGTTCAAGCTTAGGACGGAGCAAAGGAAGACCTTTGTGGAACTCATGAAAAGAGGAG
ATCTTCCTGTTTGGCTGGCCTATCAGGTTGCATCTGCCGGAATAACCTACACAGATAGAAGATGGTGCTTTGATGGCACGA
CCAACAACACCATAATGGAAGACAGTGTGCCGGCAGAGGTGTGGACCAGACACGGAGAGAAAAGAGTGCTCAAACCGA
GGTGGATGGACGCCAGAGTTTGTTCAGATCATGCGGCCCTGAAGTCATTCAAGGAGTTTGCCGCTGGGAAAAGAGGAGC
GGCTTTTGGAGTGATGGAAGCCCTGGGAACACTGCCAGGACACATGACAGAGAGATTCCAGGAAGCCATTGACAACCTC
GCTGTGCTCATGCGGGCAGAGACTGGAAGCAGGCCTTACAAAGCCGCGGCGGCCCAATTGCCGGAGACCCTAGAGACCA
TTATGCTTTTGGGGTTGCTGGGAACAGTCTCGCTGGGAATCTTTTTCGTCTTGATGAGGAACAAGGGCATAGGGAAGATG
GGCTTTGGAATGGTGACTCTTGGGGCCAGCGCATGGCTCATGTGGCTCTCGGAAATTGAGCCAGCCAGAATTGCATGTGT
CCTCATTGTTGTGTTCCTATTGCTGGTGGTGCTCATACCTGAGCCAGAAAAGCAAAGATCTCCCCAGGACAACCAAATGGC
AATCATCATCATGGTAGCAGTAGGTCTTCTGGGCTTGATTACCGCCAATGAACTCGGATGGTTGGAGAGAACAAAGAGTG
ACCTAAGCCATCTAATGGGAAGGAGAGAGGAGGGGGCAACCATAGGATTCTCAATGGACATTGACCTGCGGCCAGCCTC
AGCTTGGGCCATCTATGCTGCCTTGACAACTTTCATTACCCCAGCCGTCCAACATGCAGTGACCACTTCATACAACAACTAC
TCCTTAATGGCGATGGCCACGCAAGCTGGAGTGTTGTTTGGTATGGGCAAAGGGATGCCATTCTACGCATGGGACTTTGG
AGTCCCGCTGCTAATGATAGGTTGCTACTCACAATTAACACCCCTGACCCTAATAGTGGCCATCATTTTGCTCGTGGCGCAC
TACATGTACTTGATCCCAGGGCTGCAGGCAGCAGCTGCGCGTGCTGCCCAGAAGAGAACGGCAGCTGGCATCATGAAGA
ACCCTGTTGTGGATGGAATAGTGGTGACTGACATTGACACAATGACAATTGACCCCCAAGTGGAGAAAAAGATGGGACA
GGTGCTACTCATAGCAGTAGCCGTCTCCAGCGCCATACTGTCGCGGACCGCCTGGGGGTGGGGGGAGGCTGGGGCCCTG
ATCACAGCGGCAACTTCCACTTTGTGGGAAGGCTCTCCGAACAAGTACTGGAACTCCTCTACAGCCACTTCACTGTGTAAC
ATTTTTAGGGGAAGTTACTTGGCTGGAGCTTCTCTAATCTACACAGTAACAAGAAACGCTGGCTTGGTCAAGAGACGTGG
GGGTGGAACAGGAGAGACCCTGGGAGAGAAATGGAAGGCCCGCTTGAACCAGATGTCGGCCCTGGAGTTCTACTCCTAC
AAAAAGTCAGGCATCACCGAGGTGTGCAGAGAAGAGGCCCGCCGCGCCCTCAAGGACGGTGTGGCAACGGGAGGCCAT
GCTGTGTCCCGAGGAAGTGCAAAGCTGAGATGGTTGGTGGAGCGGGGATACCTGCAGCCCTATGGAAAGGTCATTGATC
TTGGATGTGGCAGAGGGGGCTGGAGTTACTACGCCGCCACCATCCGCAAAGTTCAAGAAGTGAAAGGATACACAAAAGG
AGGCCCTGGTCATGAAGAACCCATGTTGGTGCAAAGCTATGGGTGGAACATAGTCCGTCTTAAGAGTGGGGTGGACGTC
TTTCATATGGCGGCTGAGCCGTGTGACACGTTGCTGTGTGACATAGGTGAGTCATCATCTAGTCCTGAAGTGGAAGAAGC
ACGGACGCTCAGAGTCCTCTCCATGGTGGGGGATTGGCTTGAAAAAAGACCAGGAGCCTTTTGTATAAAAGTGTTGTGCC
CATACACCAGCACTATGATGGAAACCCTGGAGCGACTGCAGCGTAGGTATGGGGGAGGACTGGTCAGAGTGCCACTCTC
CCGCAACTCTACACATGAGATGTACTGGGTCTCTGGAGCGAAAAGCAACACCATAAAAAGTGTGTCCACCACGAGCCAGC
TCCTCTTGGGGCGCATGGACGGGCCCAGGAGGCCAGTGAAATATGAGGAGGATGTGAATCTCGGCTCTGGCACGCGGG
CTGTGGTAAGCTGCGCTGAAGCTCCCAACATGAAGATCATTGGTAACCGCATTGAAAGGATCCGCAGTGAGCACGCGGA

AACGTGGTTCTTTGACGAGAACCACCCATATAGGACATGGGCTTACCATGGAAGCTATGAGGCCCCCACACAAGGGTCAG
CGTCCTCTCTAATAAACGGGGTTGTCAGGCTCCTGTCAAAACCCTGGGATGTGGTGACTGGAGTCACAGGAATAGCCATG
ACCGACACCACACCGTATGGTCAGCAAAGAGTTTTCAAGGAAAAAGTGGACACTAGGGTGCCAGACCCCCAAGAAGGCA
CTCGTCAGGTTATGAGCATGGTCTCTTCCTGGTTGTGGAAAGAGCTAGGCAAACACAAACGGCCACGAGTCTGTACCAAA
GAAGAGTTCATCAACAAGGTTCGTAGCAATGCAGCATTAGGGGCAATATTTGAAGAGGAAAAAGAGTGGAAGACTGCAG
TGGAAGCTGTGAACGATCCAAGGTTCTGGGCTCTAGTGGACAAGGAAAGAGAGCACCACCTGAGAGGAGAGTGCCAGA
GTTGTGTGTACAACATGATGGGAAAAAGAGAAAAGAAACAAGGGGAATTTGGAAAGGCCAAGGGCAGCCGCGCCATCT
GGTATATGTGGCTAGGGGCTAGATTTCTAGAGTTCGAAGCCCTTGGATTCTTGAACGAGGATCACTGGATGGGGAGAGA
GAACTCAGGAGGTGGTGTTGAAGGGCTGGGATTACAAAGACTCGGATATGTCCTAGAAGAGATGAGTCGCATACCAGGA
GGAAGGATGTATGCAGATGACACTGCTGGCTGGGACACCCGCATCAGCAGGTTTGATCTGGAGAATGAAGCTCTAATCA
CCAACCAAATGGAGAAAGGGCACAGGGCCTTGGCATTGGCCATAATCAAGTACACATACCAAAACAAAGTGGTAAAGGT
CCTTAGACCAGCTGAAAAAGGGAAGACAGTTATGGACATTATTTCGAGACAAGACCAAAGGGGGAGCGGACAAGTTGTC
ACTTACGCTCTTAACACATTTACCAACCTAGTGGTGCAACTCATTCGGAATATGGAGGCTGAGGAAGTTCTAGAGATGCAA
GACTTGTGGCTGCTGCGGAGGTCAGAGAAAGTGACCAACTGGTTGCAGAGCAACGGATGGGATAGGCTCAAACGAATG
GCAGTCAGTGGAGATGATTGCGTTGTGAAGCCAATTGATGATAGGTTTGCACATGCCCTCAGGTTCTTGAATGATATGGG
AAAAGTTAGGAAGGACACACAAGAGTGGAAACCCTCAACTGGATGGGACAACTGGGAAGAAGTTCCGTTTTGCTCCCAC
CACTTCAACAAGCTCCATCTCAAGGACGGGAGGTCCATTGTGGTTCCCTGCCGCCACCAAGATGAACTGATTGGCCGGGC
CCGCGTCTCTCCAGGGGCGGGATGGAGCATCCGGGAGACTGCTTGCCTAGCAAAATCATATGCGCAAATGTGGCAGCTC
CTTTATTTCCACAGAAGGGACCTCCGACTGATGGCCAATGCCATTTGTTCATCTGTGCCAGTTGACTGGGTTCCAACTGGG
AGAACTACCTGGTCAATCCATGGAAAGGGAGAATGGATGACCACTGAAGACATGCTTGTGGTGTGGAACAGAGTGTGGA
TTGAGGAGAACGACCACATGGAAGACAAGACCCCAGTTACGAAATGGACAGACATTCCCTATTTGGGAAAAAGGGAAGA
CTTGTGGTGTGGATCTCTCATAGGGCACAGACCGCGCACCACCTGGGCTGAGAACATTAAAAACACAGTCAACATGGTGC
GCAGGATCATAGGTGATGAAGAAAAGTACATGGACTACCTATCCACCCAAGTTCGCTACTTGGGTGAAGAAGGGTCTACA
CCTGGAGTGCTGTAAGCACCAATCTTAGTGTTGTCAGGCCTGCTAGTCAGCCACAGCTTGGGGAAAGCTGTGCAGCCTGT
GACCCCCCCAGGAGAAGCTGGGAAACCAAGCCTATAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCT
GTGAGCCCCTCAGAGGACACTGAGTCAAAAAACCCCACGCGCTTGGAGGCGCAGGATGGGAAAAGAAGGTGGCGACCT
TCCCCACCCTTCAATCTGGGGCCTGAACTGGAGATCAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAGACCC
CCCGGAAAACGCAAAACAGCATATTGACGCTGGGAAAGACCAGAGACTCCATGAGTTTCCACCACGCTGGCCGCCAGGC
ACAGATCGCCGAATAGCGGCGGCCGGTGTGGGG

SEQ ID NO: 73

AHZ13508.1, Zika virus polyprotein from Polynesian outbreak (H/PF/2013)

MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIKPSLGLINRWGSVGKKEAMEIIKKFKK
DLAAMLRIINARKEKKRRGADTSVGIVGLLLTTAMAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDA
TMSYECPMLDEGVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREYTKHLIRVENWI
FRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIEL
VTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTG
KSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKH
WLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLK
CRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKM
MLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLF
GGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSADVGCSVDFSKKETRCGTGVFVYNDVEAWRDRYKYHP
DSPRRLAAAVKQAWEDGICGISSVSRMENIMWRSVEGELNAILEENGVQLTVVVGSVKNPMWRGPQRLPVPVNELPHGWK
AWGKSYFVRAAKTNNSFVVDGDTLKECPLKHRAWNSFLVEDHGFGVFHTSVWLKVREDYSLECDPAVIGTAVKGKEAVHSDL
GYWIESEKNDTWRLKRAHLIEMKTCEWPKSHTLWTDGIEESDLIIPKSLAGPLSHHNTREGYRTQMKGPWHSEELEIRFEECPG
TKVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGCWYGMEIRPRKEPESNLVRSMVTAGSTDHMDHFSLG
VLVILLMVQEGLKKRMTTKIIISTSMAVLVAMILGGFSMSDLAKLAILMGATFAEMNTGGDVAHLALIAAFKVRPALLVSFIFRA
NWTPRESMLLALASCLLQTAISALEGDLMVLINGFALAWLAIRAMVVPRTDNITLAILAALTPLARGTLLVAWRAGLATCGGFM
LLSLKGKGSVKKNLPFVMALGLTAVRLVDPINVVGLLLLTRSGKRSWPPSEVLTAVGLICALAGGFAKADIEMAGPMAAVGLLIV
SYVVSGKSVDMYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEDDGPPMREIILKVVLMTICGMNPIAIPFAAGAWYVY
VKTGKRSGALWDVPAPKEVKKGETTDGVYRVMTRRLLGSTQVGVGVMQEGVFHTMWHVTKGSALRSGEGRLDPYWGDVK
QDLVSYCGPWKLDAAWDGHSEVQLLAVPPGERARNIQTLPGIFKTKDGDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGVVIK
NGSYVSAITQGRREEETPVECFEPSMLKKKQLTVLDLHPGAGKTRRVLPEIVREAIKTRLRTVILAPTRVVAAEMEEALRGLPVRY
MTTAVNVTHSGTEIVDLMCHATFTSRLLQPIRVPNYNLYIMDEAHFTDPSSIAARGYISTRVEMGEAAAIFMTATPPGTRDAFP
DSNSPIMDTEVEVPERAWSSGFDWVTDHSGKTVWFVPSVRNGNEIAACLTKAGKRVIQLSRKTFETEFQKTKHQEWDFVVTT

DISEMGANFKADRVIDSRRCLKPVILDGERVILAGPMPVTHASAAQRRGRIGRNPNKPGDEYLYGGGCAETDEDHAHWLEAR
MLLDNIYLQDGLIASLYRPEADKVAAIEGEFKLRTEQRKTFVELMKRGDLPVWLAYQVASAGITYTDRRWCFDGTTNNTIMEDS
VPAEVWTRHGEKRVLKPRWMDARVCSDHAALKSFKEFAAGKRGAAFGVMEALGTLPGHMTERFQEAIDNLAVLMRAETGS
RPYKAAAAQLPETLETIMLLGLLGTVSLGIFFVLMRNKGIGKMGFGMVTLGASAWLMWLSEIEPARIACVLIVVFLLLVVLIPEPE
KQRSPQDNQMAIIIMVAVGLLGLITANELGWLERTKSDLSHLMGRREEGATIGFSMDIDLRPASAWAIYAALTTFITPAVQHAV
TTSYNNYSLMAMATQAGVLFGMGKGMPFYAWDFGVPLLMIGCYSQLTPLTLIVAIILLVAHYMYLIPGLQAAAARAAQKRTAA
GIMKNPVVDGIVVTDIDTMTIDPQVEKKMGQVLLIAVAVSSAILSRTAWGWGEAGALITAATSTLWEGSPNKYWNSSTATSLC
NIFRGSYLAGASLIYTVTRNAGLVKRRGGGTGETLGEKWKARLNQMSALEFYSYKKSGITEVCREEARRALKDGVATGGHAVSR
GSAKLRWLVERGYLQPYGKVIDLGCGRGGWSYYAATIRKVQEVKGYTKGGPGHEEPMLVQSYGWNIVRLKSGVDVFHMAAE
PCDTLLCDIGESSSSPEVEEARTLRVLSMVGDWLEKRPGAFCIKVLCPYTSTMMETLERLQRRYGGGLVRVPLSRNSTHEMYWV
SGAKSNTIKSVSTTSQLLLGRMDGPRRPVKYEEDVNLGSGTRAVVSCAEAPNMKIIGNRIERIRSEHAETWFFDENHPYRTWAY
HGSYEAPTQGSASSLINGVVRLLSKPWDVVTGVTGIAMTDTTPYGQQRVFKEKVDTRVPDPQEGTRQVMSMVSSWLWKELG
KHKRPRVCTKEEFINKVRSNAALGAIFEEEKEWKTAVEAVNDPRFWALVDKEREHHLRGECQSCVYNMMGKREKKQGEFGKA
KGSRAIWYMWLGARFLEFEALGFLNEDHWMGRENSGGGVEGLGLQRLGYVLEEMSRIPGGRMYADDTAGWDTRISRFDLE
NEALITNQMEKGHRALALAIIKYTYQNKVVKVLRPAEKGKTVMDIISRQDQRGSGQVVTYALNTFTNLVVQLIRNMEAEEVLEM
QDLWLLRRSEKVTNWLQSNGWDRLKRMAVSGDDCVVKPIDDRFAHALRFLNDMGKVRKDTQEWKPSTGWDNWEEVPFC
SHHFNKLHLKDGRSIVVPCRHQDELIGRARVSPGAGWSIRETACLAKSYAQMWQLLYFHRRDLRLMANAICSSVPVDWVPTG
RTTWSIHGKGEWMTTEDMLVVWNRVWIEENDHMEDKTPVTKWTDIPYLGKREDLWCGSLIGHRPRTTWAENIKNTVNMV
RRIIGDEEKYMDYLSTQVRYLGEEGSTPGVL

SEQ ID NO: 74
9320_Zika_PF_1F
ttaggatccGTTGTTGATCTGTGTGAAT

SEQ ID NO: 75
9321_Zika_PF_1R
taactcgagCGTACACAACCCAAGTT

SEQ ID NO: 76
9322_Zika_PF_2F
ttaggatccTCACTAGACGTGGGAGTG

SEQ ID NO: 77
9323_Zika_PF_2R
taactcgagAAGCCATGTCYGATATTGAT

SEQ ID NO: 78
9324_Zika_PF_3F
ttaggatccGCATACAGCATCAGGTG

SEQ ID NO: 79
9325_Zika_PF_3R
taactcgagTGTGGAGTTCCGGTGTCT

SEQ ID NO: 80
9326_Zika_PF_4F
ttaggatccGAATAGAGCGAARGTTGAGATA

SEQ ID NO: 81
9327_Zika_PF_4R
taactcgAGTGGTGGGTGATCTTCTTCT

SEQ ID NO: 82
9328_Zika_PF_5F
ttaggatcCAGTCACAGTGGAGGTACAGTAC

SEQ ID NO: 83
9329_Zika_PF_5R
taactcgagCRCAGATACCATCTTCCC

SEQ ID NO: 84
9330_Zika_PF_6F
ttaggatCCCTTATGTGCTTGGCCTTAG

SEQ ID NO: 85
9331_Zika_PF_6R
taactcgagTCTTCAGCCTCCATGTG

SEQ ID NO: 86
9332_Zika_PF_7F
ttaggatccAATGCCCACTCAAACATAGA

SEQ ID NO: 87
9333_Zika_PF_7R
taactcgagTCATTCTCTTCTTCAGCCCTT

SEQ ID NO: 88
9334_Zika_PF_8F
ttaggatccAAGGGTGATCGAGGAAT

SEQ ID NO: 89
9335_Zika_PF_8R
taactcgagTTCCCTTCAGAGAGAGGAGC

SEQ ID NO: 90
9336_Zika_PF_9F
ttaggatccTCTTTTGCAAACTGCGATC

SEQ ID NO: 91
9337_Zika_PF_9R
taactcgagTCCAGCTGCAAAGGGTAT

SEQ ID NO: 92
9338_Zika_PF_10F
ttaggatccGTGTGGACATGTACATTGA

SEQ ID NO: 93
9339_Zika_PF_10R
taactcgagCCCATTGCCATAAAGTC

SEQ ID NO: 94
9340_Zika_PF_11F
ttaggatccTCATACTGTGGTCCATGGA

SEQ ID NO: 95
9341_Zika_PF_11R
taactcgagGCCCATCTCAACCCTTG

SEQ ID NO: 96
9342_Zika_PF_12F

ttaggatccTAGAGGGCTTCCAGTGC

SEQ ID NO: 97
9343_Zika_PF_12R
taactcgAGATACTCATCTCCAGGTTTGTTG

SEQ ID NO: 98
9344_Zika_PF_13F
ttaggatccGAAAACAAAACATCAAGAGTG

SEQ ID NO: 99
9345_Zika_PF_13R
taactcgagGAATCTCTCTGTCATGTGTCCT

SEQ ID NO: 100
9346_Zika_PF_14F
ttaggatccTTGATGGCACGACCAAC

SEQ ID NO: 101
9347_Zika_PF_14R
ttaggatccGTTGTTGATCTGTGTGAAT

SEQ ID NO: 102
9348_Zika_PF_15F
taactcgagCAGGTCAATGTCCATTG

SEQ ID NO: 103
9349_Zika_PF_15R
ttaggatccTGTTGTGTTCCTATTGCTGGT

SEQ ID NO: 104
9350_Zika_PF_16F
taactcgaGTGATCAGRGCCCCAGC

SEQ ID NO: 105
9351_Zika_PF_16R
ttaggatccTGCTGCCCAGAAGAGAA

SEQ ID NO: 106
9352_Zika_PF_17F
taactcgaGCACCAACAYGGGTTCTT

SEQ ID NO: 107
9353_Zika_PF_17R
ttaggatcCTCAAGGACGGTGTGGC

SEQ ID NO: 108
9354_Zika_PF_18F
taactcgagCAATGATCTTCATGTTGGG

SEQ ID NO: 109
9355_Zika_PF_18R
ttaggatccTATGGGGGAGGACTGGT

SEQ ID NO: 110

9356_Zika_PF_19F
taactcGAGCCCAGAACCTTGGATC

SEQ ID NO: 111
9357_Zika_PF_19R
ttaggatcCAGACCCCCAAGAAGGC

SEQ ID NO: 112
9358_Zika_PF_20F
taactcgagCCCCTTTGGTCTTGTCT

SEQ ID NO: 113
9359_Zika_PF_20R
ttaggatccAGGAAGGATGTATGCAGATG

SEQ ID NO: 114
9360_Zika_PF_21F
taactcgagACATTTGCGCATATGATTTTG

SEQ ID NO: 115
9361_Zika_PF_21R
ttaggatccAGGAAGGACACACAAGAGT

SEQ ID NO: 116
9362_Zika_PF_22F
taactcgagACAGGCTGCACAGCTTT

SEQ ID NO: 117
9363_Zika_PF_22R
ttaggatccTCTCTCATAGGGCACAGAC

In some embodiments, the Zika virus has polyprotein, including an envelope (E) protein, with an amino acid sequence provided by any one of SEQ ID NOs: 14-69 or 72. In some embodiments, the polyprotein or E protein sequence is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%. 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-69 or 72.

The terms "identical" or percent "identity" in the context of two or more nucleic acids or amino acid sequences refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*e.g.*, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) over a specified region or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more

amino acids) in length. In some embodiments, the identity exists over the length of a protein, such as the E protein.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. Methods of alignment of sequences for comparison are well known in the art. See, *e.g.,* by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, J. MoI. Biol. 48:443, 1970, by the search for similarity method of Pearson and Lipman. Proc. Natl. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, Jalview and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group. 575 Science Dr., Madison. WI), by multi sequence alignment implementation using e.g. CLUSTALW (Larkin et al., (2007). Bioinformatics, 23, 2947-2948.) or MAFFT (Katoh & Toh 2008 Briefings in Bioinformatics 9:286-298), or by manual alignment and visual inspection (see. *e.g.,* Brent et al., Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (Ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977 and Altschul et al., J. MoI. Biol. 215:403-410, 1990, respectively.

EXAMPLES

Example 1: Production of a Zika drug substance suitable for application as a vaccine in humans and animals

Materials and Methods:

For the production of ZikaV the JEV process platform (Srivastava et al., Vaccine 19 (2001) 4557-4565; US 6,309,650B1) was used as a basis. Small changes of certain process steps were adapted to ZikaV properties and to improve purity. A short summary of the process steps is outlined below (see also Figure 9A and B). Briefly, the unexpected and novel purification properties of protamine sulphate (PS) were evaluated in purification processes for Zika Virus. As shown in Figure 10, non-infectious virus particle aggregates, HCP and other LMW impurities were removed by PS precipitation as shown by removal of aggregate shoulder in SEC-HPLC and no loss of infectious virus titer by PS treatment. Further optimization of the Zika purification protocol is provided below.

Upstream:

- Roller Bottle based Vero cell expansion (25x850cm2 CellBind):
- 5% CO_2, 35°C, MEM+2mM L-Glutamine + 10% FBS
- Infection with ZikaV research Master Seed Bank (rMSB) at MOI 0.01
- Virus Production without serum
- 5% CO_2, 35°C, MEM+2mM L-Glutamine
- Multiple harvests (days 2, 3, 5 and 7) with re-feed
- Sterile filtration of harvests and storage at 2-8°C until further processing

Downstream:

- Pooling of harvests and concentration by ultrafiltration (100kDa)
- Stabilization of concentrated harvest (Tris/10% sucrose) for storage if required (-80°C)
- Removal of hcDNA by Protamine Sulphate (2 mg/mL)
- Sucrose Gradient Purification (optimized three layered gradient)
- Formaldehyde Inactivation (0.02%, 22°C, 10 days), neutralization with Na-metabisulfite
- Dilution to DS antigen target content and formulation with Aluminium hydroxide (0.5mg Al/mL)

Zika Virus Strain H/PF/2013 was originally isolated from a 51-year-old woman (accession number KJ776791.1, also SEQ ID NO: 13 herein) from French Polynesia. A sample was obtained from the European Virus Archive (EVAg; Ref-SKU: 001v-EVA1545). Based on this material, a research master seed bank (rMSB) was prepared on Vero cells as the cell substrate and the genomic sequence was checked by sequencing. Because the genomic sequence at the 5´and 3´flanking sequences of Zika virus strain H/PF/2013 was unknown, primers for sequencing were designed in those regions based on other Zika virus strains whereas the internal primers were designed from the published sequence (SEQ ID NOs: 74 to 117, see also Table A). The sequence obtained from the rMSB by use of these primers is provided by SEQ ID NO: 72. There was 100% overlap of the sequence with the published sequence of Zika Virus Strain H/PF/2013 (SEQ ID NO: 13). However, we sequenced additional regions 5´ (an additional 40 bp) and 3' (an additional 160 bp) represented in SEQ ID NO: 72. In a preferred embodiment, the Zika virus of the invention comprises SEQ ID NO: 72. The genomic RNA is somewhat longer than the sequence according to SEQ ID NO: 72 (perhaps an additional 200 bp). Additionally, a Zika virus adapted to a host cell such as e.g. Vero cells may be expected to contain one or more mutations. For these reasons, the Zika virus of the current invention comprises the sequence of SEQ ID NO: 72 or, preferably, a sequence with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 72. Furthermore, because the viral genome is likely to contain even further flanking regions to SEQ ID NO: 72; in one embodiment, the Zika virus of the invention contains the sequence of SEQ ID NO: 72 and optionally further comprises

extensions at the 5' and/or 3' ends of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120 or at least 130 nucleotides. In a preferred embodiment, the Zika virus comprises at least the coding sequence for the entire polyprotein of Zika Virus Strain H/PF/2013 of the invention i.e. the amino acid sequence of SEQ ID NO: 73 or a polyprotein with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 73. Furthermore, the Zika virus comprises at least the coding sequence for the E-protein of Zika Virus Strain H/PF/2013 of the invention SEQ ID NO: 47 or an E-protein thereof with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 47.

Virus growth on Vero Cells

Vero cells were grown in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS). Roller bottle cultures of Vero cell monolayers were infected with Zika Virus Strain H/PF/2013 at a multiplicity of infection (moi) of 0.01 plaque forming units (pfu) per cell. After 2 hours of virus adsorption, the cultures were washed 3 times with PBS and fed with EMEM without FBS and incubated at +35°C with 5% CO_2. Infected Vero cell cultures were incubated until the virus titer reaches a desired level.

The culture medium was harvested at days 2, 3, 5 and 7 and were pooled from those harvest days and then centrifuged in a standard centrifuge. The supernatants were then filtered. Virus culture supernatants were concentrated by TFF ultrafiltration to remove cell culture media components and to reduce batch volume.

Evaluation of harvest Procedure

The current JEV harvest process has scheduled harvests on days 3, 5, 7 and 9 post infection. To mimic the JEV process roller bottles were infected with ZIKV bank P4-FBS at an MOI of 0.01 in infection medium (MEM with 2% FBS + 2mM L-glutamine) for 2 hours. After removing the inoculum the cells were washed twice with PBS and 200 mL production medium (MEM+ 2mM L-glutamine) was added.

After taking a sample on day 2 the first virus harvest was conducted on day 3 after infection. At this point significantly higher CPE could be observed compared to cells where virus was removed on day 2. Plaque assay analysis showed that the viral titers on day 2 were in the same range as for the standard harvesting schedule. However, starting with the day 3 harvest, the observed titers were significantly lower correlating with the increased CPE observed compared to the standard harvest

schedule. On day 5 post infection no more living cells could be observed at all and the experiment was terminated with a final day 5 harvest.

Table 3: The calculated titers per plaque assay are summarized in the list below.

	Log 10 PFU/mL
sample day2	7.02
harvest day 3	6.66
harvest day 5	6.26

This finding led to an optimized harvest schedule to better control of CPE and allow additional harvest day 5 and 7, see Figure 15. For both harvest days the optimized ZikaV protocol yield significant higher virus titers compared to the modified protocol showing that the time of the first harvest is crucial for production yields. Additionally first harvesting at day 3 results in maximum 2 harvest points whereas first harvesting at day 2 allows for 4 harvest points further increasing the yield gain.

Downstream Purification of Zika virus

The purification process was carried out at room temperature (18-22°C) unless stated otherwise. Virus purification started with concentration of filtered combined harvest using 100 kDa cut-off TFF ultrafiltration modules to remove cell culture media components and reduce batch volume. After concentration, the pooled filtered harvest material was adjusted to a final concentration of 25 mM Tris pH 7.5 and 10% sucrose (w/w) using stock solution of both components (see Figure 11 for SEC-HPLC of different harvests prior to PS treatment). This allowed for freezing the concentrated harvest at <-65°C if required.

Host cell DNA and protein reduction as well reduction of non-infectious virus aggregates in the concentrated material was achieved by precipitation with protamine sulphate (2 mg/mL) followed by sucrose density centrifugation (2-8°C) as final polishing step (see Figure 20 for SEC-HPLC of different harvests post PS treatment). The purification process was designed to be completed within 2 working days with SGC starting on end of day 1 followed by fractionation and SDS-PAGE analysis on day 2. The sucrose gradient fractions were stored at 2-8 °C during the SDS-PAGE analysis (Silver staining) to identify the pure fractions containing ZikaV (see Figure 21). After pooling the relevant fractions, the pool was diluted and inactivated by Formalin. After pooling the relevant fractions of sucrose gradient centrifugation, the pool was diluted 1:3 in PBS and inactivated by Formalin (0.02% v/v, 200ppm). Fractions were subjected to analysis by SDS-PAGE.

Effect of PS treatment on virus recovery

Samples of individual 30x concentrated harvests days 2, 3, 5 and 7 were analysed before (Figure 11) and after PS (Figure 12) treatment by SEC-HPLC and plaque assay. SEC-HPLC was used for determination of relative total ZikaV content (active + inactive) expressed as peak area, whereas the rel. ZikaV peak purity is given as relative content of virus monomer population to total virus peak. Plaque assay states the content of total active virus particles in each sample. Experimental results are summarized in Table 4. The virus peak recovery by SEC-HPLC was only between 12 to 36% with peak purity after PS treatment in the range of >90% (no virus aggregates detected). The recovery of active virus particles by plaque assay was all > 100% (130-700%, range within the variability of the assay) showing that no active virus particles were lost during PS treatment. These results show that during PS treatment only non-infective (immature and/or aggregated virus) particles were removed.

Table 4: ZikaV recovery by SEC-HPLC and plaque assay before and after PS treatment.

SEC-HPLC

	Peak area mAU*min			
Harvest day	30x conc	30x + PS	SEC Recovery (%)	rel. virus monomer content after PS (%)
Day 2	101.36	18.63	18	89%
Day 3	144.51	17.48	12	90%
Day 5	19.97	5.92	30	96%
Day 7	68.80	24.43	36	99%

Plaque Assay

	PFU/mL		
Harvest day	30x conc	30x + PS	Plaque Recovery (%)
Day 2	3E+08	5E+08	179
Day 3	2E+08	4E+08	193
Day 5	1E+08	9E+08	700
Day 7	3E+08	4E+08	132

Sucrose gradient centrifugation

The PS treated harvest was split in two parts and loaded on two centrifuge bottles. Sucrose density gradient centrifugation (SGC) was used for final concentration and polishing of the ZikaV material. The ZikaV PS treated concentrated harvest was loaded on top of a solution consisting of three layers of sucrose with different densities. The three sucrose layers were selected based on a preliminary study which showed the formation of a linear sucrose gradient and complete separation of the virus

particles from residual contaminants as demonstrated for ChikV (Fig. 15D). The optimal volumes of the sucrose solutions were determined empirically. The volumes of individual layers for a centrifugation in 100 mL bottle scale are shown in Table 5.

Table 5: Individual layers/volumes for a centrifugation in bottle.

Solution	**Volume (mL)**
PS treated harvest in 10% sucrose (L)	40
15% sucrose (J)	15
35% sucrose (I)	15
50% sucrose (H)	20
Total volume	90

The sucrose gradient bottles were prepared by stratifying the individual sucrose layers. A plastic tube was attached to peristaltic pump tubing. The plastic tube was mounted on a laboratory stand using a clamp and placed into the centrifuge bottle. The nozzle of the plastic tube was touching the bottom of the bottle. Using a peristaltic pump the ZikaV material and the sucrose solutions were pumped into the cylinder. A measuring cylinder was used as feed vessel. The first solution pumped was the ZikaV material as it represented the solution of lowest density (10 % sucrose (w/w)). After the ZikaV material the sucrose solutions were pumped in ascending order starting with the 15 % (w/w) solution J, followed by 35 % sucrose solution I and finishing with the highest density sucrose solution H (50 % (w/w)). The described setup is shown in Figure 14. After all sucrose solutions were transferred the plastic tubing was carefully removed in order not to disturb the layers.

Prior to centrifugation the centrifuge was pre-cooled to 4°C. The prepared SG bottles were carefully transferred into the pre-cooled rotor. (Note: Sudden movement of the bottles during transfer to the rotor must be avoided in order not to disturb the sucrose layers.) The bottles were centrifuged at ~11.000 RCF max at 4 °C for at least 20 hours, no brake/deceleration activated. In case a different centrifuge system with a different rotor is used the necessary speed and centrifugation times need to be calculated based on the k-factor in order to achieve comparable centrifugation efficiency.

Harvesting of the sucrose gradient was done manually using a peristaltic pump. A plastic tube attached to peristaltic pump tubing was used for harvesting the sucrose gradient. The bottle containing the gradient was mounted onto a laboratory stand in a tilted position (~12°) using a clamp. The plastic tubing was then placed into the bottle touching the bottom edge of the bottle and was fastened in

position using a clamp. This resulted in a small gap of 1-2 mm between the tubing inlet and the bottom of the bottle (see Figure 14).

Using a peristaltic pump set to a flow rate of 30 mL per minute the gradient was harvested and manually split into 2 mL fractions. A total number of 32 fractions per bottle were harvested (~ 64 mL) and the remaining volume was discarded. The fractions were immediately tested by SDS-PAGE / silver stain to identify the virus containing fractions with sufficient high purity. Representative SDS-PAGE is shown in Figure 14. Fraction 10-14 were pooled and further processed.

The purified viral solution was inactivated by incubation with 0.02% formaldehyde over a period of ten days in a 22°C controlled-temperature incubator. The formaldehyde is neutralized by addition of sodium metabisulphite on the tenth day.

The sucrose gradient pool (~17mL after sampling) was further diluted 3-fold with PBS to a final volume of 51 mL in a PETG container. A volume of 1% formaldehyde (10,000 ppm) solution equivalent to 1/50 of the final volume of the pre-formaldehyde pool was added to this pool resulting in an effective concentration of 200 ppm. The formaldehyde-treated solution was mixed on a magnetic stirrer for 10 minutes. After sampling, the formaldehyde-treated viral solution was placed within a cooled incubator at 22°C ± 2°C. On Day 5 post addition of formaldehyde, the formaldehyde-treated viral solution was filtered through a 0.2 µm filter and then placed in the incubator at 22°C ± 2°C again. On Day 10, after removing the 10-Day inactivation final sample, a volume of 1 % (of the weight of the final formaldehyde-treated viral solution) of 200 mM-sodium metabisulphite solution (2mM final concentration) was aseptically transferred into the PETG container containing the formaldehyde-treated viral solution. After mixing for 5 minutes on a magnetic stirrer, the neutralized inactivated viral solution is held at room temperature (20 to 25°C) for a minimum of 30 minutes. After sampling, the neutralized inactivated viral solution is stored at 5°C ± 3°C until further processing.

Inactivation by formaldehyde

Critical parameters for this step are final formalin concentration, temperature, mixing and transfer into a new container. A preliminary acceptance criterion for maximum pfu/mL (determined by plaque assay) has been set on the diluted pool pre formaldehyde treatment.

The quality of the neutralized inactivated viral solution was monitored by the following parameters: Plaque assay on Day 10, SEC-HPLC, SDS-PAGE/Western Blot.

Interestingly, SEC-HPLC analysis of samples taken during the inactivation period followed by neutralization with bisulfite showed more or less constant peak area throughout the inactivation period. This is in contrast to JEV where losses of viral particles up to 60% are observed using the process disclosed by Srivastava et al. Vaccine 19 (2001) 4557-4565. In a scale-down model the viral losses were even much higher due to surface/area ratio at smaller scale and high losses due to unspecific adsorption. Differences of the ZikaV inactivation experiment and JEV inactivation were noticed as follows:

A) Much higher purity of ZikaV SGP pool with regard to residual PS (<2µg/mL) compared to JEV. The 3-fold ZikaV inactivated sample contained therefore <<1µg/mL of residual PS. Commercial JEV SGP pool contains on average ~120µg/mL (up to 152 µg/mL possible). The average dilution to inactivation solution of ~14-fold results in a residual PS content up to ~11 µg/mL. It may be that higher amount of residual PS could cause virus precipitation due to cross-linking / reaction with formalin.

B) ZikaV inactivation sample contained ~10% sucrose (3-fold dilution of SGP pool containing ~30-35% sucrose). Sucrose might have stabilizing effect of viral ZikaV particles during treatment with formalin.

Dilution to DS and Formulation with Aluminium hydroxide (DP)

For preparation of ZikaV drug substance used in mouse potency assay an antigen content (expressed as total viral particles or SEC peak area) of 5 times higher compared to Ixiaro was targeted. The basis for determination of antigen content was SEC-HPLC. Briefly, a Superose 6 10/300 Increase column (GE Healthcare) equilibrated with PBS + 250 mM NaCl, pH 7.4 at 1 ml/min and 25°C, was used to detect ZikaV at 214 nm detection wavelength in harvest samples and throughout the downstream process. In the current JEV process the antigen content in NIV is determined by a specific ELISA. A good correlation was observed between antigen content determined by ELISA and SEC-HPLC. On average, the antigen content in commercial NIV samples is in the range of 33 AU/mL corresponding to ~5.2mAU JEV peak area, see Figure 21.

ZikaV NIV day10 (Zika peak ~36 mAU, analysed on Waters HPLC/Superose6 Increase column) was diluted with PBS to a target of 6.3 (~5.7x dilution). Aluminium hydroxide was added to a final concentration of 0.5 mg/mL Aluminium (1/20 v/v Alum 2% stock solution added) to prepare ZikaV Drug Product (DP). The DP was gently mixed for 5 min. An aliquot of the DP was removed, Alum sedimented by centrifugation and the clear supernatant analysed by SEC-HPLC. No ZikaV peak was detected in the supernatant indicating complete adsorption (estimated as >95%) of viral particles on the mineral adjuvant. Formulated ZikaV DP was stored at 2-8°C.

The impurity profile of the inactivated Zika virus DS is comparable to the profile of JEV DS with the exception of a lower PS content (Table 6).

Table 6: Determination of impurity profile in Zika and JEV DS samples:

	Specification (JEV DS)	**JEV**	**Zika**
HCP (ng/mL)	<100 LOQ 12 ng/mL	<LOQ	<LOQ
DNA (pg/mL)	<200 LOQ 40 pg/mL	<40	<40
Aggregates by SEC-MALLS (%)	Not specified, part of characterization LOQ 5%	<LOQ	<LOQ
PS (µg/mL)	Specification only at SGP pool to demonstrate consistent process performance (19-152µg/mL), *PS content in DS calculated based on PS content in SGP pool (~100 µg/mL) and average dilution factor (~28x) to DS; LOQ 2 µg/mL	~4*	<<LOQ

*Typical PS impurity in a JEV sample produced in accordance with protocol disclosed in Srivastava et al. Vaccine 19 (2001) 4557-4565.

SEC-MALLS Results

A representative SEC-HPLC elution profile of ZikaV NIV at 214 nm detection wave length is shown in Figure 16. Note that BSA (50µg/mL) was added to the sample to minimize losses in HPLC glass vial due to unspecific surface adsorption. ZikaV monomer content was estimated as ~98% with a multimer content of ~2%.

SEC-MALLS analysis (Figure 17) of the sample confirmed the radius Rz of the monomer ZikaV population peak 1 as 21.6 nm and ~49 nm for the multimer peak 2. Cumulative particle size distribution showed that 89% of all viral particles are within a radius range between 18 to 25nm (Figure 18).

Results confirm purity and homogeneity of ZikaV NIV.

Viral titer by plaque assay

Table 7: Active ZikaV pfus were quantified by plaque assay throughout the process.

Sample	**Pfu/mL**
Harvest day 2 (filtered)	$6.4x10^7$
Harvest day 3 (filtered)	$1.0x10^8$
Harvest day 5 (filtered)	$1.5x10^8$

Harvest day 7 (filtered)	$1.1x10^8$
PS treated harvest 300x concentrate (=SGP load)	$9.0x10^8$
SGP pool	$8.9x10^8$
Inactivation start (SGP pool 1:3 diluted)	$3.4x10^8$
Inactivation day 5	<LOD
Inactivation day 10	<LOD

Comparison of PS and Benzonase on process performance

A direct comparison of DNA removal method of concentrated ZikaV harvest pool was done. One aliquot was treated with PS (2 mg/mL, 15min at room temperature), the other aliquot was treated with Benzonase (50 U/mL, 2 mM MgCl2, 4h RT, 48h 2-8°C). Both samples were further purified by sucrose gradient as described in this report. Interestingly, the Benzonase treated samples did not yield any pure fractions after sucrose gradient centrifugation of the treated ZikaV harvest. In those fractions where the specific virus bands were detected, a high amount of host cell protein was detected throughout the collected fractions. The PS treated material resulted in pure ZikaV containing fractions as expected. This finding may suggest that PS is not only effective for DNA removal by precipitation; in addition it improves the recovery of virus particles in the gradient by disrupting interaction of DNA (fragments) and virus particles. Benzonase treatment does not remove DNA, it only results in its fragmentation. Residual DNA fragments might still interact with virus particles and residual HCPs resulting in cross-contamination and co-purification in the sucrose gradient. Pooled SGP fractions were also analysed by SEC-HPLC. Although a large peak was detected, SDS-PAGE confirmed that this sample was highly contaminated with HCPs. A large peak might be detected at UV214 and 280nm after SEC-HPLC analysis due to possible interaction of HCPs with large virus particles, changing the UV absorbance.

Immunogenicity of Vero grown Zika virus

Immunization of mice

Prior to immunization, groups of ten 6-week-old female CD1 mice were bled via vena facialis and pre-immune sera were prepared. One intraperitoneal immunizations of 200 µL were administered. A dose titration (12 µg, 3 µg, 1 µg, 0.33 µg, 0.11 µg, 0.037 µg and 0.012 µg, equivalent to the protein amount in IXIARO) of inactivated Zika virus formulated with aluminium hydroxide (Al(OH)3) at a final concentration of 0.7%. Three weeks after immunization, blood was collected and immune sera were prepared. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by "Magistratsabteilung 58".

Plaque reduction neutralization test (PRNT)

Twelve well plates were used for PRNT. Each well was seeded with 1 mL medium containing $4x10^5$ Vero cells and incubated 35°C with 5% CO_2 overnight. Pools of heat inactivated sera from each dose group were tested in triplicate. The target viruses (H/PF/2013 (SEQ ID NO: 13) or MR766 (SEQ ID NO: 11)) were diluted to 100 pfu/165 µL. Equal volumes of target virus and serum dilution were incubated at 35°C with 5% CO_2 for 1 hour. The cell culture medium was aspirated from the Vero cells and 330 µL of the mixture target virus/serum dilution were added to each well and the plates were rocked back and forth 5 times before incubating for 2 hours at 35°C with 5% CO_2. To each well 1 mL of a 2% methylcellulose solution containing EMEM and nutrients was added, the plates were then incubated for 5 days at 35°C with 5% CO_2 before staining the cells for 1 hour with crystal violet/5% formaldehyde and subsequently washed 3 times with deionized water. The plates were air dried and the numbers of plaques in each well were manually counted.

Results

Neutralization was observed with serum pools from mice immunized with inactivated Zika virus vaccine (H/PF/2013) down to 37 ng (dosing equivalent to the amount protein in IXIARO®) against Zika viruses of both the Asian (H/PF/2013) and African (MR766) lineages (Figures 19 and 20, respectively). Complete inhibition was seen at the 1:20 serum dilution with an immunization dose down to 110 ng (dosing equivalent to the amount protein in IXIARO®). The neutralization of both the Asian (H/PF/2013) and African (MR766) lineages of the Zika virus was equivalent, which indicates high cross-neutralization between different Zika virus strains of the inactivated Zika virus vaccine (H/PF/2013).

Another neutralization assay was performed using the microneutralization assay as described by Larocca, et al. (2016, Nature doi:10.1038/nature18952). It was found that the inactivated Zika virus of the current invention had an MN50 (microneutralization) titer of 90 at 1 µg of inactivated purified virus.

Further methods: The immunogenicity of inactivated Zika virus preparations is assessed using a mouse model of Zika infection. Groups of adult mice are immunized subcutaneously (s.c.) with 500, 50, or 5 ng of inactivated Zika virus with adjuvant (*e.g.* aluminium hydroxide with or without IC31®), or without adjuvant. An additional group of mice receive PBS as a negative control. Each group is administered the indicated inoculum at t=0 and in some cases also at three to four weeks later (t=3/4). Beginning approximately three weeks after administration of the last immunization, serum samples are obtained from each of the mice at regular intervals. The serum samples are tested for the presence of neutralizing antibodies using PRNT.

The *in vivo* protective efficacy of the inactivated Zika virus preparations is also assessed using a mouse model of Zika infection, *i.e.* IFN-alpha/beta receptor knock-out mice (A129) (see *e.g.* Dowall et al., 4. March 2016, dx.doi.org/10.1101/042358) or blocking of the IFN-alpha/beta receptor by administration of anti-IFN-alpha/beta receptor monoclonal antibodies to C57BL/6 or BALB/c mice (see e.g. Pinto et al., 7. December 2011, DOI: 10.1371/journal.ppat.1002407). For protection assays, groups of 10 three- to eight-weeks-old A129, C57BL/6 of BALB/c mice are inoculated subcutaneously in the hindquarters with inactivated Zika virus with adjuvant (aluminium hydroxide) or without adjuvant at t=0. Age-matched controls are inoculated with PBS or non-specific antigens in alum. Mice are optionally boosted with a second administration of the indicated inoculation three to four weeks later. The mice are then challenged subcutaneously at three to eight weeks post immunization by inoculation with a deadly dose of live Zika virus. One day prior to challenge of C57BL/6 and BALB/c mice, they are passively administered (intraperitoneally) anti-IFN-alpha/beta receptor monoclonal antibodies. Challenged mice are monitored daily for morbidity and mortality for up to twenty-one days. Another alternative is to challenge intracranially adult vaccinated/non-vaccinated adult mice and observe protection.

It is expected that the Zika virus produced by the process of the invention will provide very similar functional read-outs in *in vitro*, *in vivo* and finally human trials as the currently licensed JEV vaccine in the EU and US and elsewhere, IXIARO®. The dosage may alter but due to the very similar impurity profile and almost identical manufacture, a very similar efficacy and safety result will be expected as was determined for the currently licensed JEV vaccine (licensed in the EU and US and elsewhere).

Discussion & Conclusion

The existing manufacturing platform for production of inactivated JEV vaccine IXIARO® was used as a basis for a manufacturing feasibility study of inactivated ZikaV vaccine candidate (Asian strain H/PF/2013). The virus was produced on Vero cells cultivated in roller bottles. The virus was purified by PS treatment followed by an optimized sucrose gradient. Inactivation was done by formalin treat (0.02 %, 10 days at 22 °C). For exploratory immunization studies in mice, a DP formulated with Alum was prepared with an estimated 5-fold higher virus particle content compared to IXIARO®, the commercial JEV Vaccine. The impurity profile of the DS met all criteria as defined in the specification for IXIARO®, the commercial JEV vaccine. The neutralization of both the Asian (H/PF/2013) and African (MR766) lineages of the Zika virus was equivalent, which indicates high

cross-neutralization between different Zika virus strains of the inactivated Zika virus vaccine (H/PF/2013).

The in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, supra.). Inactivated viruses are among the safest vaccines and especially preferred for deliver to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Further more detailed aspects of the invention:

A1. A Zika virus vaccine comprising an inactivated Zika virus particle, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability.

A2. The Zika virus vaccine of A1, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability.

A3. The vaccine of A1 or A2, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13 or 72, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 or 72 and able to pack a virulent Zika virus.

A4. The vaccine of any one of A1-A3, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.

A5. The vaccine of any one of A1-A4, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

A6. The vaccine of A5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent to completely inactivate the Zika virus as measured by plaque assay.

A7. The vaccine of A6, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

A8. The vaccine of A7, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

A9. The vaccine of any one of A5-A8, wherein the chemical activation is performed at about +4°C or about +22°C.

A10. The vaccine of any one of A1-A9, further comprising an adjuvant.

A11. The vaccine of A10, wherein the adjuvant is an aluminum salt adjuvant.

A12. The vaccine of A11, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

A13. The vaccine of any one of A10-A12, wherein the vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).

A14. The vaccine of A13, wherein the peptide comprises the sequence KLKL5KLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 70).

A15. The vaccine of any one of A1-A14, further comprising one or more pharmaceutically acceptable excipient.

A16. The vaccine of any one of A1-A15, wherein the vaccine contains protamine sulphate or fragments or break-down products of PS at amounts too low to detect by HPLC, i.e., below 1 µg/mL, especially below 100 ng/mL.

A17. The vaccine of A16, wherein said protamine sulphate or fragments or break-down products of PS can be detected by mass spectroscopy or another sensitive method.

B1. A kit comprising a Zika virus vaccine of any one of A1-A15.

B2. The kit of B1, further comprising a second vaccine.

B3. The kit of B2, wherein the second vaccine is a West Nile virus vaccine, a Japanese Encephalitis virus vaccine, a Yellow Fever virus vaccine, a Dengue virus vaccine or a Chikungunya virus vaccine.

C1. A method, comprising administering a first dose of a therapeutically effective amount of the Zika virus vaccine of any one of A1-A15 to a subject in need thereof.

C2. The method of C1, further comprising administering a second dose of a therapeutically effective amount of the Zika virus vaccine.

C3. The method of C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

C4. The method of C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

C5. The method of C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

C6. The method of any one of C1-C5, wherein the administering results in production of Zika virus neutralizing antibodies.

D1. A method of producing a Zika virus vaccine, comprising

(i) passaging a Zika virus on Vero cells, thereby producing a culture supernatant comprising the Zika virus;

(ii) harvesting the culture medium of (i);

(iii) precipitating the harvested culture medium of (ii), thereby producing a Zika virus supernatant; and

(iv) optimally inactivating the Zika virus in the Zika virus supernatant of (iii) thereby producing an inactivated Zika virus.

D2. The method of D1, further comprising concentrating the culture medium of (ii) prior to step (iii).

D3. The method of D1 or D2, wherein the precipitating of (iii) comprises contacting the culture medium of (ii) with protamine sulfate or benzoate.

D4. The method of any one of D1-D3, further comprising (v) dialyzing the inactivated Zika virus of (iv), thereby producing a dialyzed Zika virus.

D5. The method of D4, further comprising (vi) filtering the dialyzed Zika virus of (v).

D6. The method of any one of D1-D5, wherein the inactivating is by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

D7. The method of D6, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for at least 4 days.

D8. The method of D6 or D7, wherein the chemical inactivation agent comprises formaldehyde.

D9. The method of any one of D6-D8, wherein the chemical activation is performed at about +4°C or about +22°C.

D10. The method of D8 or D9, further comprising neutralizing the formaldehyde.

D11. The method of D10, wherein the neutralizing is performed with sodium metabisulfite.

E1. The use of the optimally inactivated Zika virus vaccine of any one of A1-A15 for the treatment and/or prevention of a Zika virus infection.

E2. The use of E1, wherein the inactivated Zika virus vaccine is administered in a first dose of a therapeutically effective amount to a subject in need thereof.

E3. The use of E2, wherein the inactivated Zika virus vaccine is administered in a second dose of a therapeutically effective amount to the subject.

E4. The use of E3, wherein the second dose of the inactivated Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

E5. The use of E3, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

E6. The use of E3, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

E7. The use of any one of E1-E6, wherein the vaccine administration results in production of Zika virus neutralizing antibodies.

F1. A pharmaceutical composition for use in the treatment or prevention of a Zika virus infection, wherein said pharmaceutical composition comprises the optimally inactivated Zika virus vaccine of any one of A1-A15.

F2. The pharmaceutical composition of F1, wherein the inactivated Zika virus vaccine is administered in a first dose of a therapeutically effective amount to a subject in need thereof.

F3. The use of F2, wherein the inactivated Zika virus vaccine is administered in a second dose of a therapeutically effective amount to the subject.

F4. The use of F3, wherein the second dose of the inactivated Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

F5. The use of F3, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

F6. The use of F3, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

F7. The use of any one of F1-F6, wherein the vaccine administration results in production of Zika virus neutralizing antibodies.

G1. Use of an optimized sucrose gradient centrifugation for removal of protamine sulphate from purified infectious Zika virus particles.

G2. The use according to G1, wherein said optimized sucrose gradient centrifugation comprises a virus comprising fraction in a 10% +/- 1% (w/w) sucrose solution and three further layers of sucrose solutions with different densities, i.e. a first sucrose solution with 15% +/- 1% (w/w) sucrose, a second sucrose solution with 35% +/- 1% (w/w) sucrose, and a third sucrose solution with a 50% +/- 1% (w/w) sucrose.

G3. A process of purification of infectious Zika virus particles, comprising the steps of:

a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);

c) further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation, wherein the optimized sucrose gradient is provided such that the protamine can be completely or almost completely separated from the virus fraction; and wherein the protamine concentration is reduced by this step to the extent that the protamine concentration in the final drug substance is below 1 µg/ml, preferably below 0.5 µg/mL, more preferably below 0.1 µg/mL, most preferably below 0.05µg/mL.

G4. The process of G3, wherein said optimized sucrose density gradient centrifugation comprises a virus comprising fraction in a 10% +/- 1% (w/w) sucrose solution and three layers of sucrose with different densities, i.e. a first sucrose solution with 15% +/- 1% (w/w) sucrose, a second sucrose solution with 35% +/- 1% (w/w) sucrose, and a third sucrose solution with a 50% +/- 1% (w/w) sucrose.

G5. The process of any one of G3 to G4, additionally comprising a further purification step of: (d) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles.

G6. The process of any of G3 to G5, wherein the residual host cell DNA content of the Zika virus preparation (c) is less than 10 ng/mL and the residual host cell protein content of the final virus preparation (c) is less than 100 ng/mL.

G7. The process of any of G3 to G6, wherein said crude harvest (a) comprising Zika virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

G8. The process of G7, wherein the one or more pre-purification step(s) comprises

a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or
b) digestion of host cell genomic DNA by enzymatic treatment; and/or
c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

G9. The process of any one of G3 to G8, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml or 2 mg/ml.

G10. The process of any one of G3 to G9, wherein the enrichment of infectious Zika virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

G11. The process of any one of G7 to G10, wherein the one or more pre-purification step(s) prior to step (b) of any of G8 to G11 is performed using a filter having a pore size equal to or less than 1 µm, preferably 0.2 µm.

G12. The process of any one of G3 to G11, wherein the residual impurity of the Zika virus preparation (c) is less than 10%.

G13. The process of any one of G3 to G12, wherein the Zika virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

G14. The process of G13, wherein said cell line is a Vero cell line.

G15. The process of any one of G3 to G14, wherein said infectious Zika virus particle is an infectious virus particle that is a live virus, a live attenuated virus, a chimeric virus, a modified live virus, or a recombinant live virus.

G16. The process of any one of G3 to G15, wherein said Zika virus is preferably a strain of the Asian lineage.

G17. The process of any one of G3 to G16, wherein said process resulting in final virus preparation (c) or (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

G18. Use of the process according to any one of G3 to G17 for manufacturing a composition for immunization against a virus infection.

G19. The use according to G18, wherein said virus infection is an infection caused by a Zika virus.

Q1. A process of purification of infectious Zika virus particles, comprising the steps of:

(a) providing a crude harvest (a) comprising Zika virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

(b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);

(c) further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation, wherein the optimized sucrose gradient is provided such that the protamine can be completely or almost completely separated from the virus fraction; and wherein the protamine concentration is reduced by this step to the extent that the protamine concentration in the final drug

substance is below 1 µg/ml, preferably below 0.5 µg/mL, more preferably below 0.1 µg/mL, most preferably below 0.05µg/mL.

Q2. The process of Q2, wherein the virus particles are from Zika virus.

Q3. The process of Q1 or Q2, additionally comprising the step of:
(d) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles.

Q4. The process of any of Q1 to 3, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.

Q5. The process of any of Q1 to 4, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

Q6. The process of Q5, wherein the one or more pre-purification step(s) comprises
(a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or
(b) digestion of host cell genomic DNA by enzymatic treatment; and/or
(c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

Q7. The process of any one of Q1 to 6, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml or 2 mg/ml.

Q8. The process of any one of Q1 to 7, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

Q9. The process of any one of Q5 to 8, wherein the one or more pre-purification step(s) prior to step (b) of any of Q5 to 8 is performed using a filter having a pore size equal to or less than 1 µm, preferably 0.2 µm.

Q10. The process of any one of Q1 to 9, wherein the residual impurity of the virus preparation (c) is less than 10%.

Q11. The process of any one of Q1 to 10, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

Q12. The process of Q11, wherein said cell line is a Vero cell line.

Q13. The process of any one of Q1 to 12, wherein the infectious virus particles is an infectious Zika virus particle that is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

Q14. The process of any one of Q1 to 13, wherein the Zika virus is a Zika virus strain of the Asian lineage or an immunogenic variant thereof.

Q15. The process of any one of Q1 to 14, wherein said process resulting in final virus preparation (c) or (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

Q16. Use of the process according to any one of Q1 to 15 for manufacturing a composition for immunization against a virus infection.

Q17. The use according to Q16, wherein the composition for immunization against a virus infection is an infection caused by Zika virus.

Q18. A composition comprising the virus particles obtainable or obtained by the process of any one of Q1 to 17 for treating and/or preventing an infection, such as e.g. a Zika virus infection.

Q19. A Zika virus vaccine comprising an inactivated Zika virus particle grown on vero cells, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus

vaccine with at least a 70% probability and comprises minor amounts of protamine sulphate, preferably below the detection limit.

Q20. The Zika virus vaccine of Q19, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability, preferably a 80% probability.

Q21. The vaccine of Q19 or 20, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 and able to pack a virulent Zika virus.

Q22. The vaccine of any one of Q19, 20 and 21, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69 or 72, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 or 72 and able to pack a virulent Zika virus.

Q23. The vaccine of any one of Q19, 20 to 22, wherein the Zika virus obtained by culturing on Vero cells is purified by protamine sulfate precipitation and sucrose gradient centrifugation.

Q24. The vaccine of Q23, wherein the sucrose gradient centrifugation is an optimized sucrose gradient centrifugation.

Q25. The vaccine of Q24, wherein the optimized sucrose gradient centrifugation comprises a virus comprising fraction in a 10% (w/w) sucrose solution and three layers of sucrose with different densities, i.e. a first sucrose solution with 15% (w/w) sucrose solution, a second sucrose solution with 35% (w/w) sucrose solution, and a third sucrose solution with a 50% (w/w) sucrose solution.

Q26. The vaccine of any one of Q19, 20 to 25, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

Q27. The vaccine of Q26, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.

Q28. The vaccine of Q27, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

Q29. The vaccine of Q28, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

Q30. The vaccine of any one of Q27-29, wherein the chemical activation is performed at about +4°C or about +22°C.

Q31. The vaccine of any one of Q19 to 30, further comprising an adjuvant.

Q32. The vaccine of Q31, wherein the adjuvant is an aluminum salt adjuvant.

Q33. The vaccine of Q32, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

Q34. The vaccine of Q32, wherein the aluminum salt adjuvant is aluminium hydroxide with less than 1.25ppb Cu based on the final pharmaceutical composition comprising the Zika virus, preferably the inactivated Zika virus.

Q35. The vaccine of any one of Q19 to 34, further comprising one or more pharmaceutically acceptable excipient.

R1. Use of protamine, preferably a protamine salt, to separate infectious and non-infectious Zika virus particles, host cell proteins and/or undefined low molecular weight materials.

R2. A process of purification of infectious Zika virus particles, comprising the steps of:

(a) providing a crude harvest (a) comprising Zika virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

(b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b), wherein the enrichment of infectious virus particles in the virus preparation (b) relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

R3. The use of R1 or the process of R2, wherein the virus particles are from Zika virus.

R4. A process of purification of infectious Zika virus particles, comprising the steps of:

(a) providing a crude harvest (a) comprising Zika virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

(b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);

(c) further purifying the virus preparation (b) by one or more size exclusion methods such as (i) a sucrose density gradient centrifugation, (ii) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles, and/or (iii) size exclusion chromatography to obtain a virus preparation (c) comprising the infectious virus particles, wherein the residual host cell DNA of the virus preparation (c) is less than 100 ng/mL and the residual host cell protein and the residual aggregates of infectious virus particles of the final virus preparation (c) is less than 1 μg/mL.

R5. The process of R4, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.

R6. The process of any of R2 to 5, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

R7. The process of R6, wherein the one or more pre-purification step(s) comprises

(a) filtration using a filter having a pore size equal to or less than 0.2 μm; and/or

(b) digestion of host cell genomic DNA by enzymatic treatment; and/or

(c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

R8. The process of any one of R2 to 7, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml.

R9. The process of any one of R2 to 8, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

R10. The process of any one of R6 to 9, wherein the one or more pre-purification step(s) prior to step (b) of any of R6 to 9 is performed using a filter having a pore size equal to or less than 1 μm, preferably 0.2 μm.

R11. The process of any one of R2 to 10, wherein the residual impurity of the virus preparation (c) is less than 10%.

R12. The process of any one of R2 to 11, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

R13. The process of R12, wherein said cell line is a Vero cell line.

R14. The process of any one of R2 to 13, wherein the Zika virus is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

R15. The process of any one of R2 to 14, wherein the Zika virus is a Zika virus strain of the Asian lineage or an immunogenic variant thereof.

R16. The process of any one of R2 to 15, wherein said process resulting in final virus preparation (c) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

R17. Use of the process according to any one of R1 to 16 for manufacturing a composition for immunization against a virus infection.

R18. The use according to R17, wherein the composition for immunization against a virus infection is an infection caused by a Zika virus.

R19. A composition comprising the virus particles obtainable or obtained by the process of any one of R2 to 16 for treating and/or preventing an infection.

CLAIMS

What is claimed is:

1. A Zika virus vaccine comprising an inactivated Zika virus particle, wherein said Zika virus vaccine is able to confer seroprotection on at least 70% of subjects that are administered the Zika virus vaccine.

2. The Zika virus vaccine of claim 1, wherein the Zika virus particle is able to confer seroprotection on at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects that are administered the Zika virus vaccine, preferably on at least 80% of subjects.

3. The vaccine of claim 1 or 2, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13 or 72, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 or 72 and able to pack a virulent Zika virus.

4. The vaccine of any one of claims 1 to 3, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.

5. The vaccine of any one of claims 1 to 4, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

6. The vaccine of claim 5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.

7. The vaccine of claim 6, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

8. The vaccine of claim 7, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

9. The vaccine of any one of claims 5 to 8, wherein the chemical activation is performed at about +4°C or about +22°C.

10. The vaccine of any one of claims 1 to 9, further comprising an adjuvant.

11. The vaccine of claim 10, wherein the adjuvant is an aluminum salt adjuvant.

12. The vaccine of claim 11, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

13. The vaccine of any one of claims 10 to 12, wherein the vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).

14. The vaccine of claim 13, wherein the peptide comprises the sequence KLKL5KLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 70).

15. The vaccine of any one of claims 1 to 14, further comprising one or more pharmaceutically acceptable excipients.

16. The vaccine of any one of claims 1 to 15, wherein the vaccine contains protamine sulphate or fragments or break-down products of PS at amounts too low to detect by HPLC, i.e., below 1 µg/mL, especially below 100 ng/mL.

17. The vaccine of claim 16, wherein said protamine sulphate or fragments or break-down products of PS can be detected by mass spectroscopy or another sensitive method.

ABSTRACT

Described herein are Zika virus vaccines and compositions and methods of producing and administering said vaccines to subjects in need thereof.

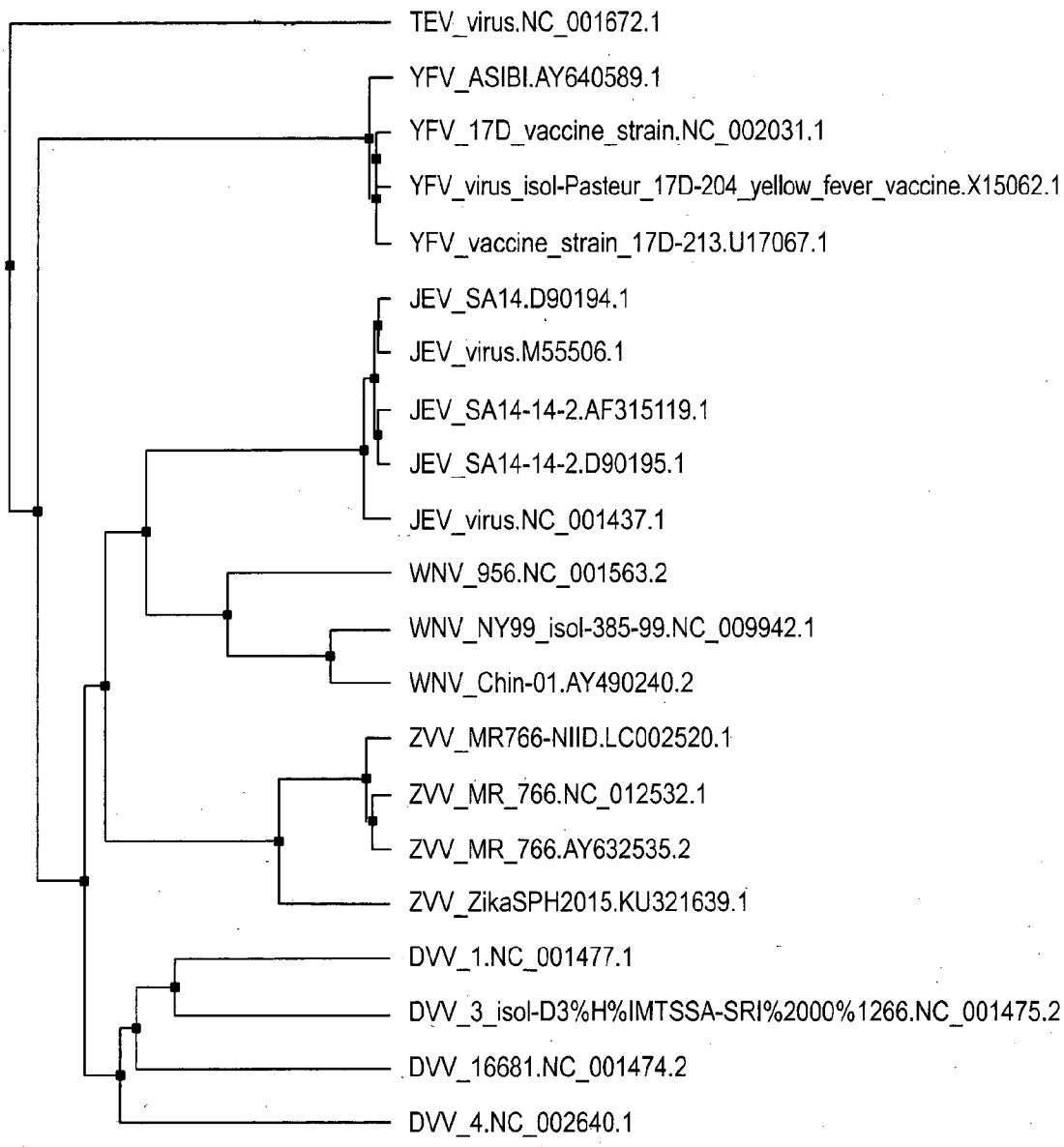

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Figure 1

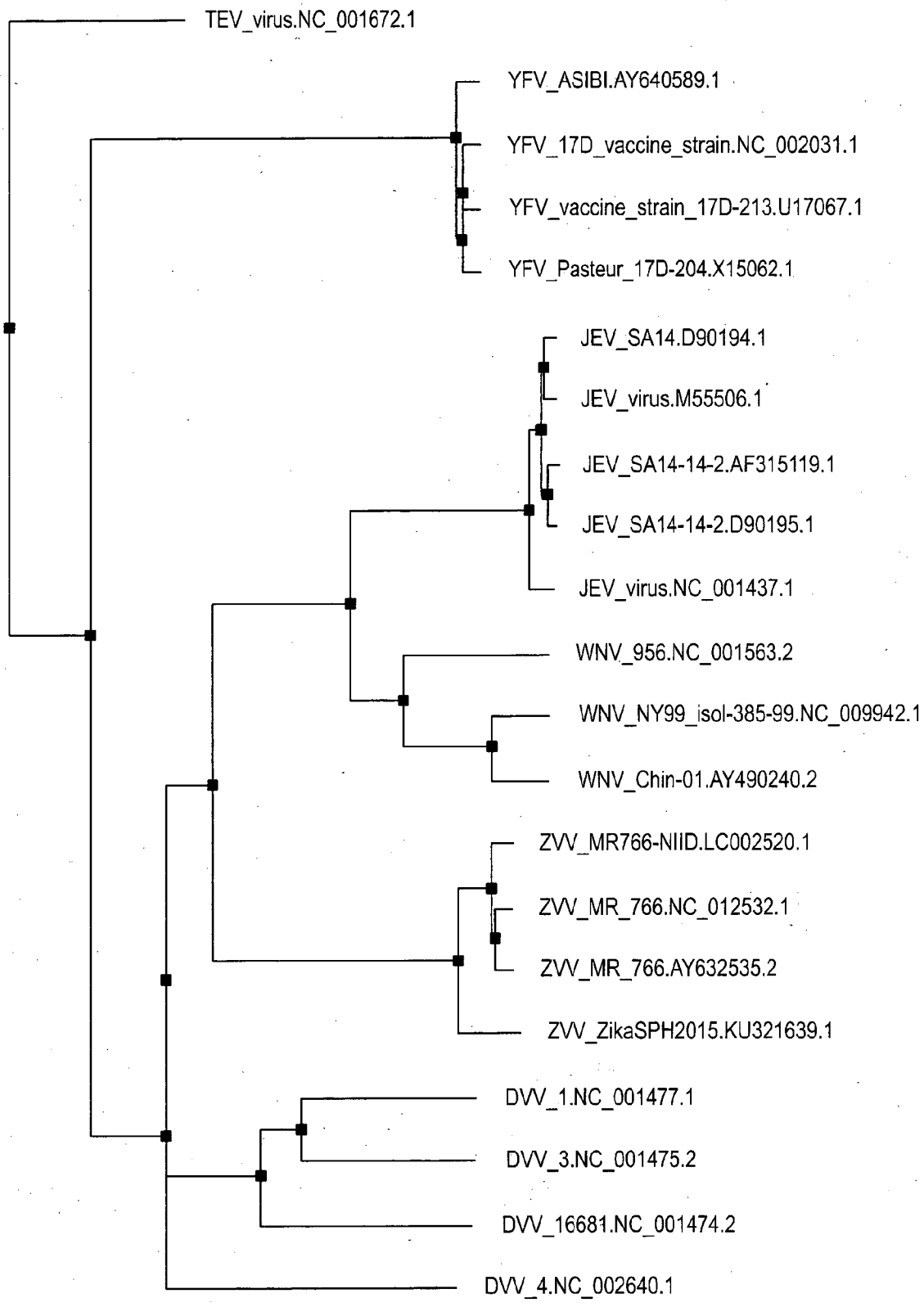

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DVV_4.NC_002640.1

Figure 2

		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DVV_1.NC_001477.1	0	100.0	70.1	73.0	67.8	60.5	60.4	60.5	60.5	60.7	55.8	60.6	60.3	60.6	58.0	57.9	57.9	58.0	62.3	62.3	62.3	61.3
DVV_16681.NC_001474.2	1	70.1	100.0	70.8	68.6	60.5	60.5	60.5	60.6	60.7	54.9	60.9	60.3	60.4	58.3	58.3	58.3	58.3	62.7	62.8	62.8	62.2
DVV_3.NC_001475.2	2	73.0	70.8	100.0	68.9	60.2	60.1	60.1	60.1	60.1	55.8	60.3	60.0	60.1	57.5	57.6	57.5	57.5	62.6	62.7	62.7	62.5
DVV_4.NC_002640.1	3	67.8	68.6	68.9	100.0	60.5	60.6	60.6	60.6	60.4	55.4	60.8	60.3	60.5	58.0	57.9	57.9	57.9	62.1	62.0	62.0	62.1
JEV_SA14-14-2.AF315119.1	4	60.5	60.5	60.2	60.5	100.0	99.8	99.4	99.5	98.1	56.5	70.6	70.3	70.3	57.9	58.0	58.0	58.0	61.9	61.8	61.8	61.4
JEV_SA14-14-2.D90195.1	5	60.4	60.5	60.1	60.6	99.8	100.0	99.4	99.5	98.2	56.6	70.6	70.4	70.3	57.9	58.0	57.9	58.0	61.9	61.8	61.8	61.5
JEV_SA14.D90194.1	6	60.5	60.5	60.1	60.6	99.4	99.4	100.0	99.8	98.6	56.6	70.8	70.5	70.4	58.0	58.0	58.0	58.0	61.9	61.8	61.8	61.6
JEV_virus.M55506.1	7	60.5	60.6	60.1	60.6	99.5	99.5	99.8	100.0	98.6	56.7	70.8	70.4	70.3	58.0	58.1	58.0	58.1	61.9	61.8	61.8	61.6
JEV_virus.NC_001437.1	8	60.7	60.7	60.1	60.4	98.1	98.2	98.6	98.6	100.0	56.6	70.6	70.5	70.6	58.3	58.3	58.4	58.3	62.1	62.0	62.0	61.7
TEV_virus.NC_001672.1	9	55.8	54.9	55.8	55.4	56.5	56.6	56.6	56.7	56.6	100.0	56.2	56.7	56.6	56.9	57.0	57.0	57.0	56.6	56.7	56.7	56.7
WNV_956.NC_001563.2	10	60.6	60.9	60.3	60.8	70.6	70.6	70.8	70.8	70.6	56.2	100.0	79.6	79.6	58.1	58.0	58.1	58.2	62.4	62.3	62.3	62.2
WNV_Chin-01.AY490240.2	11	60.3	60.3	60.0	60.3	70.3	70.4	70.5	70.4	70.5	56.7	79.6	100.0	95.3	58.2	58.2	58.2	58.2	62.0	62.1	62.1	62.7
WNV_NY99_isol-385-99.NC_009942.1	12	60.6	60.4	60.1	60.5	70.3	70.3	70.4	70.3	70.6	56.6	79.6	95.3	100.0	57.8	57.8	57.8	57.8	62.0	62.0	62.0	62.4
YFV_17D_vaccine_strain.NC_002031.1	13	58.0	58.3	57.5	58.0	57.9	57.9	58.0	58.0	58.3	56.9	58.1	58.2	57.8	100.0	99.4	99.9	99.9	58.5	58.5	58.5	58.2
YFV_ASIB.AY640589.1	14	57.9	58.3	57.6	57.9	58.0	58.0	58.0	58.1	58.3	57.0	58.0	58.2	57.8	99.4	100.0	99.4	99.4	58.4	58.4	58.4	58.1
YFV_Pasteur_17D-204.X15062.1	15	57.9	58.3	57.5	57.9	58.0	57.9	58.0	58.0	58.4	57.0	58.1	58.2	57.8	99.9	99.4	100.0	99.9	58.4	58.4	58.4	58.2
YFV_vaccine_strain_17D-213.U17067.1	16	58.0	58.3	57.5	57.9	58.0	58.0	58.0	58.1	58.3	57.0	58.2	58.2	57.8	99.9	99.4	99.9	100.0	58.4	58.4	58.4	58.1
ZVV_MR766-NIID.LC002520.1	17	62.3	62.7	62.6	62.1	61.9	61.9	61.9	61.9	62.1	56.6	62.4	62.0	62.0	58.5	58.4	58.4	58.4	100.0	99.7	99.7	88.9
ZVV_MR_766.AY632535.2	18	62.3	62.8	62.7	62.0	61.8	61.8	61.8	61.8	62.0	56.7	62.3	62.1	62.0	58.5	58.4	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_MR_766.NC_012532.1	19	62.3	62.8	62.7	62.0	61.8	61.8	61.8	61.8	62.0	56.7	62.3	62.1	62.0	58.5	58.4	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_ZikaSPH2015.KU321639.1	20	61.3	62.2	62.5	62.1	61.4	61.5	61.6	61.6	61.7	56.7	62.2	62.7	62.4	58.2	58.1	58.2	58.1	88.9	88.8	88.8	100.0

60 % Identity and higher
70 % Identity and higher
80 % Identity and higher

Figure 3

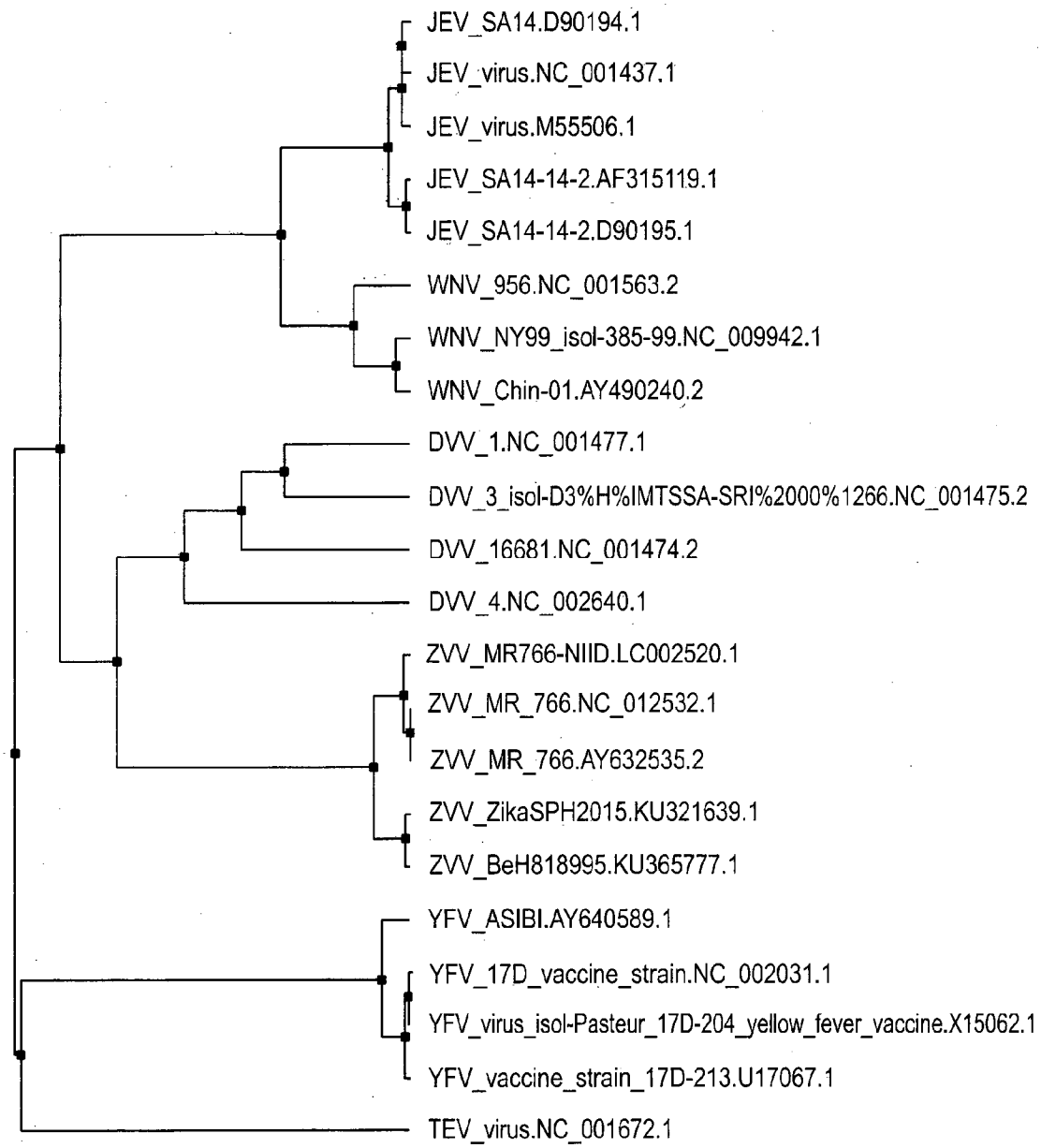

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Figure 4

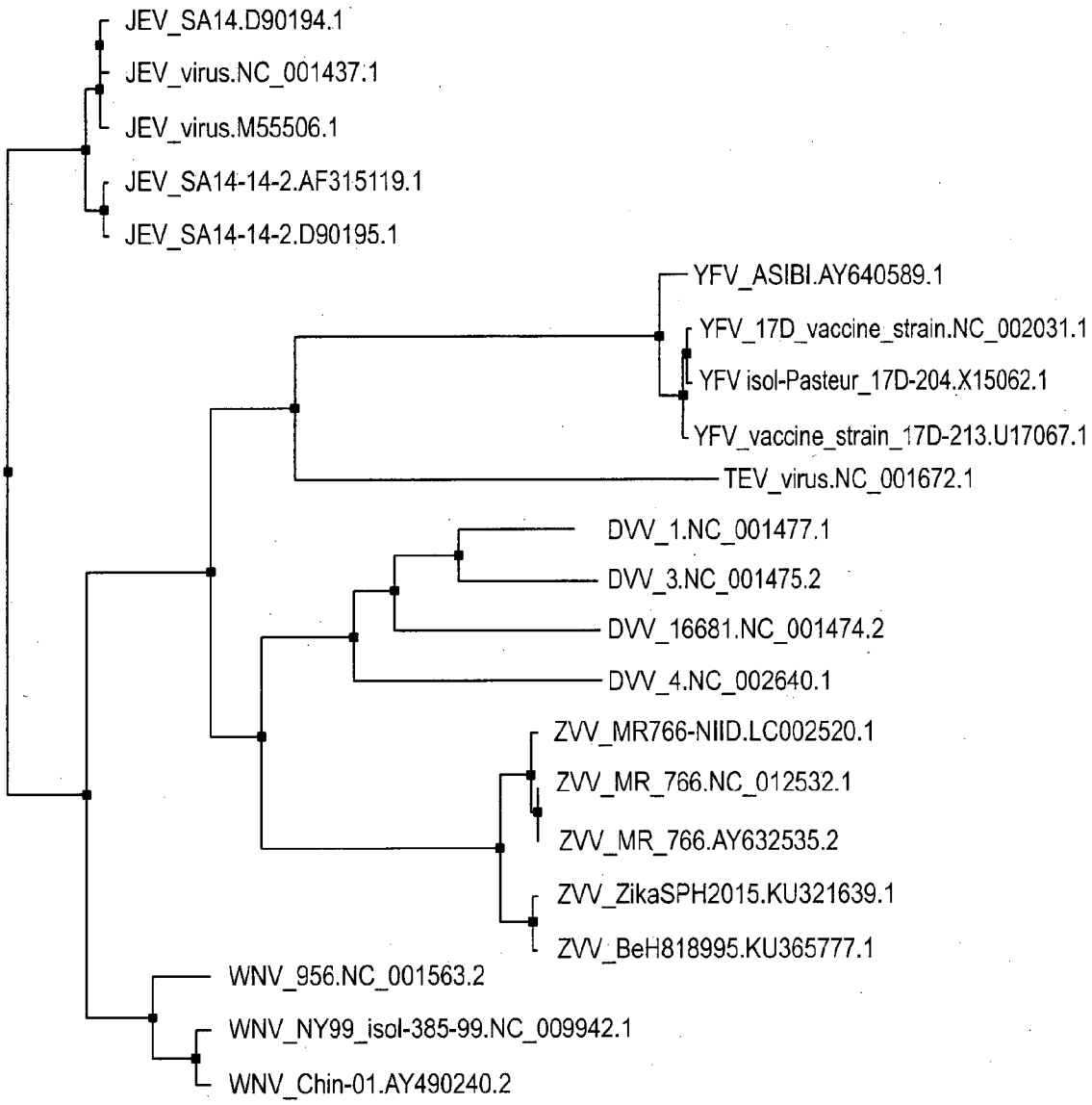

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Figure 5

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DVV_1.NC_001477.1	0	100.0	68.7	77.4	63.8	49.7	49.7	50.1	49.9	50.5	38.3	51.5	50.5	50.9	43.0	43.8	43.0	43.2	57.3	57.9	58.4	58.4	57.1
DVV_16681.NC_001474.2	1	68.7	100.0	68.7	64.0	46.7	46.7	47.1	46.9	47.5	38.9	48.4	47.6	47.6	43.6	44.4	43.6	43.6	53.6	53.8	54.2	54.2	53.8
DVV_3.NC_001475.2	2	77.4	68.7	100.0	62.8	48.0	48.0	48.2	48.2	48.6	38.2	46.8	46.7	46.3	41.6	42.2	41.6	41.7	57.3	57.7	58.2	58.2	57.1
DVV_4.NC_002640.1	3	63.8	64.0	62.8	100.0	47.5	47.5	47.7	47.5	48.1	39.7	49.6	49.6	49.8	40.0	40.6	40.0	40.0	55.8	56.4	56.4	56.4	55.8
JEV_SA14-14-2.AF315119.1	4	49.7	46.7	48.0	47.5	100.0	99.8	98.0	98.6	97.8	39.6	76.1	76.5	76.7	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV_SA14-14-2.D90195.1	5	49.7	46.7	48.0	47.5	99.8	100.0	98.2	98.8	98.0	39.8	76.3	76.7	76.9	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV_SA14.D90194.1	6	50.1	47.1	48.2	47.7	98.0	98.2	100.0	99.4	99.4	40.0	77.5	77.8	78.0	43.3	43.3	43.3	43.3	54.4	55.4	55.2	55.2	54.4
JEV_virus.M55506.1	7	49.9	46.9	48.2	47.5	98.6	98.8	99.4	100.0	99.2	40.0	77.3	77.6	77.8	43.3	43.3	43.3	43.3	54.2	55.2	55.0	55.0	54.2
JEV_virus.NC_001437.1	8	50.5	47.5	48.6	48.1	97.8	98.0	99.4	99.2	100.0	40.4	78.0	78.4	78.6	43.5	43.5	43.5	43.5	54.8	55.4	55.2	55.2	54.8
TEV_virus.NC_001672.1	9	38.3	38.9	38.2	39.7	39.6	39.8	40.0	40.0	40.4	100.0	41.3	41.6	41.0	42.3	41.9	42.3	42.3	39.9	39.9	39.6	39.6	39.9
WNV_956.NC_001563.2	10	51.5	48.4	46.8	49.6	76.1	76.3	77.5	77.3	78.0	41.3	100.0	94.6	94.0	43.8	43.6	43.8	43.8	55.0	55.0	54.8	54.8	55.0
WNV_Chin-01.AY490240.2	11	50.5	47.6	46.7	49.6	76.5	76.7	77.8	77.6	78.4	41.6	94.6	100.0	98.8	43.9	43.5	43.9	43.9	54.4	54.2	54.0	54.0	54.4
WNV_NY99_isol-385-99.NC_009942.1	12	50.9	47.6	45.3	49.8	76.7	76.9	78.0	77.8	78.6	41.0	94.0	98.8	100.0	43.9	43.5	43.9	43.9	54.0	53.8	53.8	53.8	54.0
YFV_17D_vaccine_strain.NC_002031.1	13	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	100.0	97.6	100.0	99.8	42.0	43.4	43.5	43.5	42.0
YFV_ASIB.AY640589.1	14	43.8	44.4	42.2	40.6	42.9	42.9	43.3	43.3	43.5	41.9	43.6	43.5	43.5	97.6	100.0	97.6	97.4	42.4	43.8	43.9	43.9	42.4
YFV_isol-Pasteur_17D-204.X15062.1	15	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	100.0	97.6	100.0	99.8	42.0	43.4	43.5	43.5	42.0
YFV_vaccine_strain_17D-213.U17067.1	16	43.2	43.6	41.7	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	99.8	97.4	99.8	100.0	42.0	43.4	43.5	43.5	42.0
ZVVBeH818995.KU365777.1	17	57.3	53.6	57.3	55.8	54.0	54.0	54.4	54.2	54.8	39.9	55.0	54.4	54.0	42.0	42.4	42.0	42.0	100.0	96.8	96.4	96.4	99.8
ZVV_MR766-NIID.LC002520.1	18	57.9	53.8	57.7	56.4	54.8	54.8	55.4	55.2	55.4	39.9	55.0	54.2	53.8	43.4	43.8	43.4	43.4	96.8	100.0	98.8	98.8	96.6
ZVV_MR_766.AY632535.2	19	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.0	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	96.4	98.8	100.0	100.0	96.2
ZVV_MR_766.NC_012532.1	20	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.0	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	96.4	98.8	100.0	100.0	96.2
ZVV_ZikaSPH2015.KU321639.1	21	57.1	53.8	57.1	55.8	54.0	54.0	54.4	54.2	54.8	39.9	55.0	54.4	54.0	42.0	42.4	42.0	42.0	99.8	96.6	96.2	96.2	100.0

50 % Identity and higher
60 % Identity and higher
70 % Identity and higher

Figure 6

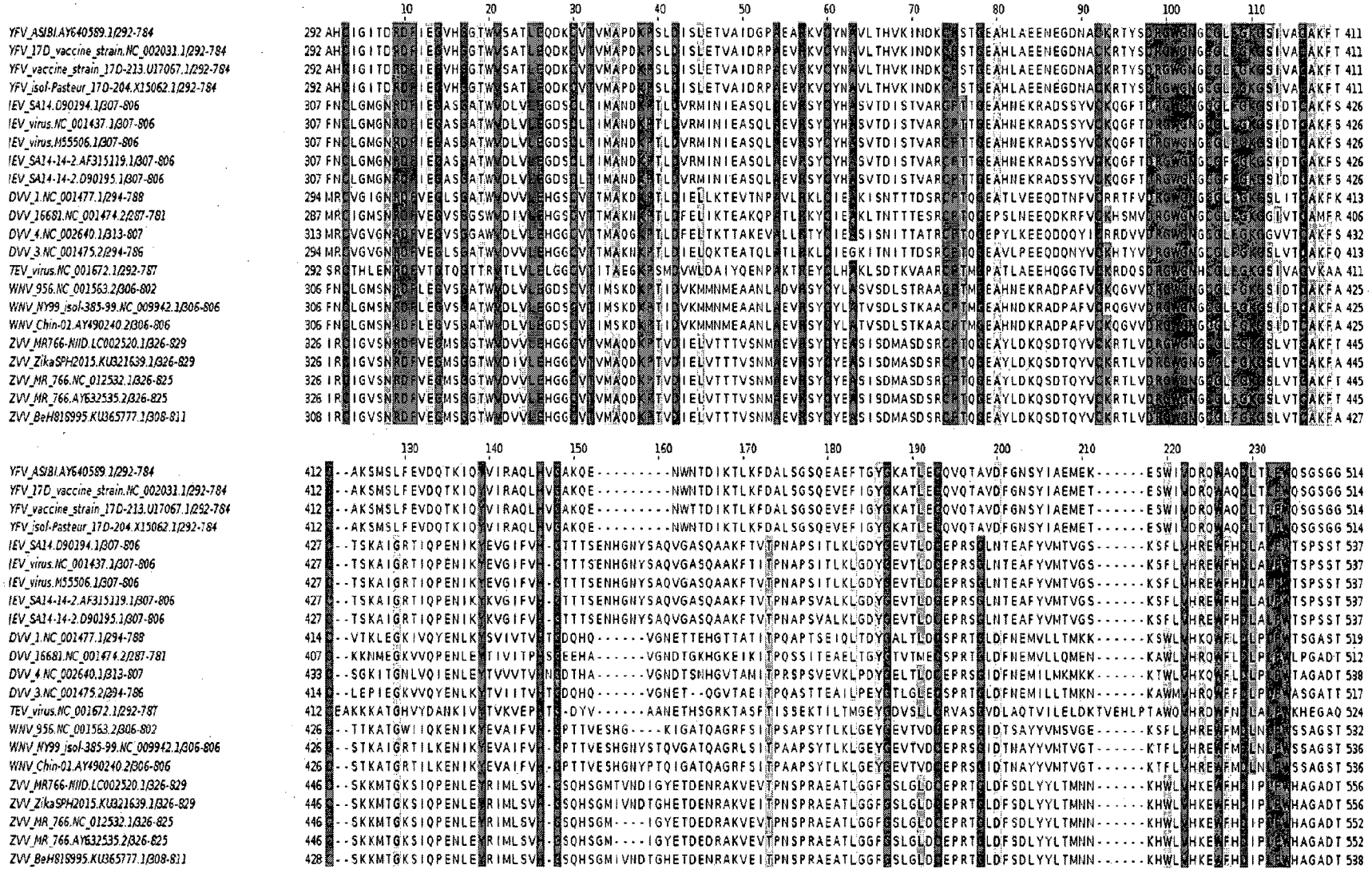

YFV_ASIBI.AY640589.1/292-784
YFV_17D_vaccine_strain.NC_002031.1/292-784
YFV_vaccine_strain_17D-213.U17067.1/292-784
YFV_isol-Pasteur_17D-204.X15062.1/292-784
IEV_SA14.D90194.1/807-806
IEV_virus.NC_001437.1/807-806
IEV_virus.M55506.1/807-806
IEV_SA14-14-2.AF315119.1/807-806
IEV_SA14-14-2.D90195.1/807-806
DVV_1.NC_001477.1/294-788
DVV_16681.NC_001474.2/287-781
DVV_4.NC_002640.1/313-807
DVV_3.NC_001475.2/294-786
TEV_virus.NC_001672.1/292-787
WNV_956.NC_001563.2/306-802
WNV_NY99_isol-385-99.NC_009942.1/306-806
WNV_Chin-01.AY490240.2/306-806
ZVV_MR766-NIID.LC002520.1/326-829
ZVV_ZikaSPH2015.KU321639.1/326-829
ZVV_MR_766.NC_012532.1/326-825
ZVV_MR_766.AY632535.2/326-825
ZVV_BeH818995.KU365777.1/308-811

Figure 7A

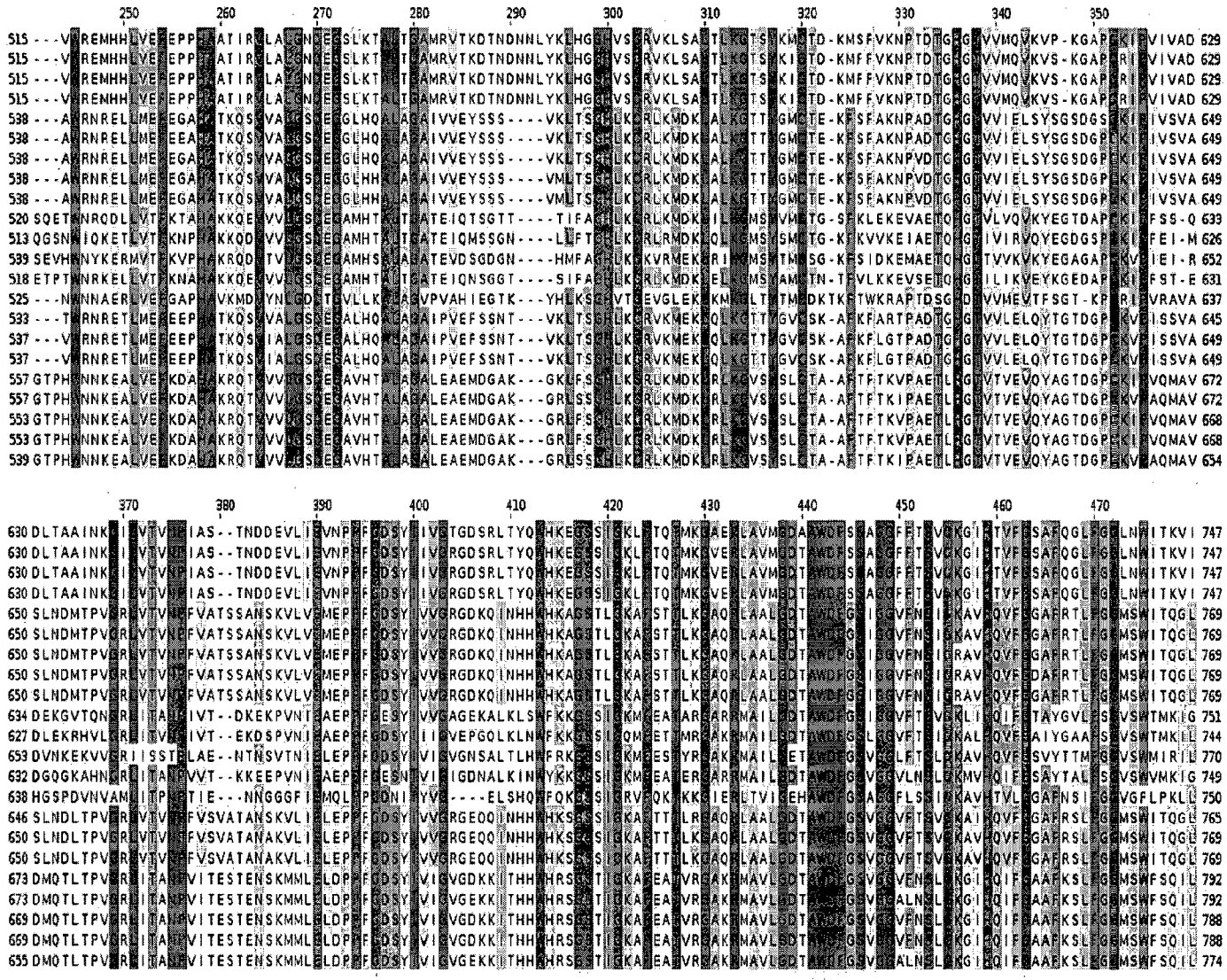

250 260 270 280 290 300 310 320 330 340 350
YFV_ASIBI.AY640589.1/292-784 515 629
YFV_17D_vaccine_strain.NC_002031.1/292-784 515 629
YFV_vaccine_strain_17D-213.U17067.1/292-784 515 629
YFV_isol-Pasteur_17D-204.X15062.1/292-784 515 629
JEV_SA14.D90194.1/307-806 538 649
JEV_virus.NC_001437.1/307-806 538 649
JEV_virus.M55506.1/307-806 538 649
JEV_SA14-14-2.AF315119.1/307-806 538 649
JEV_SA14-14-2.D90195.1/307-806 538 649
DVV_1.NC_001477.1/294-788 520 639
DVV_16681.NC_001474.2/287-781 513 626
DVV_4.NC_002640.1/313-807 539 652
DVV_3.NC_001475.2/294-786 518 631
TEV_virus.NC_001672.1/292-787 525 637
WNV_956.NC_001563.2/306-802 533 645
WNV_NY99_isol-385-99.NC_009942.1/306-806 537 649
WNV_Chin-01.AY490240.2/306-806 537 649
ZVV_MR766-NIID.LC002520.1/326-829 557 672
ZVV_ZikaSPH2015.KU321639.1/326-829 557 672
ZVV_MR_766.NC_012532.1/326-825 553 668
ZVV_MR_766.AY632535.2/326-825 553 668
ZVV_BeH818995.KU365777.1/308-811 539 654
370 380 390 400 410 420 430 440 450 460 470
YFV_ASIBI.AY640589.1/292-784 630 747
YFV_17D_vaccine_strain.NC_002031.1/292-784 630 747
YFV_vaccine_strain_17D-213.U17067.1/292-784 630 747
YFV_isol-Pasteur_17D-204.X15062.1/292-784 630 747
JEV_SA14.D90194.1/307-806 650 769
JEV_virus.NC_001437.1/307-806 650 769
JEV_virus.M55506.1/307-806 650 769
JEV_SA14-14-2.AF315119.1/307-806 650 769
JEV_SA14-14-2.D90195.1/307-806 650 769
DVV_1.NC_001477.1/294-788 634 751
DVV_16681.NC_001474.2/287-781 627 744
DVV_4.NC_002640.1/313-807 653 770
DVV_3.NC_001475.2/294-786 632 749
TEV_virus.NC_001672.1/292-787 638 750
WNV_956.NC_001563.2/306-802 646 765
WNV_NY99_isol-385-99.NC_009942.1/306-806 650 769
WNV_Chin-01.AY490240.2/306-806 650 769
ZVV_MR766-NIID.LC002520.1/326-829 673 792
ZVV_ZikaSPH2015.KU321639.1/326-829 673 792
ZVV_MR_766.NC_012532.1/326-825 669 788
ZVV_MR_766.AY632535.2/326-825 669 788
ZVV_BeH818995.KU365777.1/308-811 655 774

Figure 7B

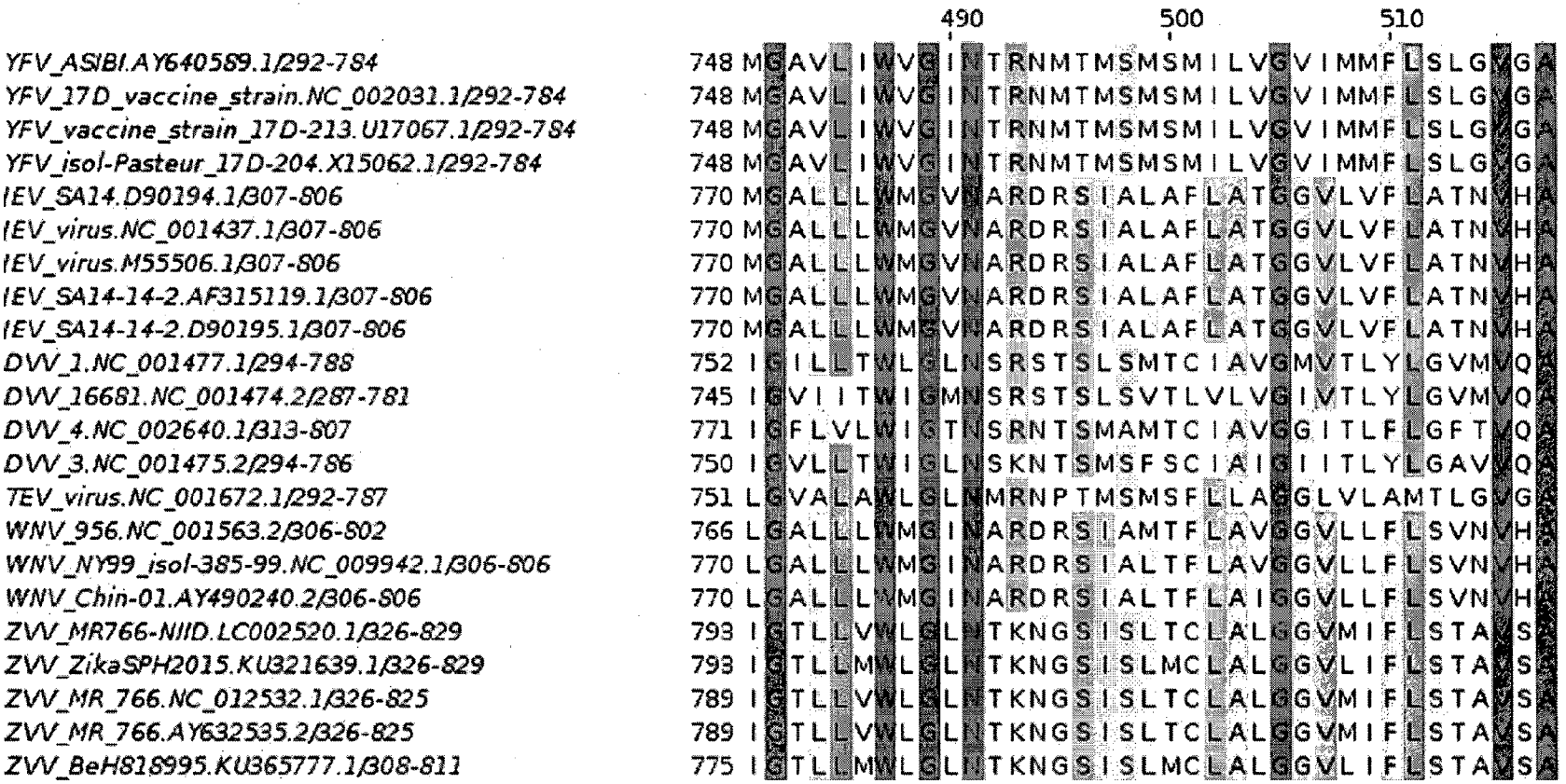

490
500
510
YFV_ASIBI.AY640589.1/292-784
YFV_17D_vaccine_strain.NC_002031.1/292-784
YFV_vaccine_strain_17D-213.U17067.1/292-784
YFV_isol-Pasteur_17D-204.X15062.1/292-784
JEV_SA14.D90194.1/307-806
JEV_virus.NC_001437.1/307-806
JEV_virus.M55506.1/307-806
JEV_SA14-14-2.AF315119.1/307-806
JEV_SA14-14-2.D90195.1/307-806
DVV_1.NC_001477.1/294-788
DVV_16681.NC_001474.2/287-781
DVV_4.NC_002640.1/313-807
DVV_3.NC_001475.2/294-786
TEV_virus.NC_001672.1/292-787
WNV_956.NC_001563.2/306-802
WNV_NY99_isol-385-99.NC_009942.1/306-806
WNV_Chin-01.AY490240.2/306-806
ZVV_MR766-NIID.LC002520.1/326-829
ZVV_ZikaSPH2015.KU321639.1/326-829
ZVV_MR_766.NC_012532.1/326-825
ZVV_MR_766.AY632535.2/326-825
ZVV_BeH818995.KU365777.1/308-811

Figure 7C

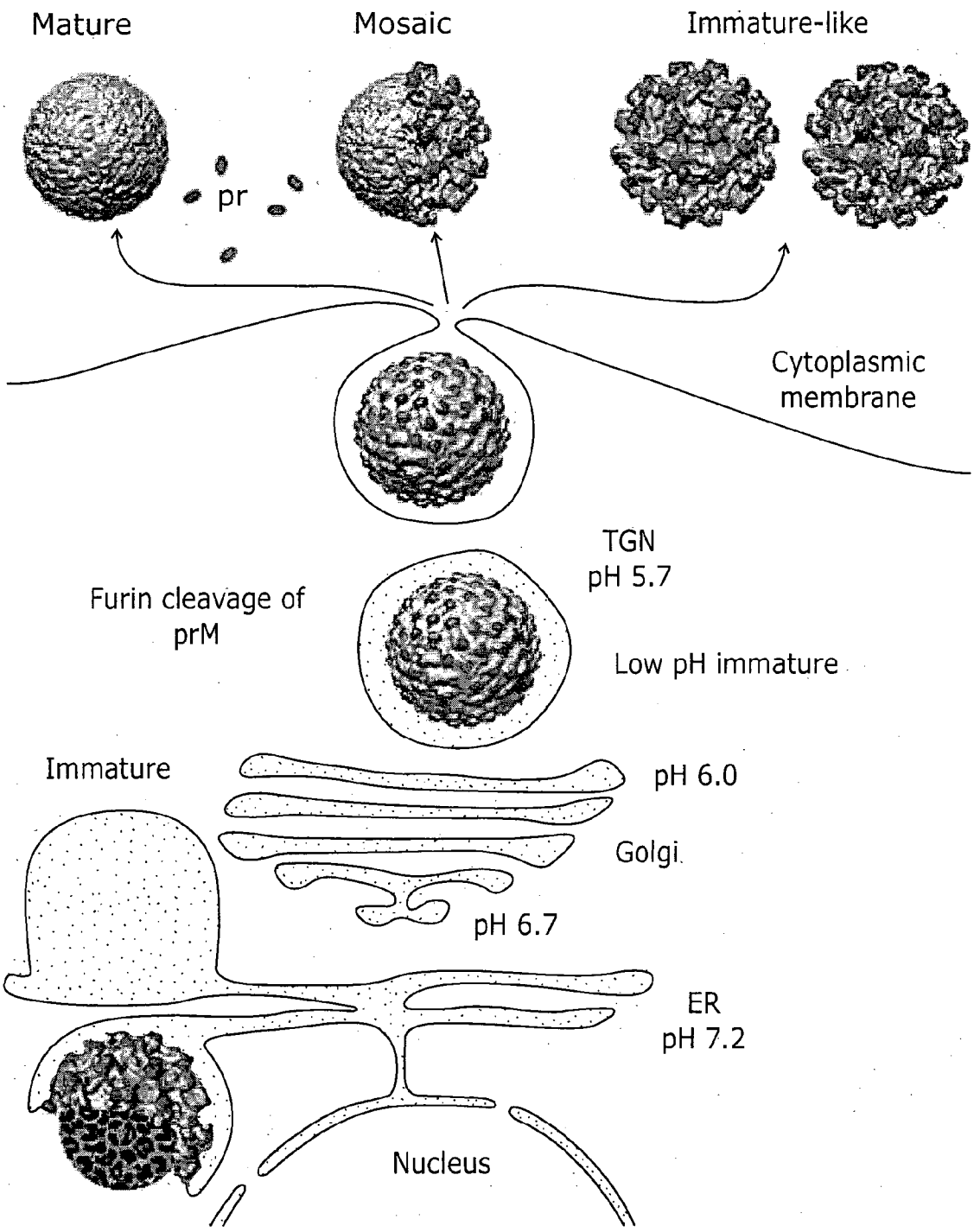

Mature
Mosaic
Immature-like
pr
Cytoplasmic membrane
TGN
pH 5.7
Furin cleavage of prM
Low pH immature
Immature
pH 6.0
Golgi
pH 6.7
ER
pH 7.2
Nucleus

Figure 8

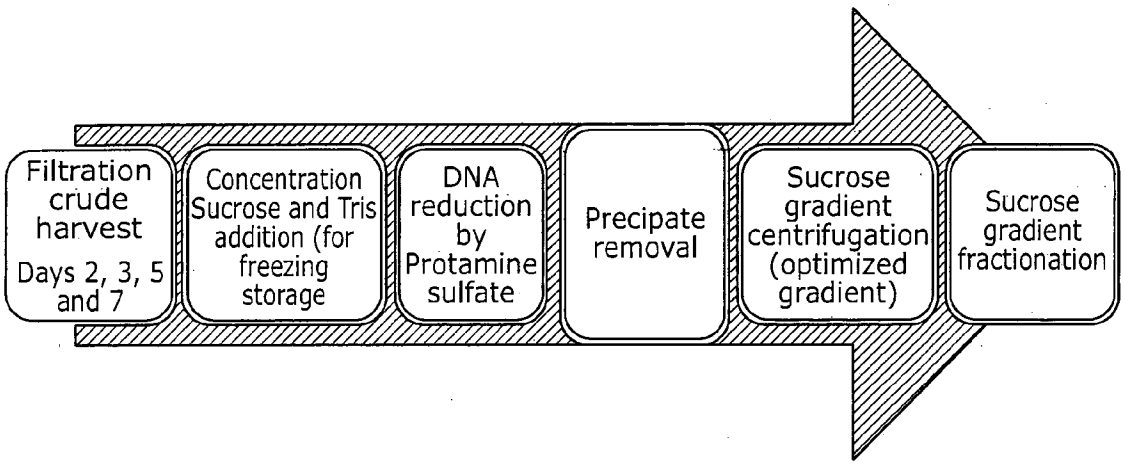


Figure 9A

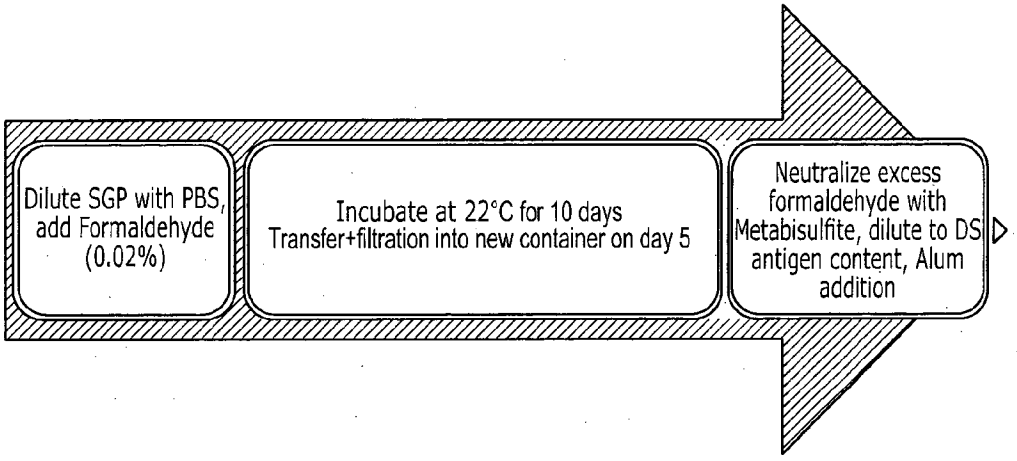


Figure 9B

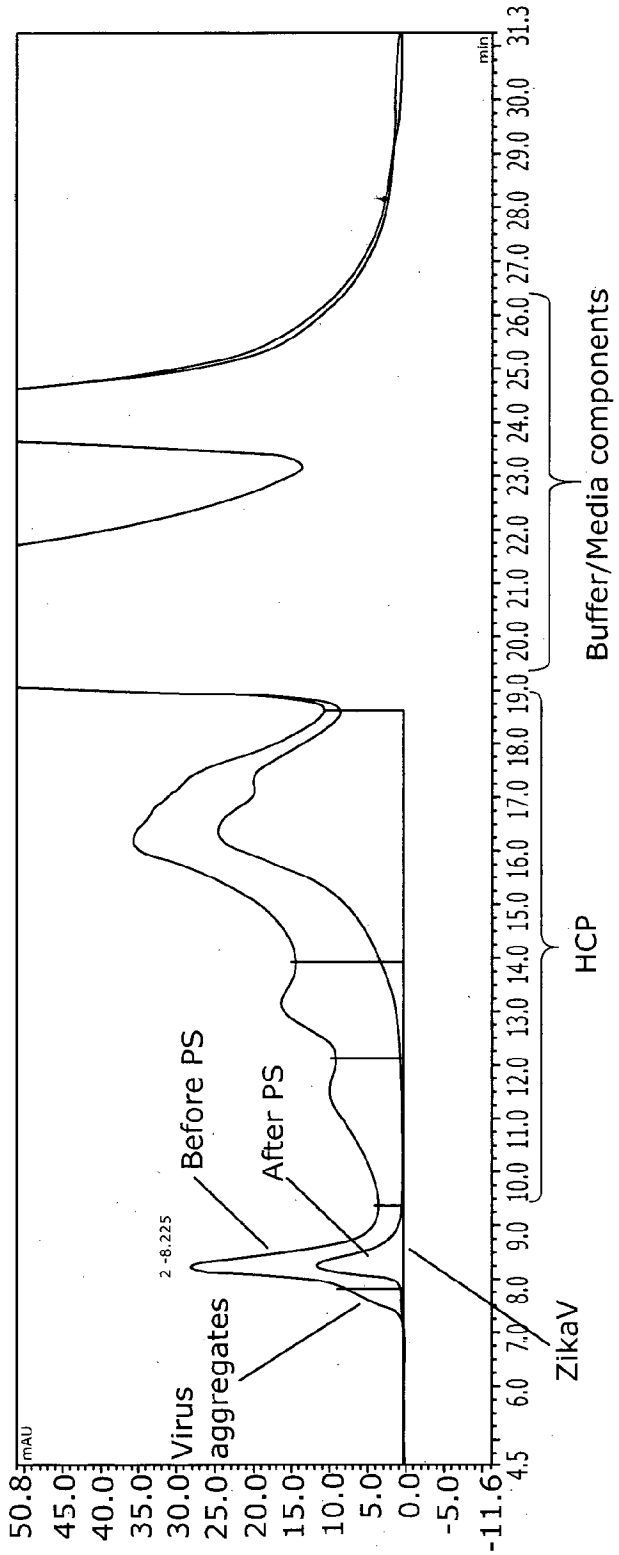
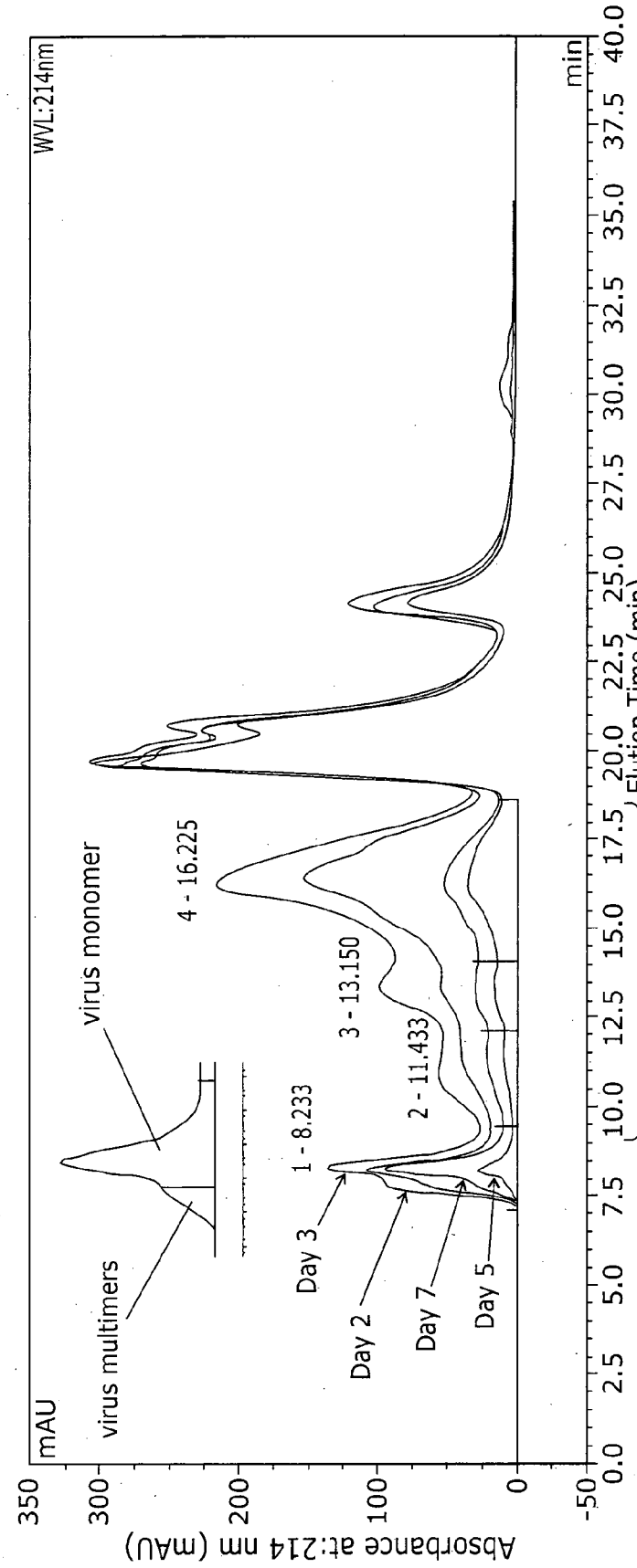
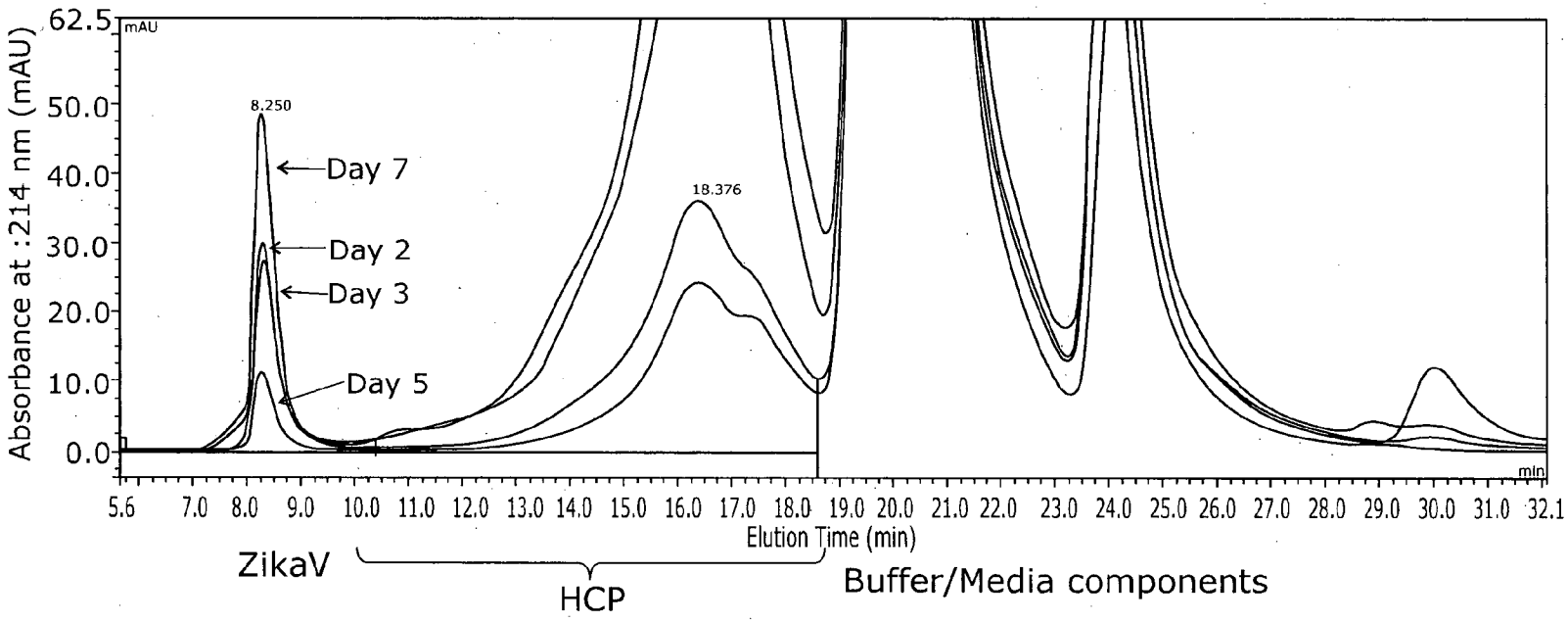

mAU
50.8
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-11.6
4.5
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0
25.0
26.0
27.0
28.0
29.0
30.0
31.3
min
2 -8.225
Virus aggregates
Before PS
After PS
ZikaV
HCP
Buffer/Media components

Figure 10



+ Similar virus concentration in harvests Day 2, Day3, Day 7; lower virus concentration in harvest Day 5
+ Day 2 and Day 3 harvests contained more HCP than Days 5/7 (correlates with observed CPE)
+ Minor amount of virus multimers observed

Figure 11



+ PS removes virus aggregates and partially host cell proteins (HCP)
+ Infectoius virus titer is not affected by PS treatment
+ Strong cytopathic effect (CPE) on day 3,drop in productivity
+ Remaining cells seem to recover well and increase in Zika productivity

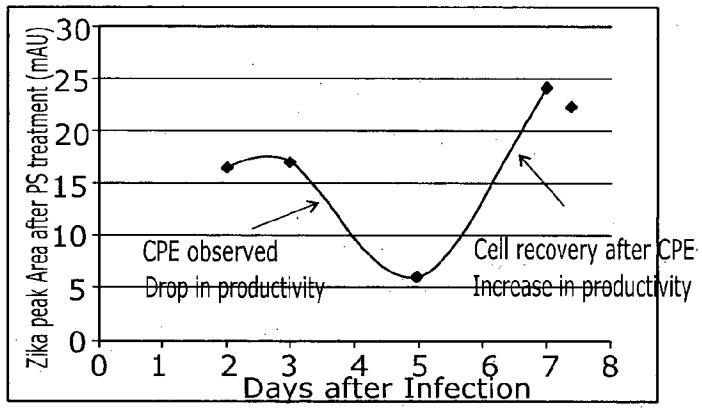


Figure 12

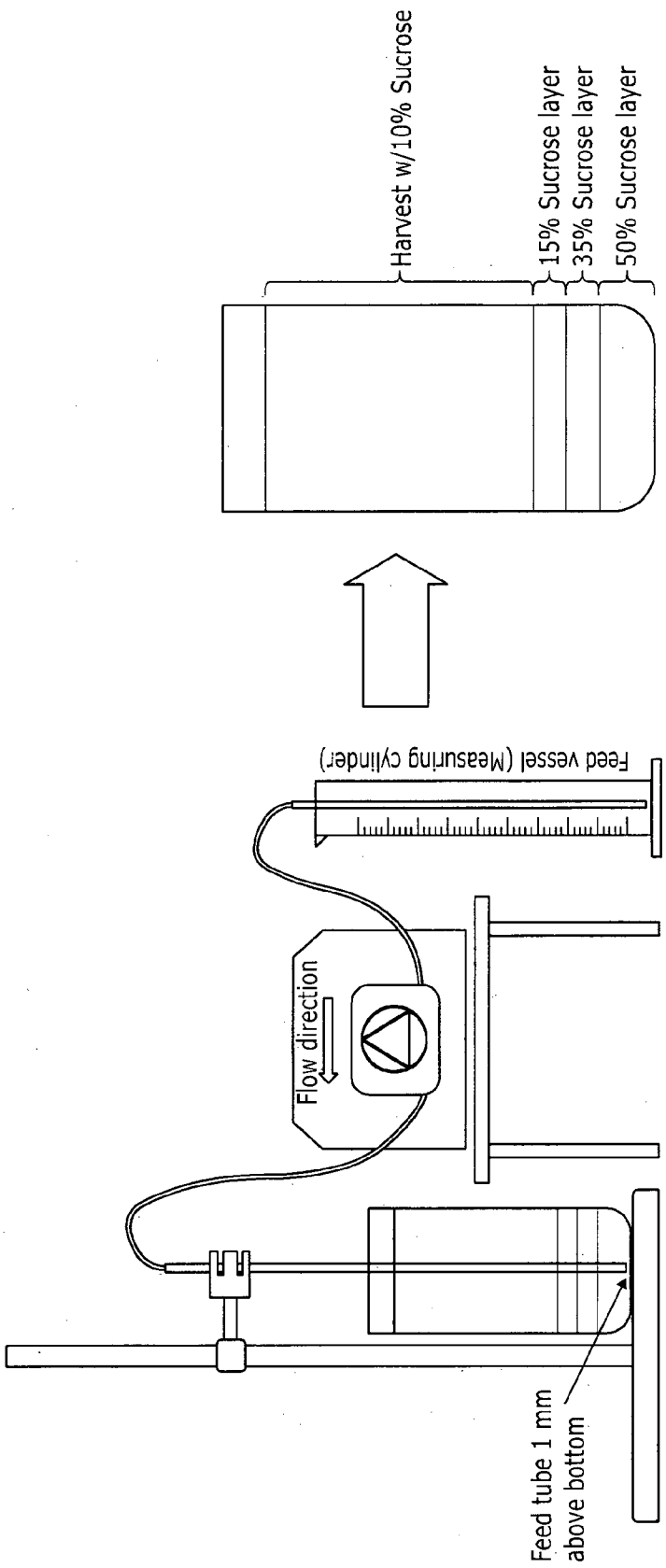
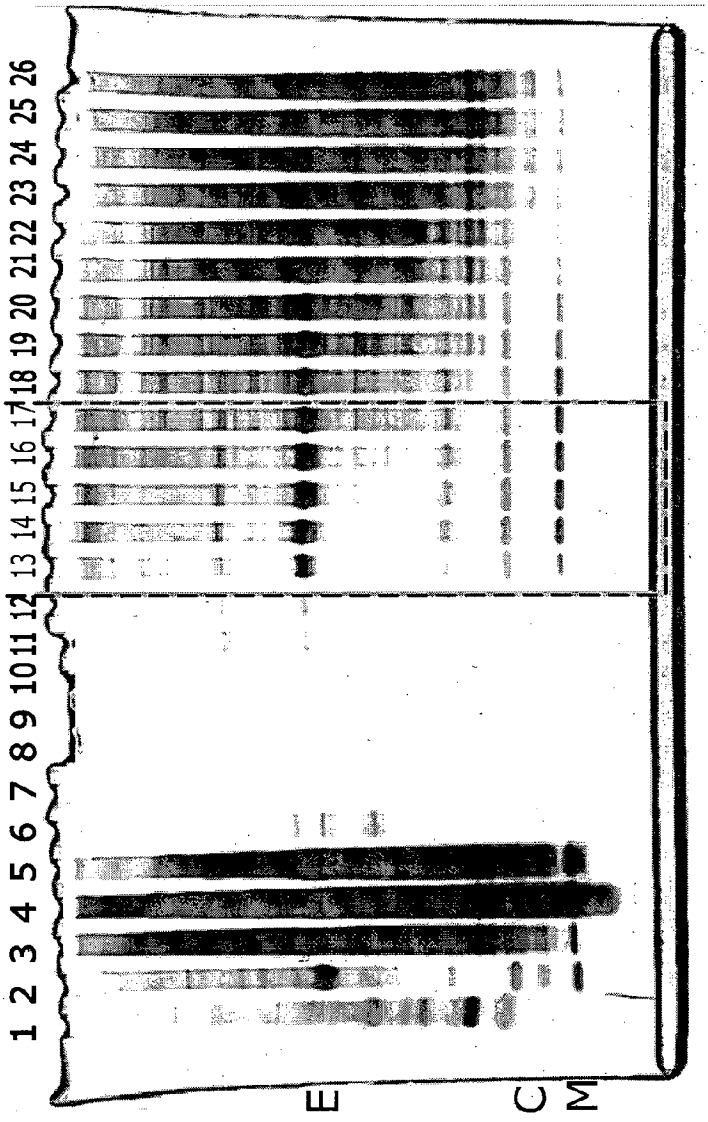

Harvest w/10% Sucrose
15% Sucrose layer
35% Sucrose layer
50% Sucrose layer
Feed vessel (Measuring cylinder)
Flow direction
Feed tube 1 mm
above bottom

Figure 13



Lane	Sample
1	Molecular weight Marker
2	JEV Sucrose Gradient Pool (Reference)
3	Zika Harvest pool (day 2,3,5,7)
4	Zika Harvest pool (day 2,3,5,7)concentrated 300x
5	Zika Harvest pool (day 2,3,5,7)concentrated 300x + Protamine sulfate
6	Ultrafiltration Permeate
7	SGC Bottle 1, Fraction 4
8	SGC Bottle 1, Fraction 5
9	SGC Bottle 1, Fraction 6
10	SGC Bottle 1, Fraction 7
11	SGC Bottle 1, Fraction 8
12	SGC Bottle 1, Fraction 9
13	SGC Bottle 1, Fraction 10
14	SGC Bottle 1, Fraction 11
15	SGC Bottle 1, Fraction 12
16	SGC Bottle 1, Fraction 13
17	SGC Bottle 1, Fraction 14
18	SGC Bottle 1, Fraction 15
19	SGC Bottle 1, Fraction 16
20	SGC Bottle 1, Fraction 17
21	SGC Bottle 1, Fraction 18
22	SGC Bottle 1, Fraction 19
23	SGC Bottle 1, Fraction 20
24	SGC Bottle 1, Fraction 21
25	SGC Bottle 1, Fraction 22
26	SGC Bottle 1, Fraction 23

Pooled fractions F10-F14
(10mL total volume pere bottle)

Figure 14

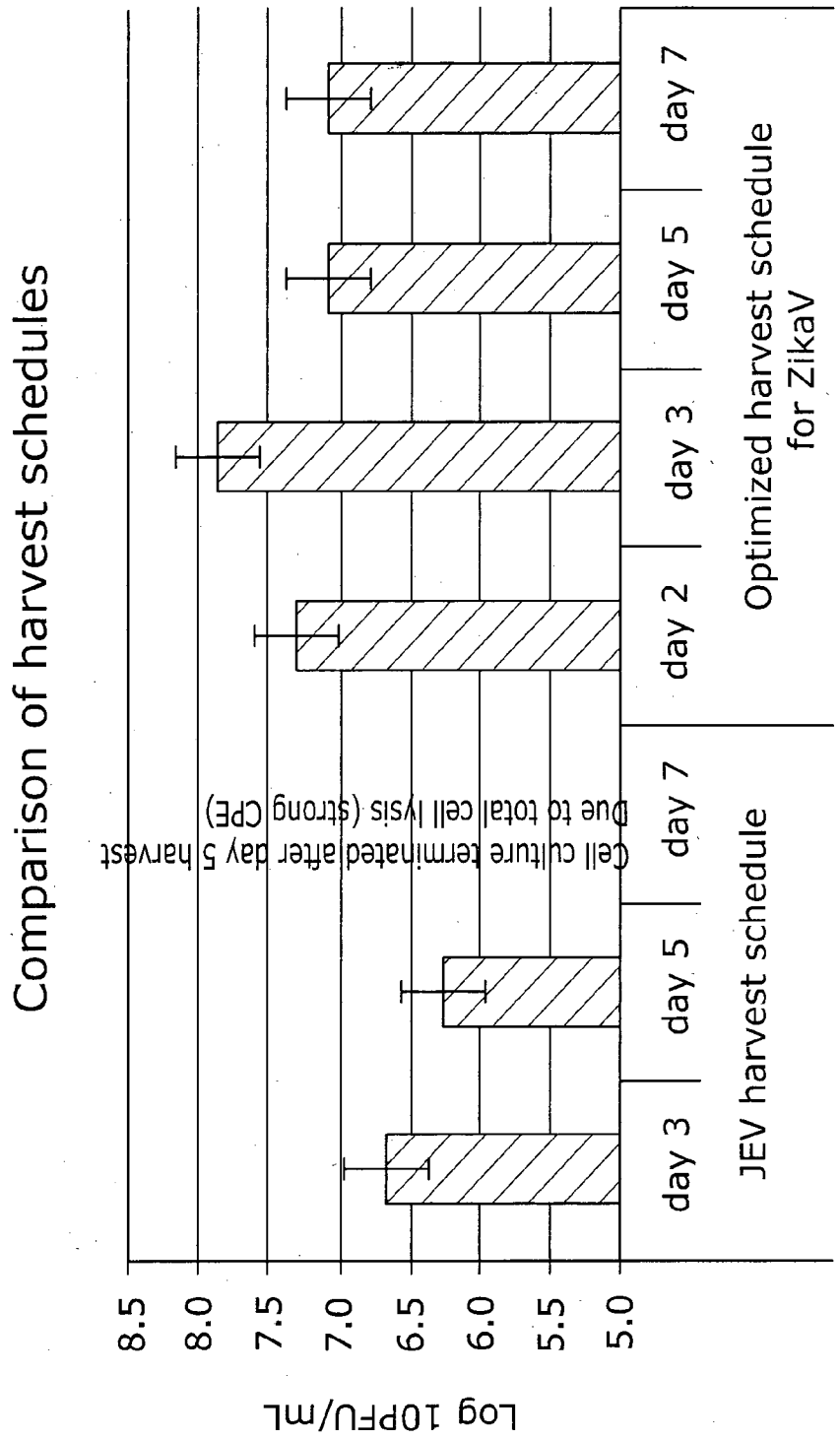

Comparison of harvest schedules
Log 10PFU/mL
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
day 3
day 5
day 7
JEV harvest schedule
day 2
day 3
day 5
day 7
Optimized harvest schedule for ZikaV
Cell culture terminated after day 5 harvest
Due to total cell lysis (strong CPE)

Figure 15

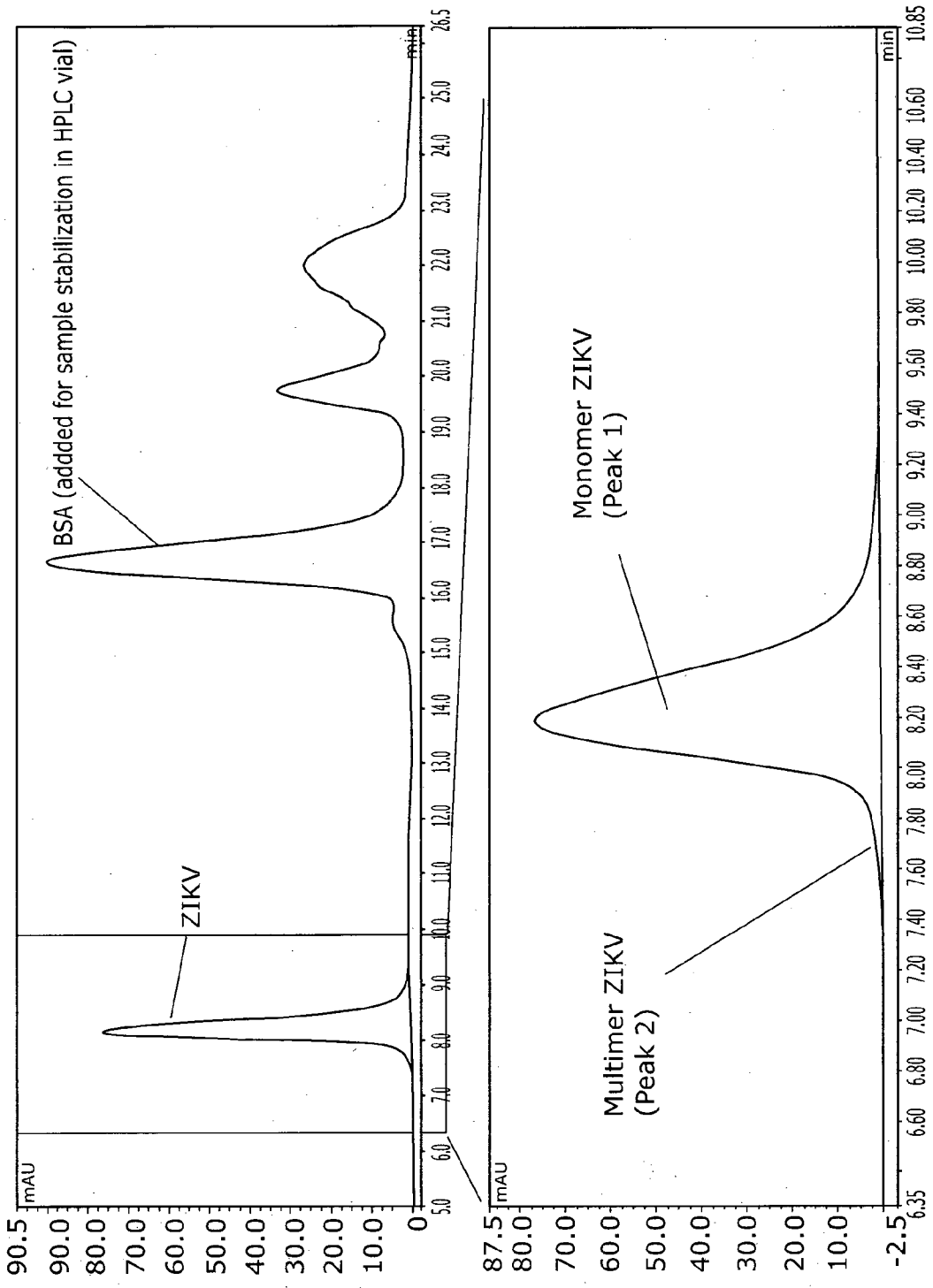

BSA (addded for sample stabilization in HPLC vial)
ZIKV
mAU
min
Monomer ZIKV
(Peak 1)
Multimer ZIKV
(Peak 2)

Figure 16

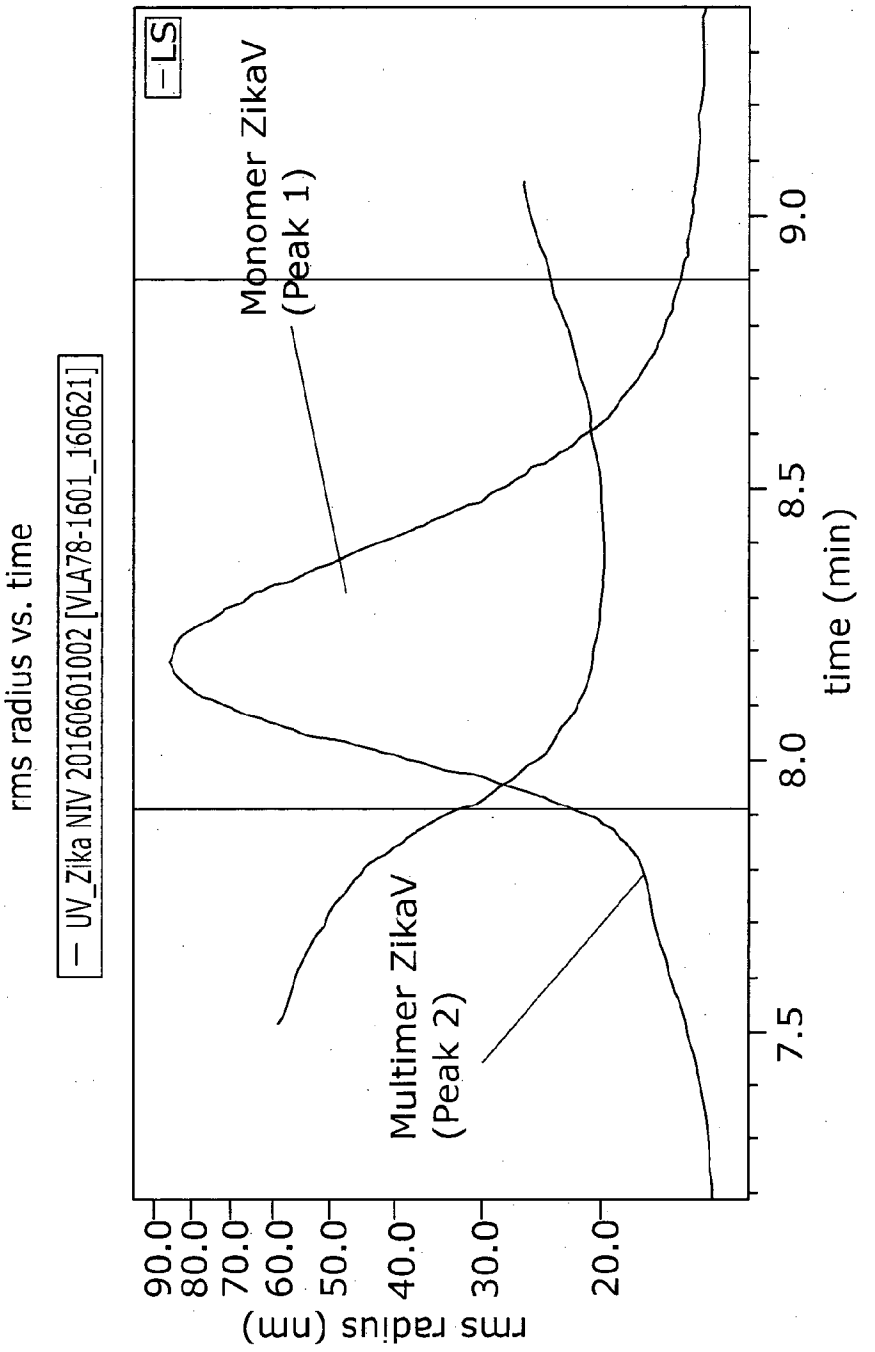

rms radius vs. time
UV_Zika NIV 20160601002 [VLA78-1601_160621]
LS
Monomer ZikaV
(Peak 1)
Multimer ZikaV
(Peak 2)
rms radius (nm)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
7.5
8.0
8.5
9.0
time (min)

Figure 17

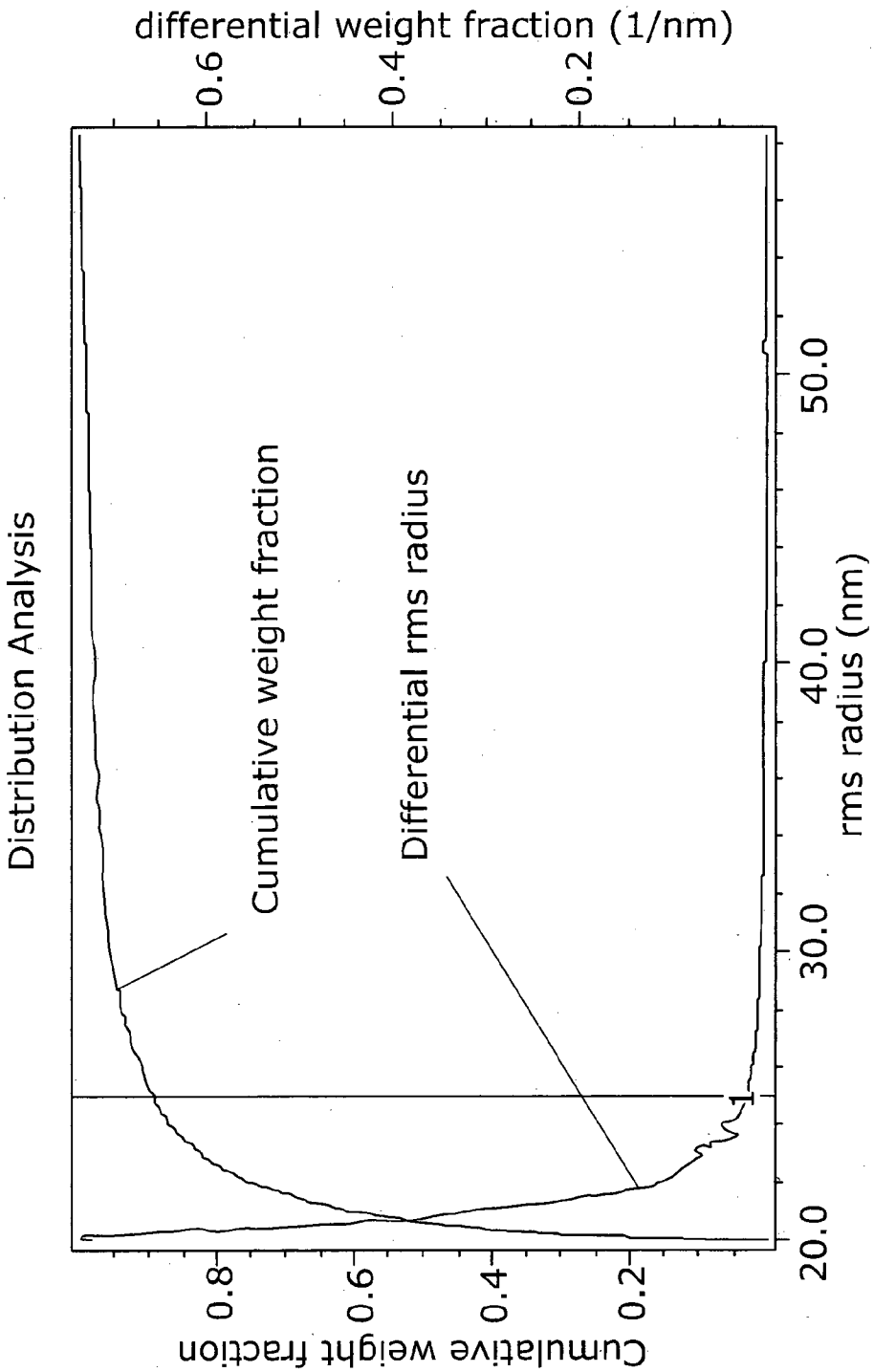
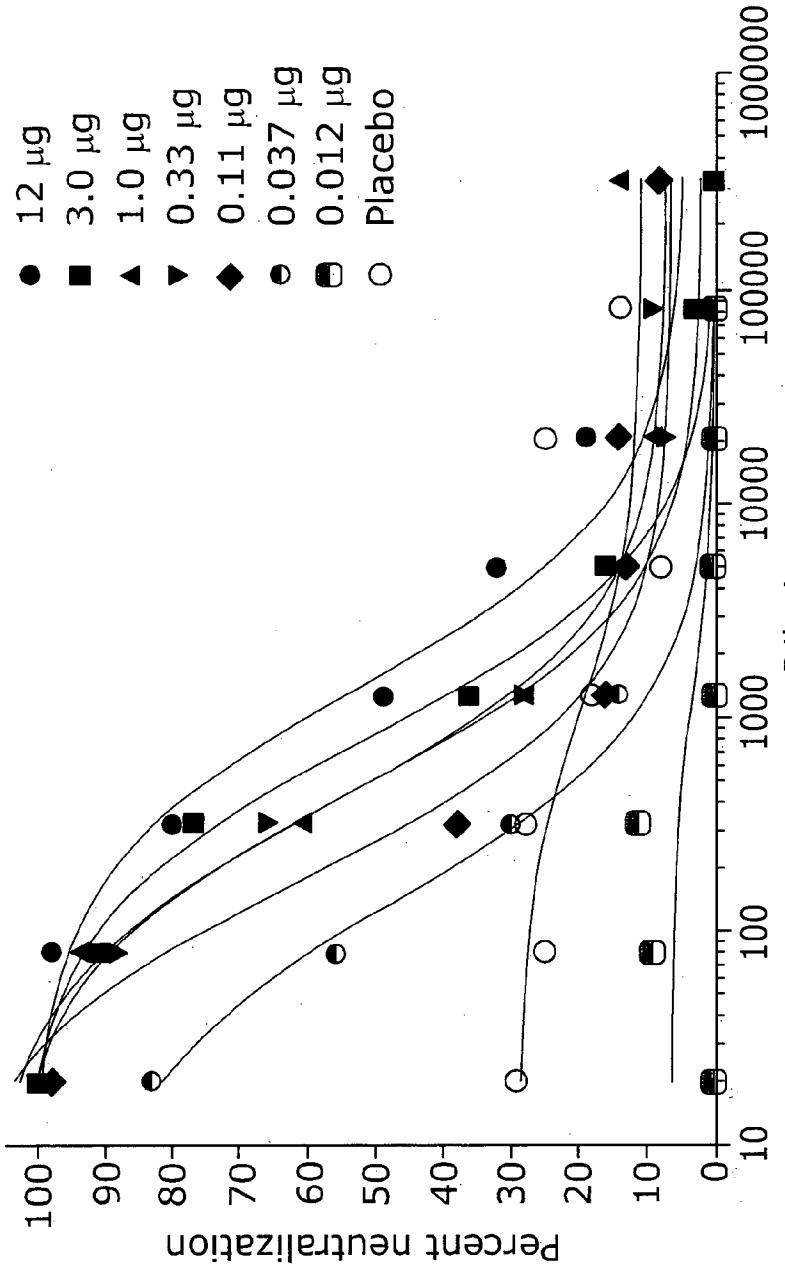
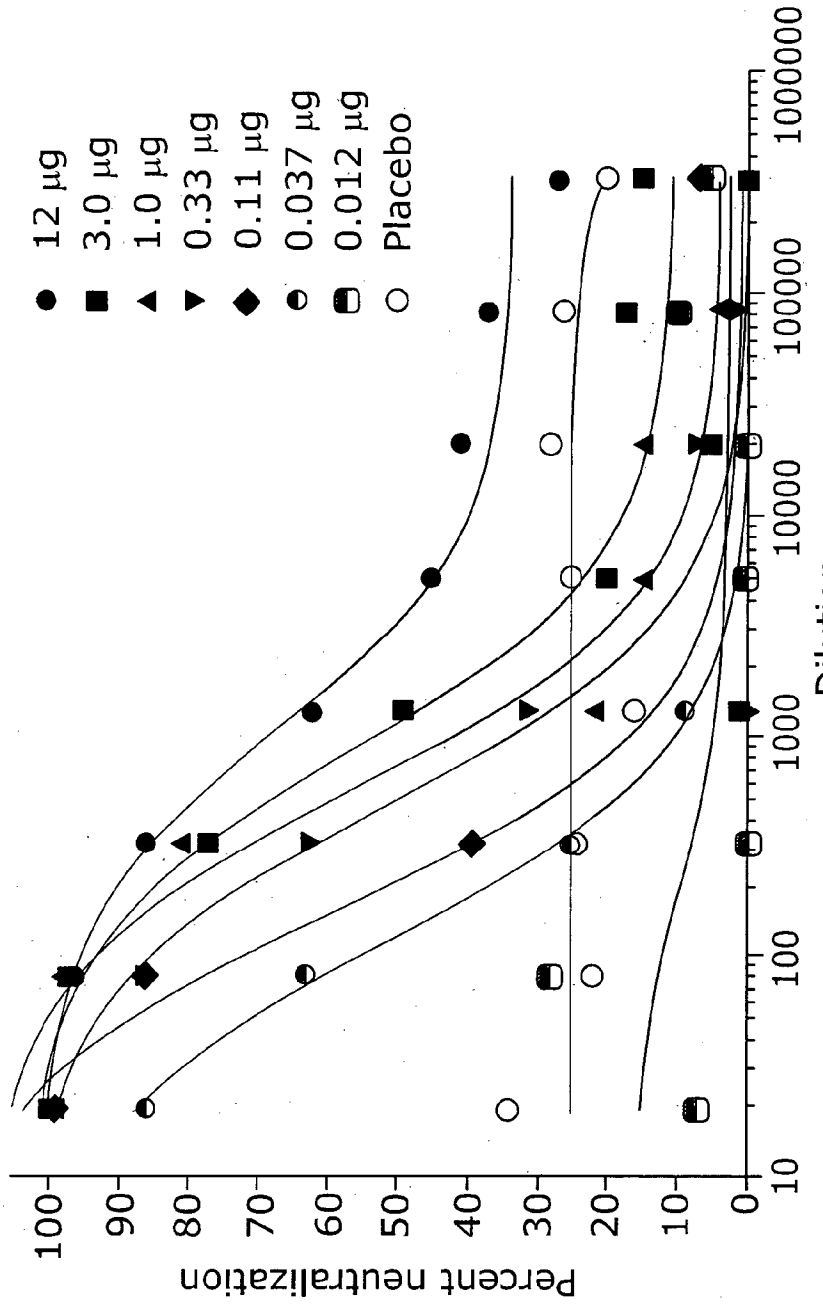

Distribution Analysis
Cumulative weight fraction
Differential rms radius
Cumulative weight fraction
0.8
0.6
0.4
0.2
20.0
30.0
40.0
50.0
rms radius (nm)
differential weight fraction (1/nm)
0.6
0.4
0.2

Figure 18



	12 µg	3.0 µg	1.0 µg	0.33 µg	0.11 µg	0.037 µg
EC50	1371	807.6	380.7	456.1	179.9	142.0

Figure 19



	12 µg	3.0 µg	1.0 µg	0.33 µg	0.11 µg	0.037 µg
EC50	1029	819.1	556.9	477.3	158.1	118.0

Figure 20

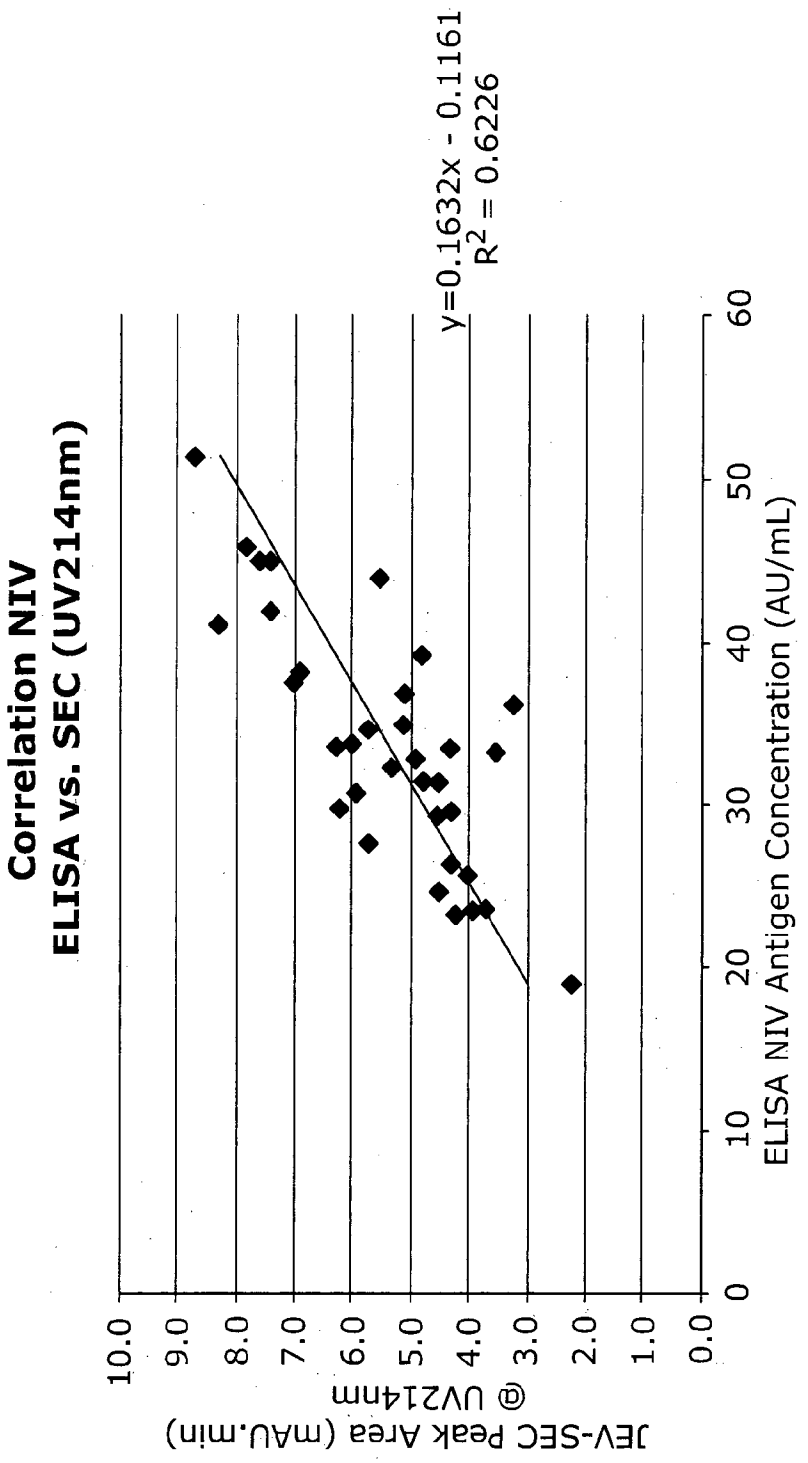

Correlation NIV
ELISA vs. SEC (UV214nm)
y=0.1632x - 0.1161
R2 = 0.6226
JEV-SEC Peak Area (mAU.min)
@ UV214nm
ELISA NIV Antigen Concentration (AU/mL)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
0
10
20
30
40
50
60

Figure 21

DECLARATION (37 C.F.R. § 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 C.F.R. § 1.76)

Title of Invention	ZIKA VIRUS VACCINE

As the below named inventor, I declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number PCT/EP2016/082664 filed on December 23, 2016 Assigned U.S. Serial No. 16/063,007

The above-identified application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I have reviewed and understand the contents of the above-identified application, including the claims.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. § 1.56.

All statements made herein of my own knowledge are true, and all statements made herein on information and belief are believed to be true.

I hereby acknowledge that any willful false statement and the like made in this declaration is punishable by fine or imprisonment of not more than five (5) years, or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the application or any patent issuing thereon.

LEGAL NAME OF SOLE OR FIRST INVENTOR

Inventor one: Jana Barbero Calzado Date: 03 AUG 2018

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor two: Mario Nebenführ Date: 03.08.2018

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor three: Robert Schlegl Date: 3-Aug-2018

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor four: Michael Weber Date: 10-Aug-2018

Signature:

☒ Additional inventors are being named on 1 Additional form(s) attached hereto.

DECLARATION FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET	ADDITIONAL INVENTOR(S) Supplemental Sheet Page 1 of 1
LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any	
Inventor five: Jürgen Heindl-Wruss	Date: 06-Apr-2009
Signature:	

Electronic Acknowledgement Receipt

EFS ID:	38816481
Application Number:	16813862
International Application Number:	
Confirmation Number:	9700
Title of Invention:	ZIKA VIRUS VACCINE
First Named Inventor/Applicant Name:	Jana Barbero Calzado
Customer Number:	23628
Filer:	Chelsea E. Witte-Garcia/Katelyn Farmer
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	I0422.70125US01
Receipt Date:	10-MAR-2020
Filing Date:	
Time Stamp:	10:37:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	I042270125US01-TRN-CEW.pdf	33230 af3a1946d2f2ab941025965d75c3787587e38975	no	1

Warnings:

Information:					
2	Application Data Sheet	I042270125US01-ADS-CEW.pdf	31948 b7a25120f52a62f0c0fb8e115eb12a76c9facede	no	7
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
3		I042270125US01-UTLAPL-CEW.pdf	521008 7f46d3f18199e6a7b78b78a87bd11de1240ff68c	yes	102

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	99
	Claims	100	101
	Abstract	102	102

Warnings:					
Information:					
4	Drawings-other than black and white line drawings	I042270125US01-DRW-CEW.pdf	1529428 87ba51b58767b4bae154ac1e940d94f3a8a9547e	no	23
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Information:					
5	Oath or Declaration filed	I042270125US01-INVDEC-CEW.pdf	1115041 305ef540beecd5e82449a3028a92acf947616314	no	2
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6	Sequence Listing (Text File)	I042270125US01-SEQ-CEW.txt	466226	no	-
Warnings:					
Information:					
		Total Files Size (in bytes):	3696881		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 16813862 Document Date: 03/10/2020

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via DAV or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 16813862 Document Date: 03/10/2020

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Sequence Listing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via DAV or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: March 1, 2019

==========

Sequence Listing could not be accepted due to errors.
See attached Validation Report.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Anjum, Durreshwar
Timestamp: [year=2020; month=3; day=10; hr=16; min=12; sec=50; ms=860;]

==========

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Reviewer Comments:
NOTE: This error report refers to the sequence listing CRF received by the USPTO on:3/10/2020

For any sequence listing-related questions or concerns the Applicant is invited to contact the USPTO Sequence Help Desk at 571-272-2510.

****

1.

W213 Artificial or Unknown found in <213> in SEQ ID (70)

For SEQ ID# 70, has a missing mandatory feature section "<220>-<223>" when using "Artificial sequence" for numeric identifier "<213>". Please insert a separate feature to explain the source of genetic material.

For all sequences using “Artificial sequence”, for numeric identifier <213>, a mandatory feature is required to explain the source of the genetic material. The feature consists of numeric identifier <220>, which remains blank and numeric identifier <223>, which states the source of the genetic material. To explain the source, if the sequence is put together from several organisms, please list those organisms. If the sequence is made in the laboratory, please indicate that the sequence is synthesized.

2.

The messages shown below do not indicate non-compliance with the sequence rules, and do not require correction.

E287 Invalid WIPO ST.2 date format; Use (YYYY-MM-DD) in <141>
W213 Artificial or Unknown found in <213> in SEQ ID (1)
W402 Undefined organism found in <213> in SEQ ID (2)
W402 Undefined organism found in <213> in SEQ ID (3)
W402 Undefined organism found in <213> in SEQ ID (4)
W402 Undefined organism found in <213> in SEQ ID (5)
W402 Undefined organism found in <213> in SEQ ID (6)
W402 Undefined organism found in <213> in SEQ ID (7)
W402 Undefined organism found in <213> in SEQ ID (8)
W402 Undefined organism found in <213> in SEQ ID (9)
W402 Undefined organism found in <213> in SEQ ID (10)
W402 Undefined organism found in <213> in SEQ ID (11)
W402 Undefined organism found in <213> in SEQ ID (12)
W402 Undefined organism found in <213> in SEQ ID (13)
W402 Undefined organism found in <213> in SEQ ID (14)
W402 Undefined organism found in <213> in SEQ ID (15)
W402 Undefined organism found in <213> in SEQ ID (16)
W447 n or Xaa used, for: SEQID(16) on line number 4760
W447 n or Xaa used, for: SEQID(16) on line number 4764
W402 Undefined organism found in <213> in SEQ ID (17)
W447 n or Xaa used, for: SEQID(17) on line number 4901
W402 Undefined organism found in <213> in SEQ ID (18)
W402 Undefined organism found in <213> in SEQ ID (19)
W402 Undefined organism found in <213> in SEQ ID (20)
W402 Undefined organism found in <213> in SEQ ID (21)
W447 n or Xaa used, for: SEQID(65) on line number 11387
W447 n or Xaa used, for: SEQID(65) on line number 11451
W447 n or Xaa used, for: SEQID(70) on line number 12034
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W213 Artificial or Unknown found in <213> in SEQ ID (71)

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W213 Artificial or Unknown found in <213> in SEQ ID (79)
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W213 Artificial or Unknown found in <213> in SEQ ID (90)

****

Note:

The errors listed above are exemplary and may not be a complete list of all errors in the sequence listing file.

To correct the sequence listing errors noted in this report - The recommended method for correction of errors is to access the sequence listing working file using the software program in which the listing was originally prepared, e.g., the project file in PatentIn, make any necessary corrections within that program, then generate a new sequence listing file. Use of a word processing program to correct errors directly in the original sequence listing file is strongly discouraged, since such programs often introduce unintended changes to the sequence listing, rendering the listing unacceptable. When the working file or original program is not available for correction, then use of a common or plain text-only editor, such as NotePad, to edit the original sequence listing file may suffice.

A sequence listing text file submitted via EFS-Web that complies with the requirements of 37 CFR 1.824(a) (2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), serves as both the paper

copy required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e).

****

Validated By CRFValidator v 1.0.5

Application No: 16813862 Version No: 1.0

Input Set:

Output Set:

Started: 2020-03-10 10:38:01.600
Finished: 2020-03-10 10:38:04.729
Elapsed: 0 hr(s) 0 min(s) 3 sec(s) 129 ms
Total Warnings: 135
Total Errors: 1
No. of SeqIDs Defined: 117
Actual SeqID Count: 117

Error code		Error Description
E	287	Invalid WIPO ST.2 date format; Use (YYYY-MM-DD) in <141>
W	213	Artificial or Unknown found in <213> in SEQ ID (1)
W	402	Undefined organism found in <213> in SEQ ID (2)
W	402	Undefined organism found in <213> in SEQ ID (3)
W	402	Undefined organism found in <213> in SEQ ID (4)
W	402	Undefined organism found in <213> in SEQ ID (5)
W	402	Undefined organism found in <213> in SEQ ID (6)
W	402	Undefined organism found in <213> in SEQ ID (7)
W	402	Undefined organism found in <213> in SEQ ID (8)
W	402	Undefined organism found in <213> in SEQ ID (9)
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W	402	Undefined organism found in <213> in SEQ ID (15)
W	402	Undefined organism found in <213> in SEQ ID (16)
W	447	n or Xaa used, for: SEQID(16) on line number 4760
W	447	n or Xaa used, for: SEQID(16) on line number 4764
W	402	Undefined organism found in <213> in SEQ ID (17)

Input Set:

Output Set:

Started: 2020-03-10 10:38:01.600
Finished: 2020-03-10 10:38:04.729
Elapsed: 0 hr(s) 0 min(s) 3 sec(s) 129 ms
Total Warnings: 135
Total Errors: 1
No. of SeqIDs Defined: 117
Actual SeqID Count: 117

Error code	Error Description
W 447	n or Xaa used, for: SEQID(17) on line number 4901
W 402	Undefined organism found in <213> in SEQ ID (18)
W 402	Undefined organism found in <213> in SEQ ID (19)
W 402	Undefined organism found in <213> in SEQ ID (20)
W 402	Undefined organism found in <213> in SEQ ID (21) This error has occured more than 20 times, will not be displayed
W 447	n or Xaa used, for: SEQID(65) on line number 11387
W 447	n or Xaa used, for: SEQID(65) on line number 11451
W 213	Artificial or Unknown found in <213> in SEQ ID (70)
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W 213	Artificial or Unknown found in <213> in SEQ ID (71)

Input Set:

Output Set:

Started: 2020-03-10 10:38:01.600
Finished: 2020-03-10 10:38:04.729
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Total Warnings: 135
Total Errors: 1
No. of SeqIDs Defined: 117
Actual SeqID Count: 117

Error code	Error Description
W 213	Artificial or Unknown found in <213> in SEQ ID (74)
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W 213	Artificial or Unknown found in <213> in SEQ ID (89)
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This error has occured more than 20 times, will not be displayed

SEQUENCE LISTING

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/813,862	03/10/2020		0.00	I0422.70125US01	17	1

CONFIRMATION NO. 9700

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

FILING RECEIPT

OC000000118406949

Date Mailed: 04/22/2020

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Assignment For Published Patent Application

Valneva Austria GmbH, Vienna, AUSTRIA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of 16/063,007 06/15/2018 PAT 10639365
which is a 371 of PCT/EP2016/082664 12/23/2016

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
EUROPEAN PATENT OFFICE (EPO) 16182845.4 08/04/2016 No Access Code Provided
EUROPEAN PATENT OFFICE (EPO) 16176049.1 06/23/2016 No Access Code Provided
EUROPEAN PATENT OFFICE (EPO) 16176025.1 06/23/2016 No Access Code Provided
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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/813,862**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No
Title

ZIKA VIRUS VACCINE

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/813,862

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	18 minus 20 =	*			OR	x 100 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 460 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							820
			TOTAL			TOTAL	2540

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/813,862	03/10/2020	Jana Barbero Calzado	I0422.70125US01

CONFIRMATION NO. 9700

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

FORMALITIES LETTER

OC000000116406950

Date Mailed: 04/22/2020

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.

Items 1) and 2) provide general guidance related to requirements for sequence disclosures.

1) 37 CFR 1.821(c) requires that patent applications which contain disclosures of nucleotide and/or amino acid sequences that fall within the definitions of 37 CFR 1.821(a) must contain a "Sequence Listing", *as a separate part of the disclosure*, which presents the nucleotide and/or amino acid sequences and associated information using the symbols and format in accordance with the requirements of 37 CFR 1.821-1.825. This "Sequence Listing" part of the disclosure may be submitted:
   a) via EFS-Web (see Section I.1 of the Legal Framework for EFS-Web (https://www.uspto.gov/patents-application-process/filing-online/legal-framework-efs-web), hereinafter

"Legal Framework") as an ASCII text file, together with an incorporation-by-reference of the material in the ASCII text file in a separate paragraph of the specification identifying:
   i) the name of the ASCII text file;
   ii) the date of creation; and
   iii) the size of the ASCII text file in bytes;
b) on compact disc(s) in duplicate according to 37 CFR 1.52(e)(1)(ii) and (4), labeled according to 37 CFR 1.52(e)(6), together with a statement that the duplicate compact discs are identical and an incorporation-by-reference of the material in the ASCII text file according to 37 CFR 1.52(e)(5) in a separate paragraph of the specification identifying:
   i) the name of the ASCII text file;
   ii) the date of creation; and
   iii) the size of the ASCII text file in bytes;
c) via EFS-Web as a PDF (not recommended); or
d) on paper.

2) 37 CFR 1.821(e) requires that a *copy* of the "Sequence Listing" must also be submitted in computer readable form (CRF) in accordance with the requirements of 37 CFR 1.824.
   a) If a "Sequence Listing" ASCII text file submitted via EFS-Web on the application filing date complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b), and applicant has not filed a "Sequence Listing" as a PDF, the text file will serve as both the "Sequence Listing" required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e), and the statement of identity under the "Legal Framework" is not required.
   b) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed via EFS-Web as a PDF, then the "Legal Framework" requires submission of a statement that the "Sequence Listing" content of the PDF copy and the ASCII text file copy submitted via EFS-Web are identical.
   c) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed on paper or compact disc, then 37 CFR 1.821(f) requires submission of a statement that the "Sequence Listing" content of the paper or compact disc copy and the CRF are identical.

Specific deficiencies and the required response to this notice are as follows:

- A copy of the "Sequence Listing" in computer readable form (CRF) has been submitted. However, the content of the CRF does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated in the "Error Report" that indicates the "Sequence Listing" could not be accepted. Refer to attachment or PAIR document "Computer Readable Form (CRF) for Sequence Listing - Defective" dated 03/10/2020.
  Applicant must provide:
  - A "Sequence Listing" part of the disclosure, as described above in any one of items 1) a), b), c), or d); **together with**
  - **An amendment specifically directing its entry into the application;**
  - A statement that the "Sequence Listing" includes no new matter; and
  - A statement that indicates support for the amendment in the application, as filed, as required by 37 CFR 1.825 and the "Legal Framework".
- If the "Sequence Listing" part of the disclosure is submitted according to item 1) a) or b) above, Applicant must also provide:
  - A substitute specification in compliance with 37 CFR 1.121(b)(3) and 1.125 inserting the required incorporation-by-reference paragraph, consisting of:
    - A copy of the previously-submitted specification, with deletions shown with strikethrough or brackets and insertions shown with underlining (marked-up version);
    - A copy of the amended specification without markings (clean version); and
    - A statement that the substitute specification contains no new matter.
- If the "Sequence Listing" part of the disclosure is submitted according to item 1) b), c), or d) above, Applicant must also provide:
  - A CRF; and
  - Statement according to item 2) b) or c) above.

For questions regarding compliance to these requirements, please contact:

- **For Rules interpretation, call (571) 272-2510**
- **For CRF submission help, call (571) 272-2510**
- **For PatentIn software program support technical assistance, call (866) 217-9197.**
- **Send e-mail correspondence for PatentIn software program help to ebc@uspto.gov**
- **PatentIn software is available at http://www.uspto.gov/patents/resources/tools/checker/patentinrel.jsp .**

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

- The statutory basic filing fee is missing.
- The application search fee must be submitted.
- The application examination fee must be submitted.
- Additional claim fees of $ **820** as an undiscounted entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. No entity status discount is in effect. If applicant is qualified for small entity status, a written assertion of small entity status must be submitted to establish small entity status. (See 37 CFR 1.27). If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **300** basic filing fee.
- $ **160** surcharge.
- $ **660** search fee.
- $ **760** examination fee.
- $ **820** for multiple dependent claim surcharge.
- $(**0**) previous unapplied payment amount.
- $ **2700** TOTAL FEE BALANCE DUE.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/sareebuddin/

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
16813862	

Applicant(s) Jana Barbero Calzado

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1					
2		1				
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Total Claims	18		0		0	

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PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT6168669

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	SECURITY INTEREST

CONVEYING PARTY DATA

Name	Execution Date
VALNEVA SE	06/23/2020
VALNEVA AUSTRIA GMBH	06/23/2020

RECEIVING PARTY DATA

Name:	WILMINGTON TRUST, NATIONAL ASSOCIATION
Street Address:	50 SOUTH SIXTH STREET, SUITE 1290
City:	MINNEAPOLIS
State/Country:	MINNESOTA
Postal Code:	55402

PROPERTY NUMBERS Total: 4

Property Type	Number
Application Number:	16815215
Application Number:	16840760
Application Number:	16641012
Application Number:	16813862

CORRESPONDENCE DATA

Fax Number:

Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.

Email: akwon@cov.com
Correspondent Name: COVINGTON & BURLING LLP
Address Line 1: ONE CITY CENTER, 850 TENTH ST NW
Address Line 4: WASHINGTON, D.C. 20001

ATTORNEY DOCKET NUMBER:	034550.00068
NAME OF SUBMITTER:	ASHLEY M. KWON
SIGNATURE:	/Ashley M. Kwon/
DATE SIGNED:	06/24/2020
	This document serves as an Oath/Declaration (37 CFR 1.63).

Total Attachments: 6
source=Valneva - Patent Security Agreement#page1.tif

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PATENT SECURITY AGREEMENT

This PATENT SECURITY AGREEMENT, dated as of June 23, 2020 (this "Agreement"), is made by the Grantors listed on the signature pages hereto (each a "Grantor" and collectively, the "Grantors"), in favor of Wilmington Trust, National Association, as Administrative Agent (in such capacity, the "Administrative Agent") for the Secured Parties.

W I T N E S S E T H :

WHEREAS, pursuant to the Credit Agreement, dated as of February 3, 2020 (as amended, restated, supplemented or otherwise modified from time to time, the "Credit Agreement"), by and among the Borrower, Holdings, the Lenders (as defined therein), and the Administrative Agent, the Lenders have extended Commitments to make Loans to the Borrower;

WHEREAS, in connection with the Credit Agreement, each Grantor and its Affiliates have executed and delivered a Pledge and Security Agreement in favor of the Administrative Agent, for the benefit of the Secured Parties, dated as of March 4, 2020 (as amended, supplemented or otherwise modified from time to time, the "Security Agreement");

WHEREAS, pursuant to the Credit Agreement and pursuant to clause (d) of Section 4.5 of the Security Agreement, each Grantor is required to execute and deliver this Agreement and to grant to the Administrative Agent, for the benefit of the Secured Parties, a continuing security interest in all of the Patent Collateral (as defined below) to secure all of the Obligations; and

WHEREAS, each Grantor has duly authorized the execution, delivery and performance of this Agreement;

NOW, THEREFORE, for good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, each Grantor agrees, for the benefit of the Administrative Agent, as follows:

Section 1 Definitions. Unless otherwise defined herein or the context otherwise requires, terms used in this Agreement, including its preamble and recitals, have the meanings provided (or incorporated by reference) in the Security Agreement.

Section 2 Grant of Security Interest. Each Grantor hereby grants to the Administrative Agent, for the benefit of the Secured Parties, a continuing security interest in all of such Grantor's right, title and interest in and to the following property, whether now or hereafter existing or acquired by such Grantor (the "Patent Collateral"):

(a) all of its letters patent and applications for letters patent throughout the world, including each patent and patent application referred to in Item A of Schedule I attached hereto;

(b) all reissues, divisions, continuations, continuations-in-part, extensions, renewals and reexaminations of any of the items described in clause (a);

(c) all patent licenses and other agreements providing such Grantor with the right to use any of items of the type referred to in clauses (a) and (b) above, including each exclusive patent license referred to in Item B of Schedule I attached; and

(d) all Proceeds of, and rights associated with, the foregoing (including royalties income, payments, claims, damages and Proceeds of infringement suits) and the right to sue third

parties for past, present or future infringements of any patent or patent application and for breach or enforcement of any patent license.

Section 3 Security Agreement. This Agreement has been executed and delivered by each Grantor for the purpose of registering the security interest of the Administrative Agent in the Patent Collateral with the United States Patent and Trademark Office. The security interest granted hereby has been granted in furtherance of, and not in limitation of, the security interest granted to the Administrative Agent for the benefit of the Secured Parties under the Security Agreement. The Security Agreement (and all rights and remedies of the Secured Parties thereunder) shall remain in full force and effect in accordance with its terms.

Section 4 Release of Liens. Upon (a) the Disposition of Patent Collateral in accordance with the Credit Agreement or (b) the occurrence of the Termination Date, the security interest granted herein shall automatically terminate with respect to (i) such Patent Collateral (in the case of clause (a)) or (ii) all Patent Collateral (in the case of clause (b)). Upon any such Disposition or termination, the Administrative Agent will, at the Grantors' sole expense, deliver to the Grantor or Grantors, as applicable, without any representations, warranties or recourse of any kind whatsoever, all Patent Collateral held by the Administrative Agent hereunder, and execute and deliver to the Grantor or Grantors, as applicable, such documents as the Grantor or Grantors shall reasonably request to evidence such termination.

Section 5 Acknowledgment. Each Grantor does hereby further acknowledge and affirm that the rights and remedies of the Secured Parties with respect to the security interest in the Patent Collateral granted hereby are more fully set forth in the Security Agreement, the terms and provisions of which (including the remedies provided for therein) are incorporated by reference herein as if fully set forth herein.

Section 6 Loan Document. This Agreement is a Loan Document executed pursuant to the Credit Agreement and shall (unless otherwise expressly indicated herein) be construed, administered and applied in accordance with the terms and provisions thereof, including Article X thereof.

Section 7 Effectiveness. This Agreement shall become effective when a counterpart hereof executed by each Grantor, shall have been received by the Administrative Agent. Delivery of an executed counterpart of a signature page to this Agreement by email (in "pdf," "tiff" or similar format) or telecopy shall be effective as delivery of a manually executed counterpart of this Agreement.

[*Signature Page Follows*]

IN WITNESS WHEREOF, each Grantor hereto has caused this Agreement to be duly executed and delivered by its Authorized Officer(s) as of the date first above written.

VALNEVA SE

By: _____
Name: Frédéric Jacotot
Title: General Counsel

[*Signature Page to Patent Security Agreement*]

VALNEVA AUSTRIA GMBH

By: _____
Name: Thomas Lingelbach
Title: Managing Director

By: _____
Name: David Lawrence
Title: Managing Director

[*Signature Page to Patent Security Agreement*]

WILMINGTON TRUST, NATIONAL ASSOCIATION,
as Administrative Agent

By: _____

Name: Jessica A. Jankiewicz
Title: Assistant Vice President

Pending Patent Applications

Valneva SE

Docket ID	Country	Application No Application Date	Publication No Publication Date
PAT012/US/1	United States of America	16/815,215 04-Apr-2016	
PAT022/US/1	United States of America	16/840,760 23-Dec-2016	
PAT028/US	United States of America	16/641,012 23-Dec-2016	WO2019/057793 28-Mar-2019

Valneva Austria GmbH

Docket ID	Country	Application No Application Date	Publication No Publication Date
PAT018/US/1	United States of America	16/813,862 23-Dec-2016	

Docket No.: I0422.70125US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Jana Barbero Calzado
Application No.:	16/813,862
Confirmation No.:	9700
Filed:	March 10, 2020
For:	ZIKA VIRUS VACCINE
Examiner:	Not Yet Assigned
Art Unit:	Not Yet Assigned

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: July 22, 2020 Electronic Signature for Taryn Bollers: /Taryn Bollers/

FIRST PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Drawings begin on page 3 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 4 of this paper.

Remarks/Arguments begin on page 8 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please enter the Substitute Specification (clean version) and Substitute Sequence Listing filed concurrently herewith into the specification. The content of the Substitute Specification and Substitute Sequence Listing, which is filed as a text file herewith, contain no new matter.

AMENDMENTS TO THE DRAWINGS

Applicant submits herewith Replacement Sheets (23 sheets), a full set of Figures including a clean version of amended Figs. 1-21. Please replace the original sheets as filed with the attached Replacement Sheets. No new matter has been added.

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows. Applicant presents a full set of claims showing markups of the claims with insertions and deletions indicated by underlining and strikethrough text (or double bracketing), respectively.

1.- 17. (Canceled)

18. (New) A Zika virus vaccine comprising a Zika virus having an RNA genome corresponding to the DNA sequence provided by SEQ ID NO: 72 or a variant nucleic acid having at least 80% identity to SEQ ID NO: 72, wherein said Zika virus vaccine is capable of stimulating a neutralizing antibody titer greater than 15 in at least 70% of vaccinated subjects, wherein the neutralizing antibody titer is determined using a microneutralization assay (MN_{50}).

19. (New) The Zika virus vaccine according to claim 18, wherein said MN_{50} is greater than 20, 25, 30, 35, 40, 45, preferably greater than 50, 55, 60, 65, 70, 75, 80, 85, most preferably greater than or equal to 90.

20. (New) The Zika virus vaccine according to claim 18, wherein the Zika virus vaccine is capable of stimulating a MN_{50} titer greater than 15 in at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects, preferably in at least 80% of subjects.

21. (New) The Zika virus vaccine according to claim 18, wherein the Zika virus comprises an E protein having an amino acid sequence provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.

22. (New) The Zika virus vaccine according to claim 18, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

23. (New) The Zika virus vaccine according to claim 22, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.

24. (New) The Zika virus vaccine according to claim 22, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

25. (New) The Zika virus vaccine according to claim 24, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

26. (New) The Zika virus vaccine according to claim 22, wherein the chemical activation is performed at about +4°C or about +22°C.

27. (New) The Zika virus vaccine according to claim 18, further comprising an adjuvant.

28. (New) The Zika virus vaccine according to claim 27, wherein the adjuvant is an aluminium salt adjuvant.

29. (New) The Zika virus vaccine according to claim 28, wherein said aluminium salt adjuvant is aluminium hydroxide with less than 1.25 parts per billion (ppb) copper (Cu) based on a final pharmaceutical composition comprising the Zika virus.

30. (New) The Zika virus vaccine according to claim 27, wherein the adjuvant comprises a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).

31. (New) The Zika virus vaccine according to claim 30, wherein the peptide comprises the sequence $KLKL_5KLK$ (SEQ ID NO: 71) and the I-ODN comprises oligo-$d(IC)_{13}$ (SEQ ID NO: 70).

32. (New) The Zika virus vaccine according to claim 18, further comprising one or more pharmaceutically acceptable excipients.

33. (New) The Zika virus vaccine according to claim 18, wherein the vaccine comprises protamine sulphate (PS) or fragments or break-down products of PS at amounts below the limits of detection by high performance liquid chromatography (HPLC), i.e., below 1 μg/mL, especially below 100 ng/mL.

34. (New) The Zika virus vaccine according to claim 33, wherein said PS or fragments or break-down products of PS are detectable by mass spectroscopy or another sensitive method.

35. (New) A method for purifying infectious Zika virus, comprising the steps of

i) providing a crude harvest (a) comprising infectious Zika virus particles, non-infectious Zika virus particles, and impurities, wherein the impurities are generated from growing said Zika virus on a cell substrate;

ii) contacting said crude harvest (a) with an agent comprising protamine to obtain a Zika virus preparation (b) comprising infectious Zika virus particles; and

iii) further purifying said Zika virus preparation (b) by one or more size exclusion methods, wherein said one or more size exclusion methods comprise

(A) sucrose density gradient centrifugation,

(B) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the pores comprise a molecular weight cut-off that excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cut-off of the pores can enter the ligand-activated core, and/or

(C) size exclusion chromatography;

to obtain a purified Zika virus preparation (c) comprising infectious Zika virus particles, less than 100 ng/mL residual host cell DNA, less than 1 μg/mL residual host cell protein, and less than 1 μg/mL residual aggregates of virus particles.

36. (New) The method according to claim 35, wherein the purified Zika virus preparation (c) comprises less than 10 ng/mL residual host cell DNA and less than 100 ng/mL residual host cell protein.

37. (New) The method according to claim 35, wherein said crude harvest (a) is subjected to one or more pre-purification step(s) prior to step ii), wherein the one or more pre-purification step(s) comprise

filtration using a filter having a pore size equal to or less than 0.2 μm,

digestion of host cell genomic DNA by enzymatic treatment, and/or

ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 100 kDa.

REMARKS

Applicant submits herewith both marked-up and clean versions of the Substitute Specification in which the brief description of the figures has been amended for consistency with the amended labeling of the Figures in the Replacement Sheets. Also submitted herewith is a Substitute Sequence Listing. No new matter has been added.

Claims 1-17 were previously pending in this application. Claims 1-17 have been canceled without prejudice or disclaimer. Claims 18-37 have been newly added. Support for the new claims can be found throughout the specification and claims as originally filed. No new matter has been added.

Upon entry of this amendment, claims 18-37 will be pending for examination, with claims 18 and 35 being independent claims. Applicant reserves the right to pursue the claims as previously pending or originally filed in one or more continuing applications.

Applicant believes no fee is due with this Amendment, other than the enclosed fees. However, if a fee is due with this Amendment, please charge our Deposit Account No. 23/2825, under Docket No. I0422.70125US01 from which the undersigned is authorized to draw.

Dated: July 22, 2020

Respectfully submitted,

Electronic signature: /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

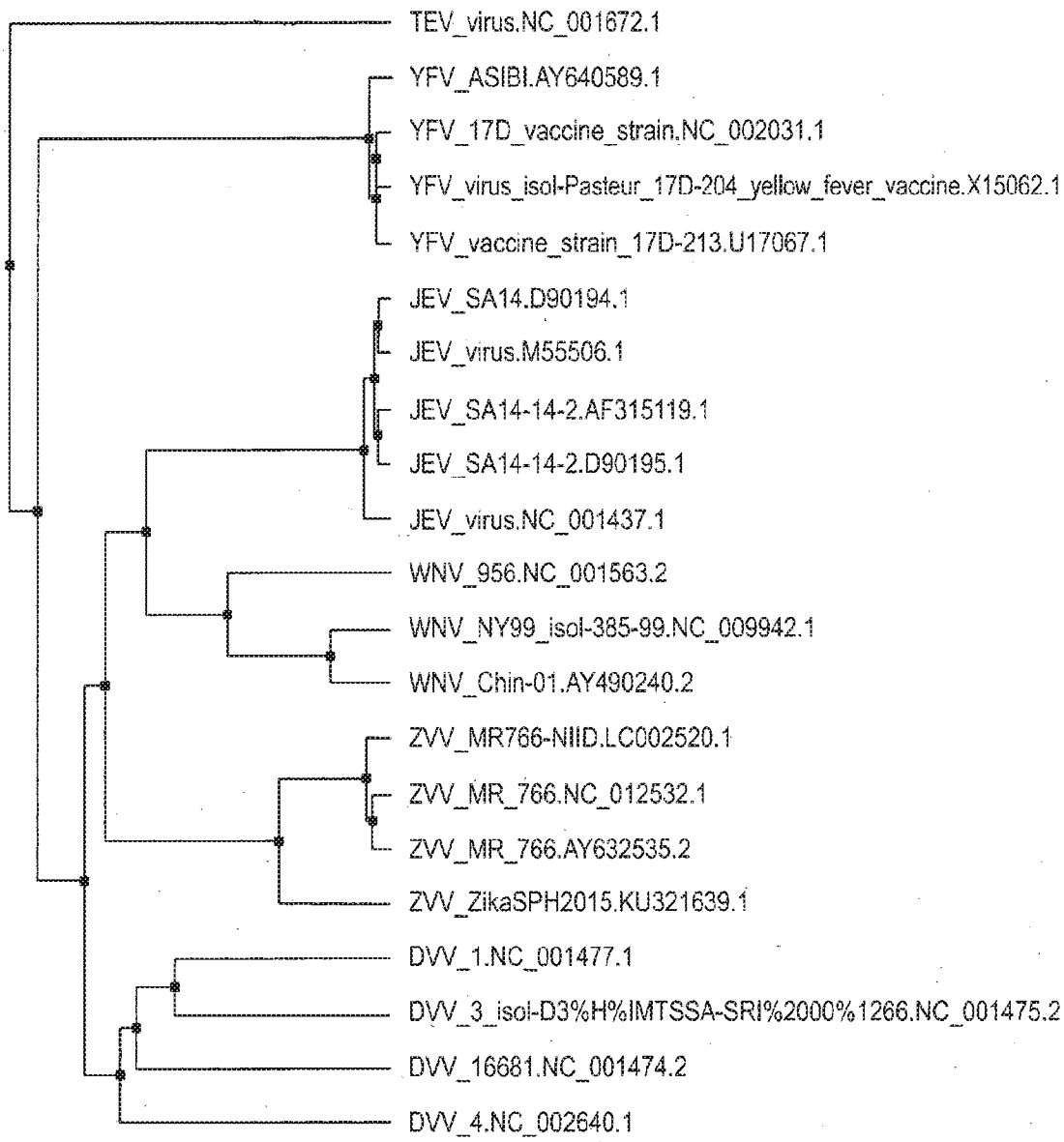

TEV_virus.NC_001672.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV_virus_isol-Pasteur_17D-204_yellow_fever_vaccine.X15062.1
YFV_vaccine_strain_17D-213.U17067.1
JEV_SA14.D90194.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
JEV_virus.NC_001437.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
DVV_1.NC_001477.1
DVV_3_isol-D3%H%IMTSSA-SRI%2000%1266.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1

FIG. 1

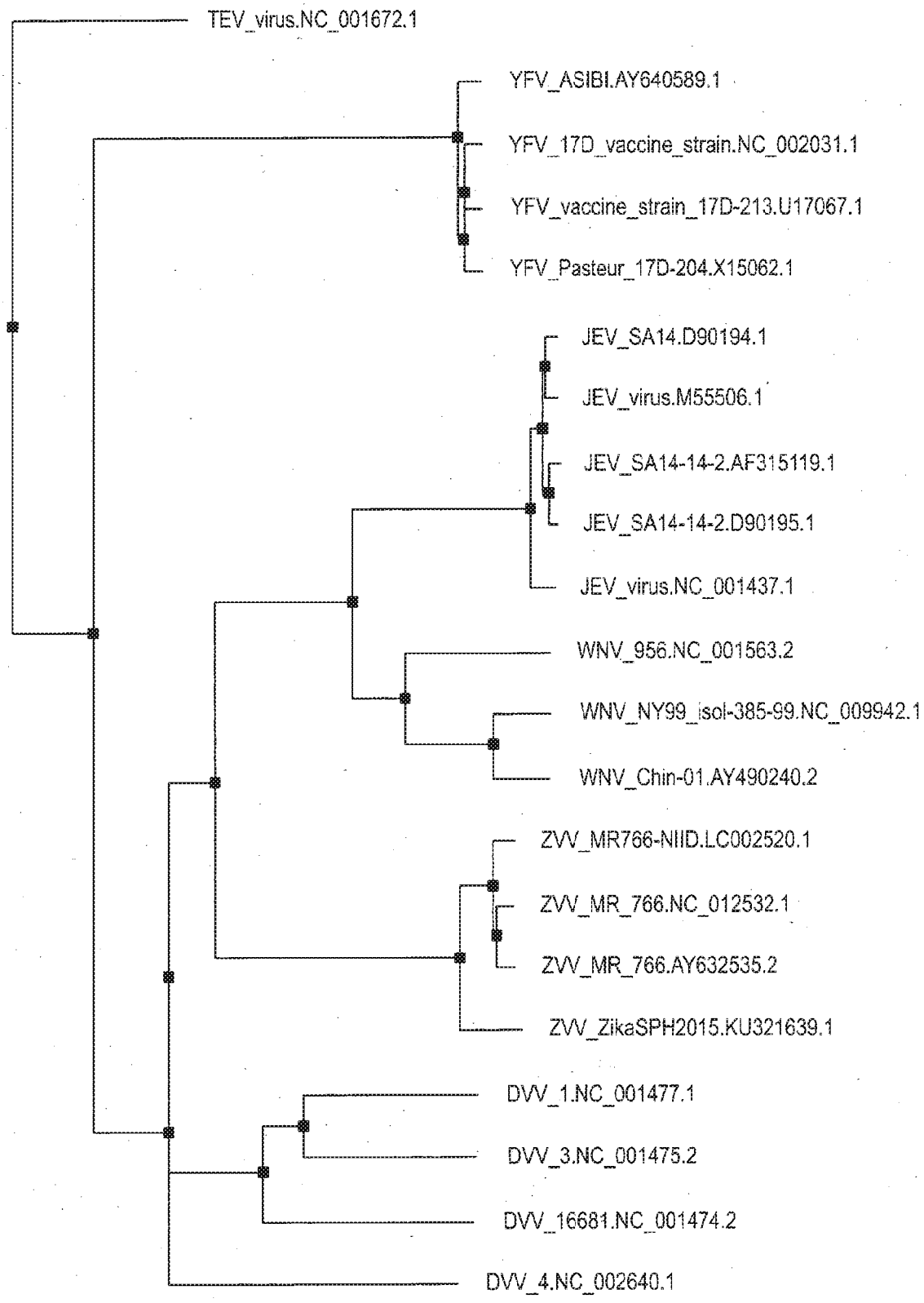
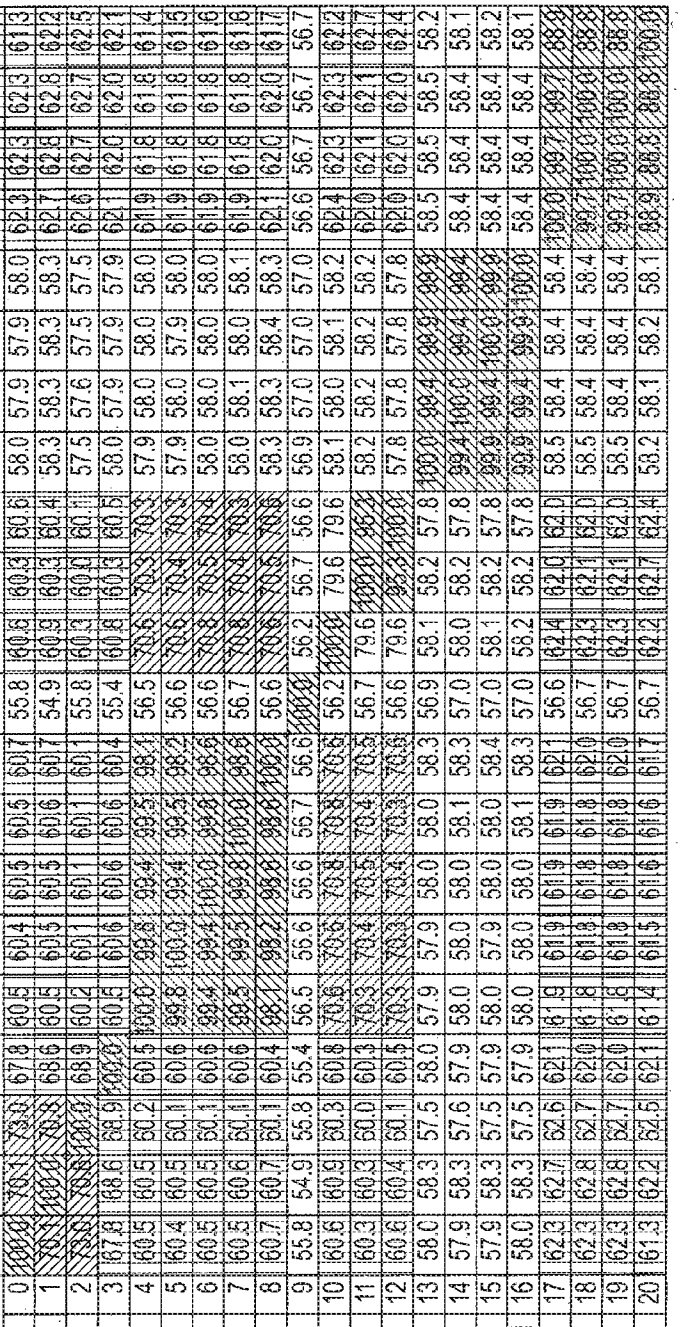

TEV_virus.NC_001672.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
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YFV_Pasteur_17D-204.X15062.1
JEV_SA14.D90194.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
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WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
DVV_1.NC_001477.1
DVV_3.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1

FIG. 2



	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DVV_1.NC_001477.1	0	100.0	70.1	73.0	67.8	60.5	60.4	60.5	60.6	60.7	55.8	60.6	60.3	60.6	58.0	57.9	57.9	58.0	62.3	62.3	61.3
DVV_16681.NC_001474.2	1	70.1	100.0	70.3	68.6	60.5	60.5	60.5	60.6	60.7	54.9	60.9	60.3	60.4	58.3	58.3	58.3	58.3	62.7	62.8	62.2
DVV_3.NC_001475.2	2	73.0	70.3	100.0	68.9	60.2	60.1	60.1	60.1	60.1	55.8	60.3	60.0	60.1	57.5	57.6	57.5	57.5	62.6	62.7	62.5
DVV_4.NC_002640.1	3	67.8	68.6	68.9	100.0	60.5	60.6	60.6	60.4	60.4	55.4	60.8	60.3	60.5	58.0	57.9	57.9	57.9	62.1	62.0	62.1
JEV_SA14-14-2.AF315119.1	4	60.5	60.5	60.2	60.5	100.0	99.6	99.4	99.5	98.4	56.5	70.6	70.3	70.3	58.0	58.0	58.0	58.0	61.9	61.8	61.4
JEV_SA14-14-2.D90195.1	5	60.4	60.5	60.1	60.6	99.8	100.0	99.4	99.5	98.2	56.6	70.5	70.4	70.3	57.9	58.0	57.9	58.0	61.9	61.8	61.5
JEV_SA14.D90194.1	6	60.5	60.5	60.1	60.6	99.4	99.4	100.0	99.8	98.6	56.6	70.8	70.5	70.4	58.0	58.0	58.0	58.0	61.9	61.8	61.6
JEV_virus.M55506.1	7	60.5	60.6	60.1	60.6	99.5	99.5	99.8	100.0	98.9	56.7	70.8	70.4	70.3	58.0	58.1	58.0	58.1	61.9	61.8	61.6
JEV_virus.NC_001437.1	8	60.7	60.7	60.1	60.4	98.1	98.2	98.6	98.9	100.0	56.6	70.6	70.5	70.5	58.3	58.4	58.3	58.3	62.1	62.0	61.7
TEV_virus.NC_001672.1	9	55.8	54.9	55.8	55.4	56.5	55.6	56.6	55.7	56.6	100.0	56.2	56.7	56.6	56.9	57.0	57.0	57.0	56.6	56.7	56.7
WNV_956.NC_001563.2	10	60.6	60.9	60.3	60.8	70.6	70.6	70.8	70.8	70.6	56.2	100.0	79.6	79.6	58.1	58.0	58.1	58.2	62.4	62.3	62.2
WNV_Chin-01.AY490240.2	11	60.3	60.3	60.0	60.3	70.3	70.4	70.5	70.4	70.5	56.7	79.6	100.0	95.2	58.2	58.2	58.2	58.2	62.0	62.1	62.7
WNV_NY99_isol-365-99.NC_009942.1	12	60.6	60.4	60.1	60.5	70.3	70.4	70.3	70.5	70.6	56.6	79.6	95.2	100.0	57.8	57.8	57.8	57.8	62.0	62.0	62.4
YFV_17D_vaccine_strain.NC_002031.1	13	58.0	58.3	57.5	58.0	57.9	57.9	58.0	58.3	56.9	58.1	58.2	57.8	100.0	99.4	99.4	99.4	58.5	58.5	58.5	58.2
YFV_ASIB.AY640589.1	14	57.9	58.3	57.6	57.9	58.0	58.0	58.1	58.3	57.0	58.0	58.2	57.8	99.4	100.0	99.4	99.4	58.4	58.4	58.4	58.1
YFV_Pasteur_17D-204.X15062.1	15	57.9	58.3	57.5	57.9	58.0	57.9	58.0	58.0	58.4	57.0	58.1	58.2	57.8	99.4	100.0	99.4	58.4	58.4	58.4	58.2
YFV_vaccine_strain_17D-213.U17067.1	16	58.0	58.3	57.5	57.9	58.0	58.0	58.1	58.3	57.0	58.2	58.2	57.8	99.4	99.4	99.4	100.0	58.4	58.4	58.4	58.1
ZVV_MR766-NIID.LC002520.1	17	62.3	62.7	62.6	62.1	61.9	61.9	61.9	62.1	56.6	62.4	62.1	62.0	58.5	58.4	58.4	58.4	100.0	99.7	99.7	88.9
ZVV_MR_766.AY632535.2	18	62.3	62.8	62.7	62.0	61.8	61.9	61.8	61.8	56.7	62.3	62.1	62.0	58.5	58.4	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_MR_766.NC_012532.1	19	62.3	62.8	62.7	62.0	61.9	61.8	61.8	61.8	56.7	62.3	62.1	62.0	58.5	58.4	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_ZikaSPH2015.KU321639.1	20	61.3	62.2	62.5	62.1	61.4	61.5	61.6	61.6	56.7	62.2	62.7	62.4	58.2	58.1	58.2	58.1	88.9	88.8	88.8	100.0

60 % Identity and higher
70 % Identity and higher
80 % Identity and higher

FIG. 3

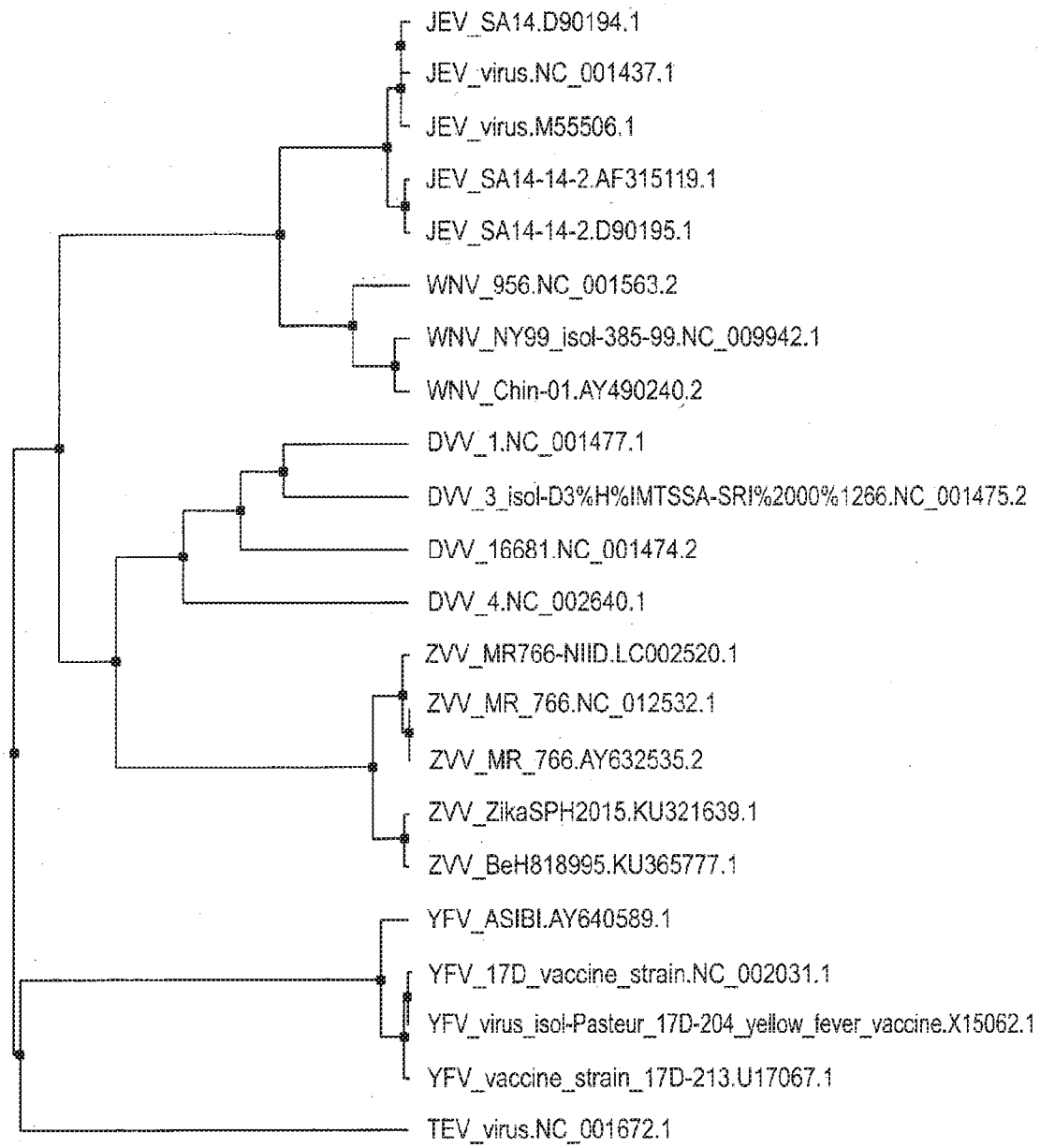

JEV_SA14.D90194.1
JEV_virus.NC_001437.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2
DVV_1.NC_001477.1
DVV_3_isol-D3%H%IMTSSA-SRI%2000%1266.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1
ZVV_MR766-NIID.LC002520.1
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ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
ZVV_BeH818995.KU365777.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV_virus_isol-Pasteur_17D-204_yellow_fever_vaccine.X15062.1
YFV_vaccine_strain_17D-213.U17067.1
TEV_virus.NC_001672.1

FIG. 4

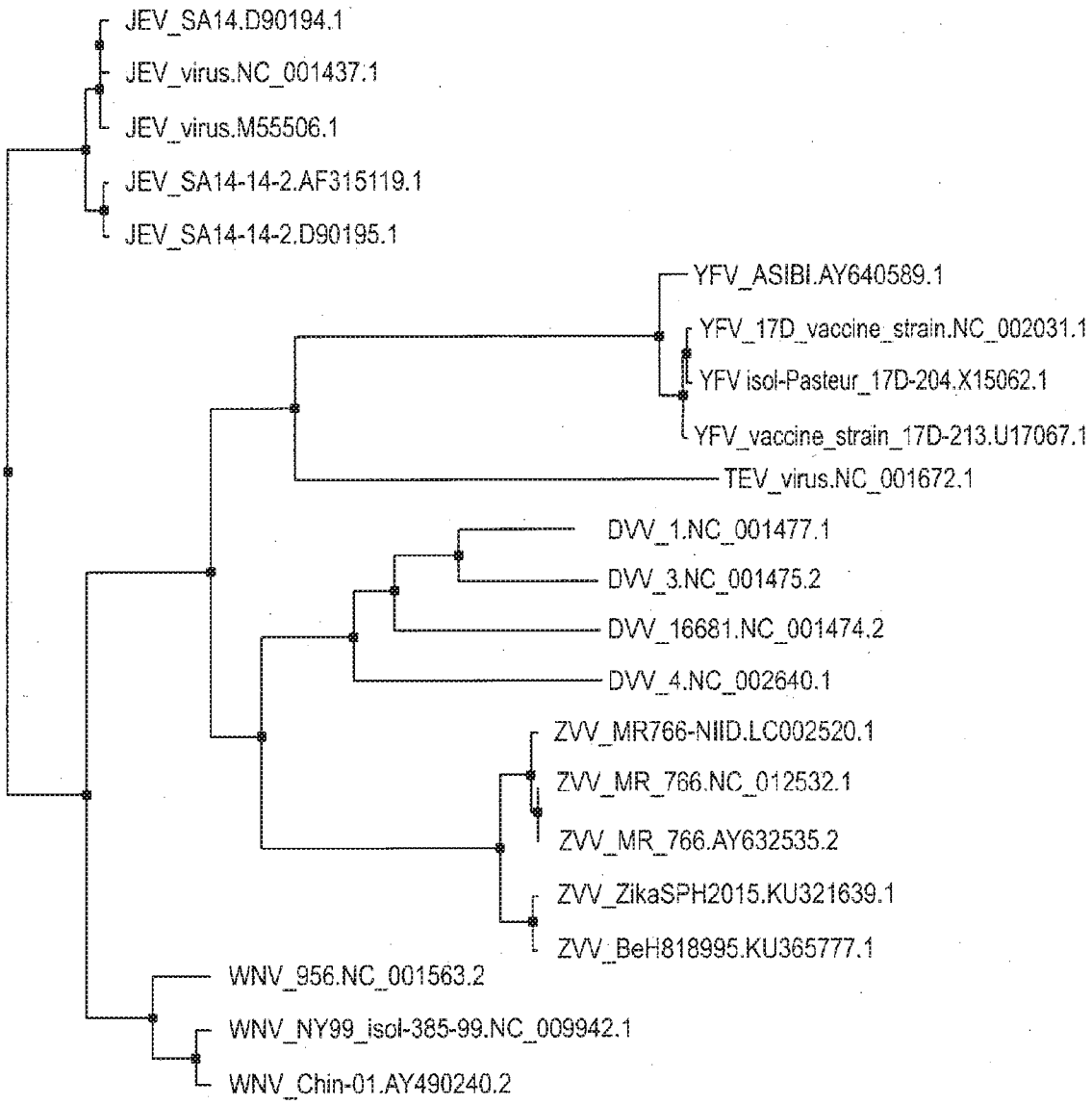

JEV_SA14.D90194.1
JEV_virus.NC_001437.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV isol-Pasteur_17D-204.X15062.1
YFV_vaccine_strain_17D-213.U17067.1
TEV_virus.NC_001672.1
DVV_1.NC_001477.1
DVV_3.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
ZVV_BeH818995.KU365777.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2

FIG. 5

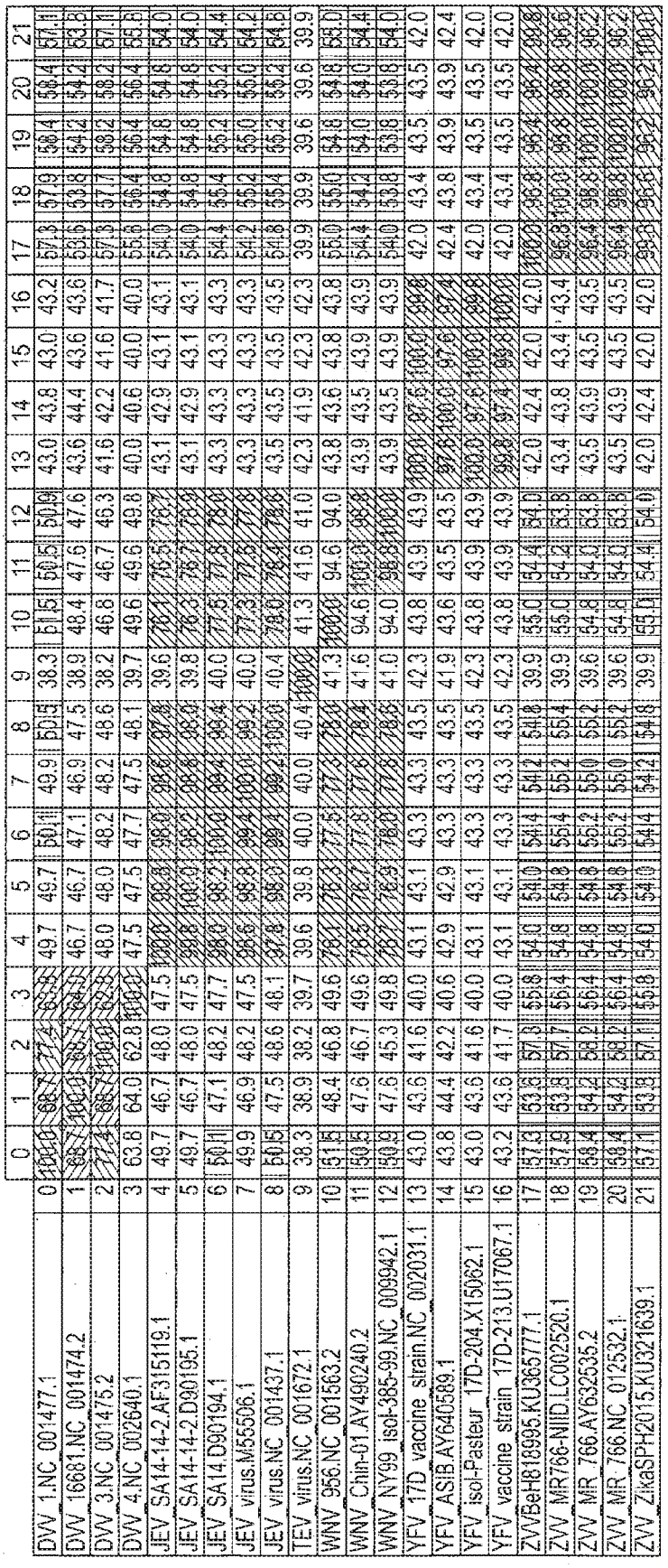

50 % Identity and higher
60 % Identity and higher
70 % Identity and higher

FIG. 6

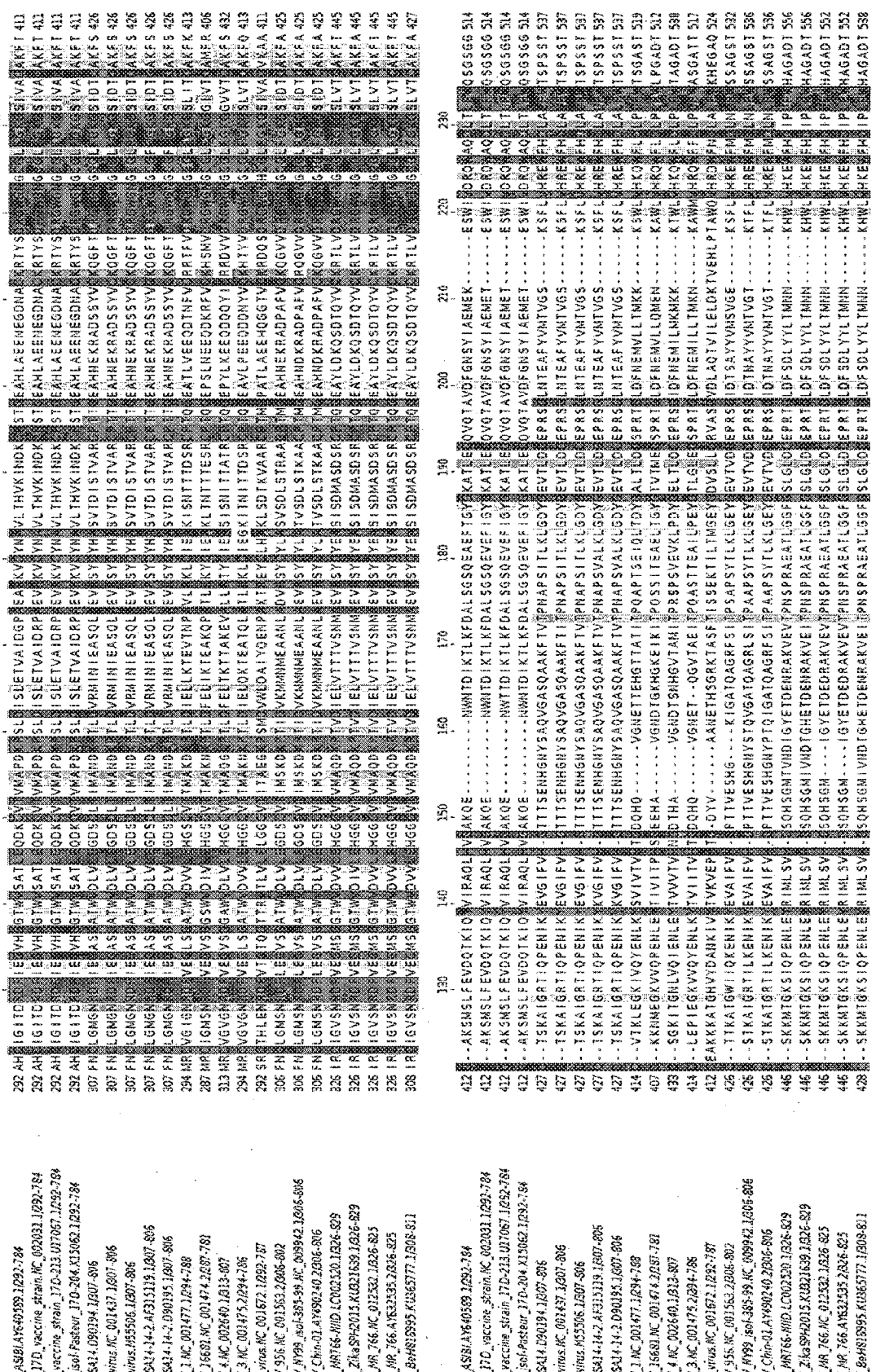


FIG. 7A

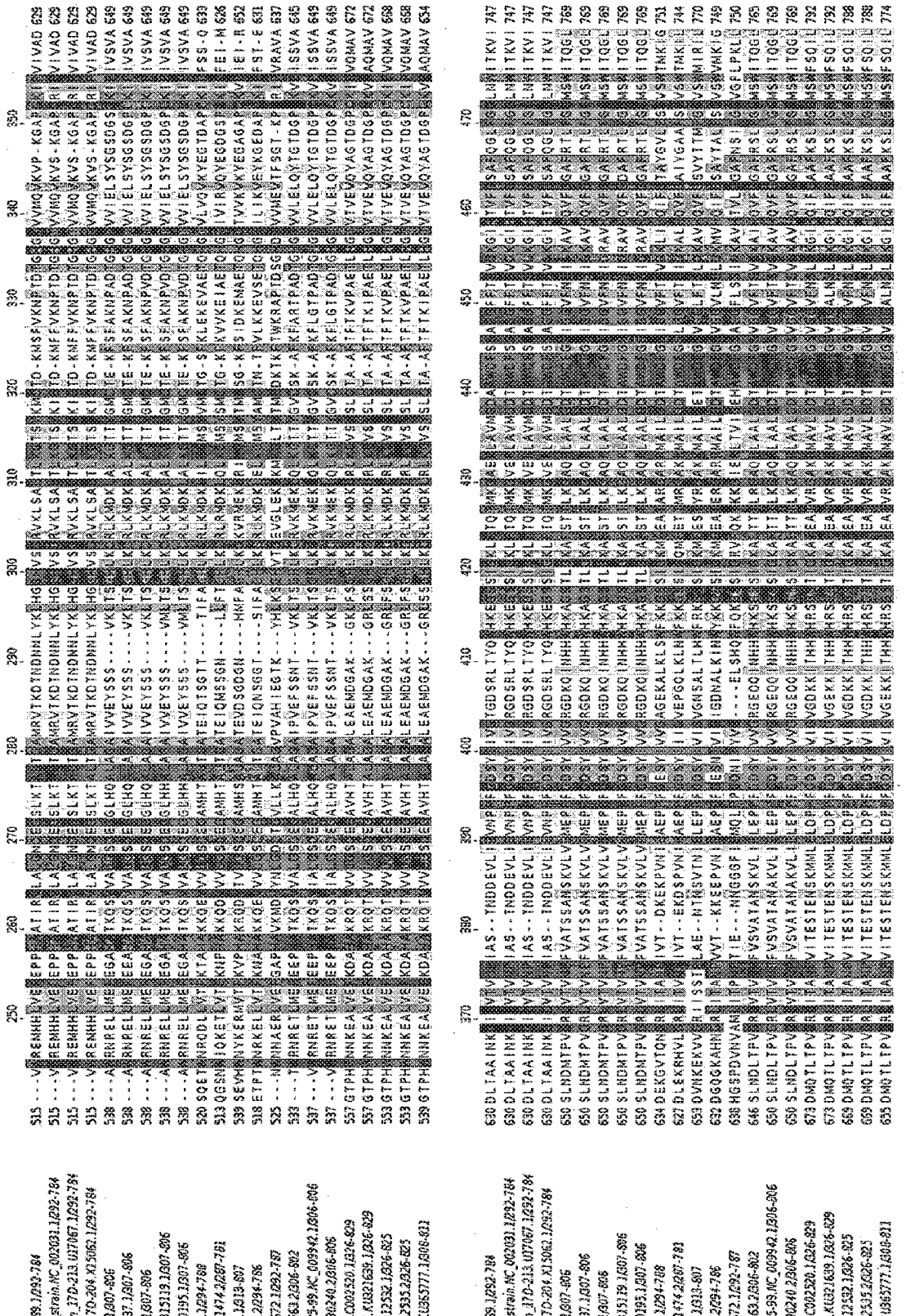


FIG. 7B

490
500
510
YFV_ASIBI.AY640589.1/292-784
YFV_17D_vaccine_strain.NC_002031.1/292-784
YFV_vaccine_strain_17D-213.U17067.1/292-784
YFV_isol-Pasteur_17D-204.X15062.1/292-784
IEV_SA14.D90194.1/307-806
IEV_virus.NC_001437.1/307-806
IEV_virus.M55506.1/307-806
IEV_SA14-14-2.AF315119.1/307-806
IEV_SA14-14-2.D90195.1/307-806
DVV_1.NC_001477.1/294-788
DVV_16681.NC_001474.2/287-781
DVV_4.NC_002640.1/313-807
DVV_3.NC_001475.2/294-786
TEV_virus.NC_001672.1/292-787
WNV_956.NC_001563.2/306-802
WNV_NY99_isol-385-99.NC_009942.1/306-606
WNV_Chin-01.AY490240.2/306-606
ZVV_MR766-NIID.LC002520.1/326-829
ZVV_ZikaSPH2015.KU321639.1/326-829
ZVV_MR_766.NC_012532.1/326-825
ZVV_MR_766.AY632535.2/326-825
ZVV_BeH818995.KU365777.1/308-811

FIG. 7C

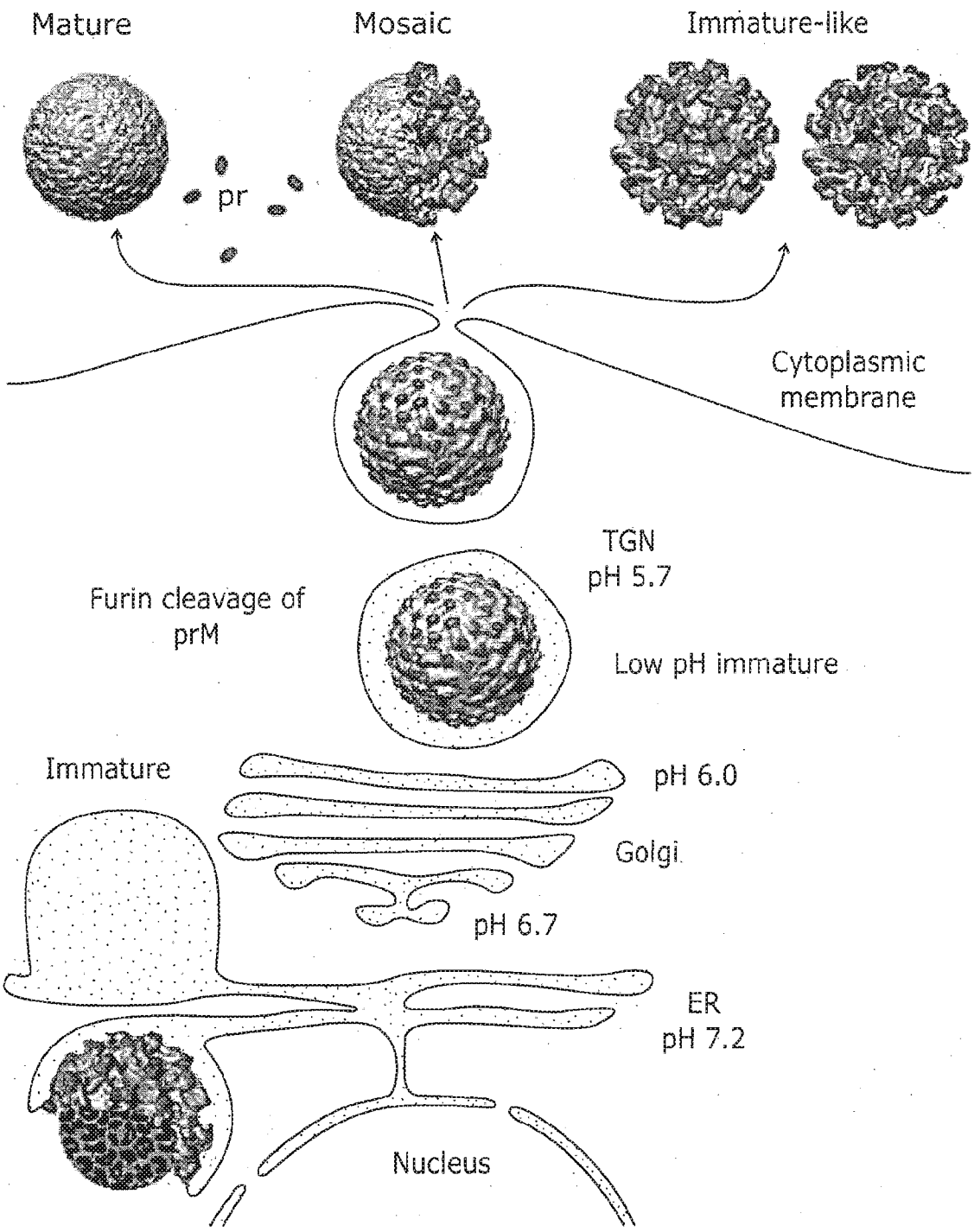

Mature
Mosaic
Immature-like
pr
Cytoplasmic membrane
TGN
pH 5.7
Furin cleavage of prM
Low pH immature
Immature
pH 6.0
Golgi
pH 6.7
ER
pH 7.2
Nucleus

FIG. 8

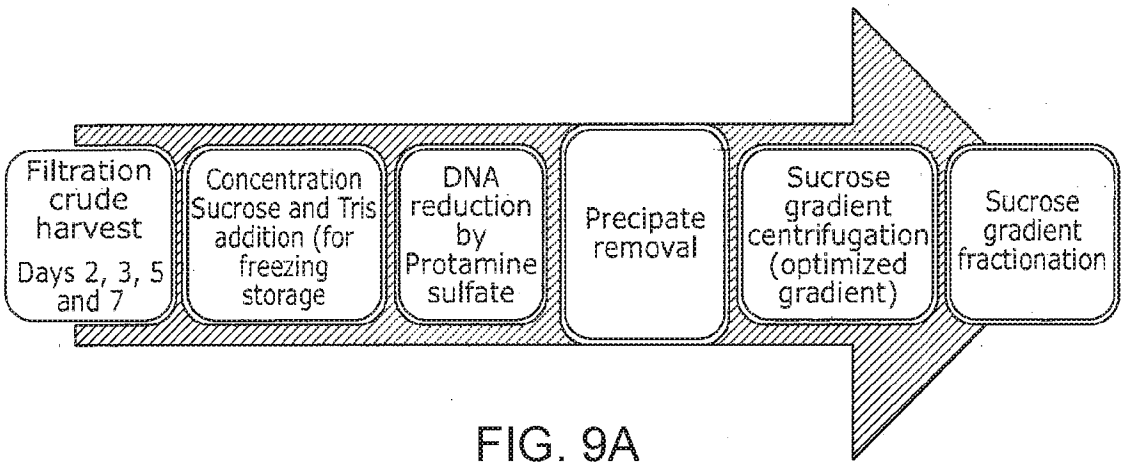

Filtration crude harvest
Days 2, 3, 5 and 7
Concentration Sucrose and Tris addition (for freezing storage
DNA reduction by Protamine sulfate
Precipate removal
Sucrose gradient centrifugation (optimized gradient)
Sucrose gradient fractionation

FIG. 9A

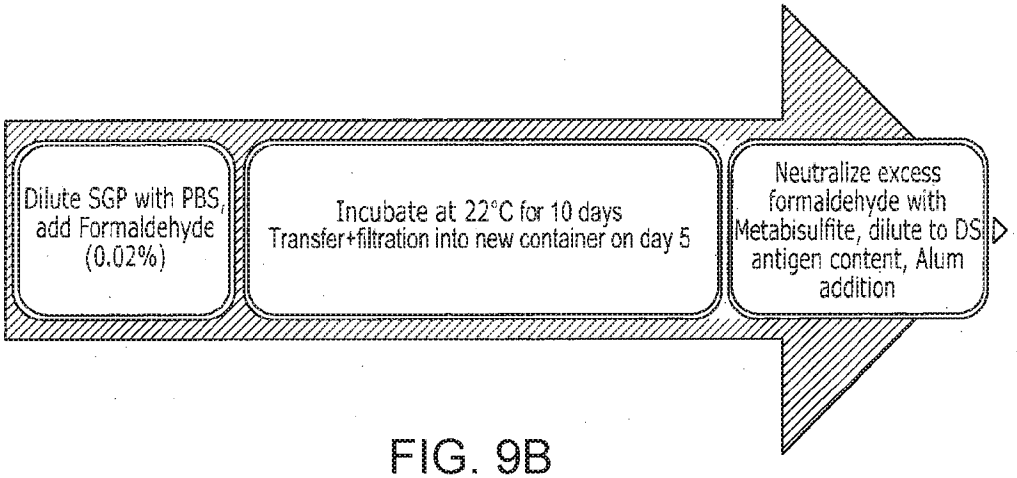

Dilute SGP with PBS, add Formaldehyde (0.02%)
Incubate at 22°C for 10 days
Transfer+filtration into new container on day 5
Neutralize excess formaldehyde with Metabisulfite, dilute to DS antigen content, Alum addition

FIG. 9B

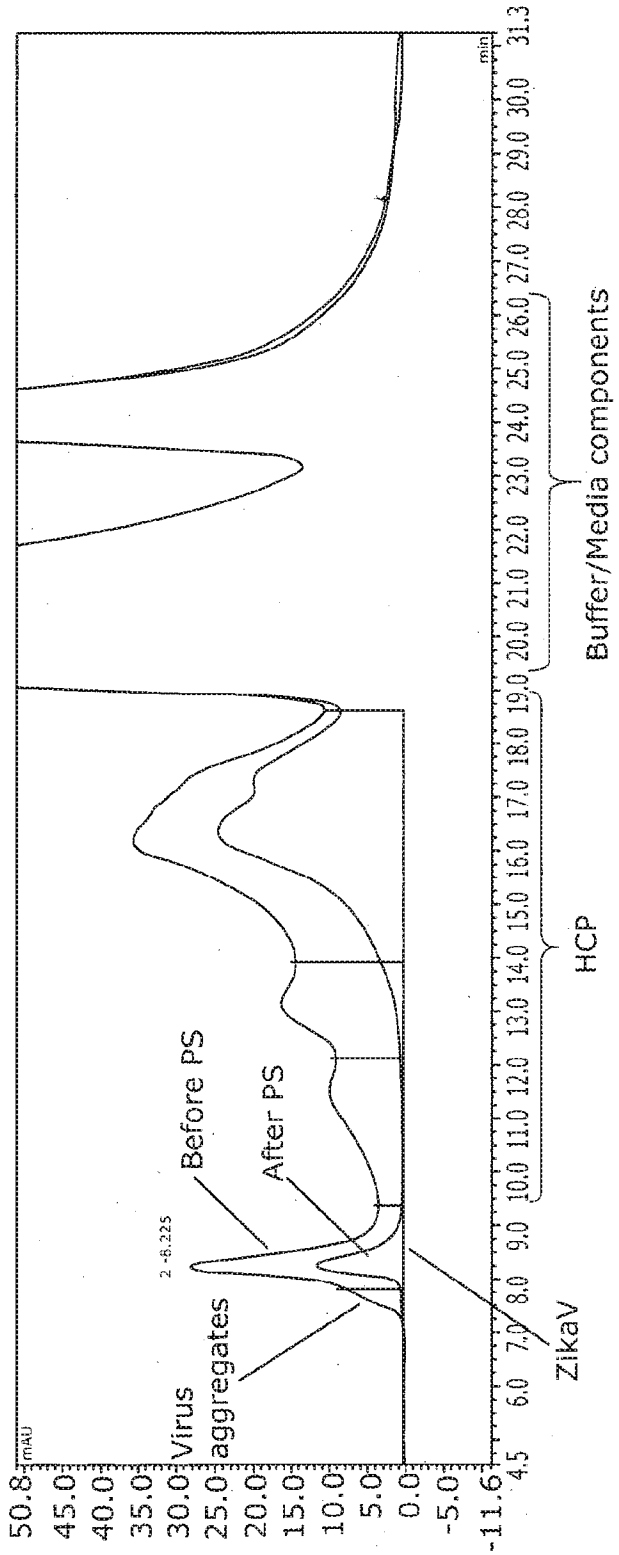
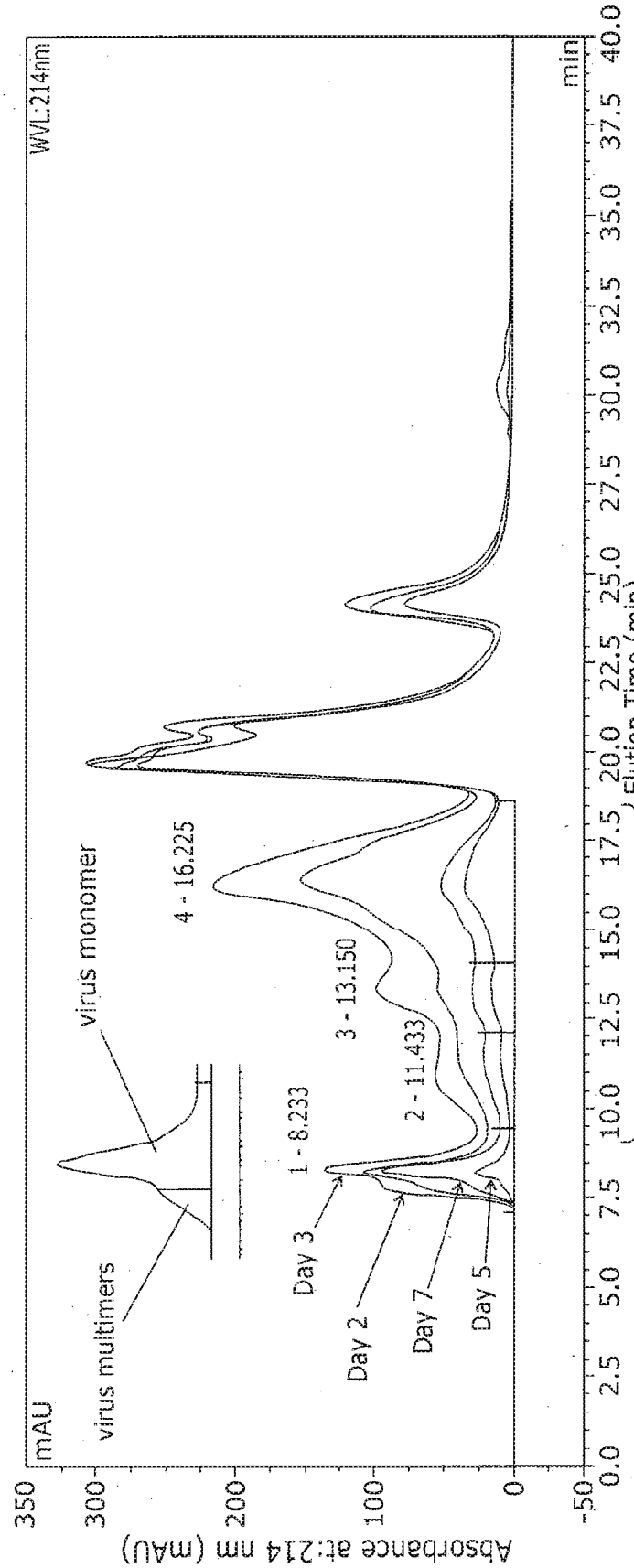

mAU
50.8
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-11.6
4.5
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0
25.0
26.0
27.0
28.0
29.0
30.0
31.3
min
2 - 8.225
Virus aggregates
Before PS
After PS
ZikaV
HCP
Buffer/Media components

FIG. 10



+ Similar virus concentration in harvests Day 2, Day3, Day 7; lower virus concentration in harvest Day 5
+ Day 2 and Day 3 harvests contained more HCP than Days 5/7 (correlates with observed CPE)
+ Minor amount of virus multimers observed

FIG. 11

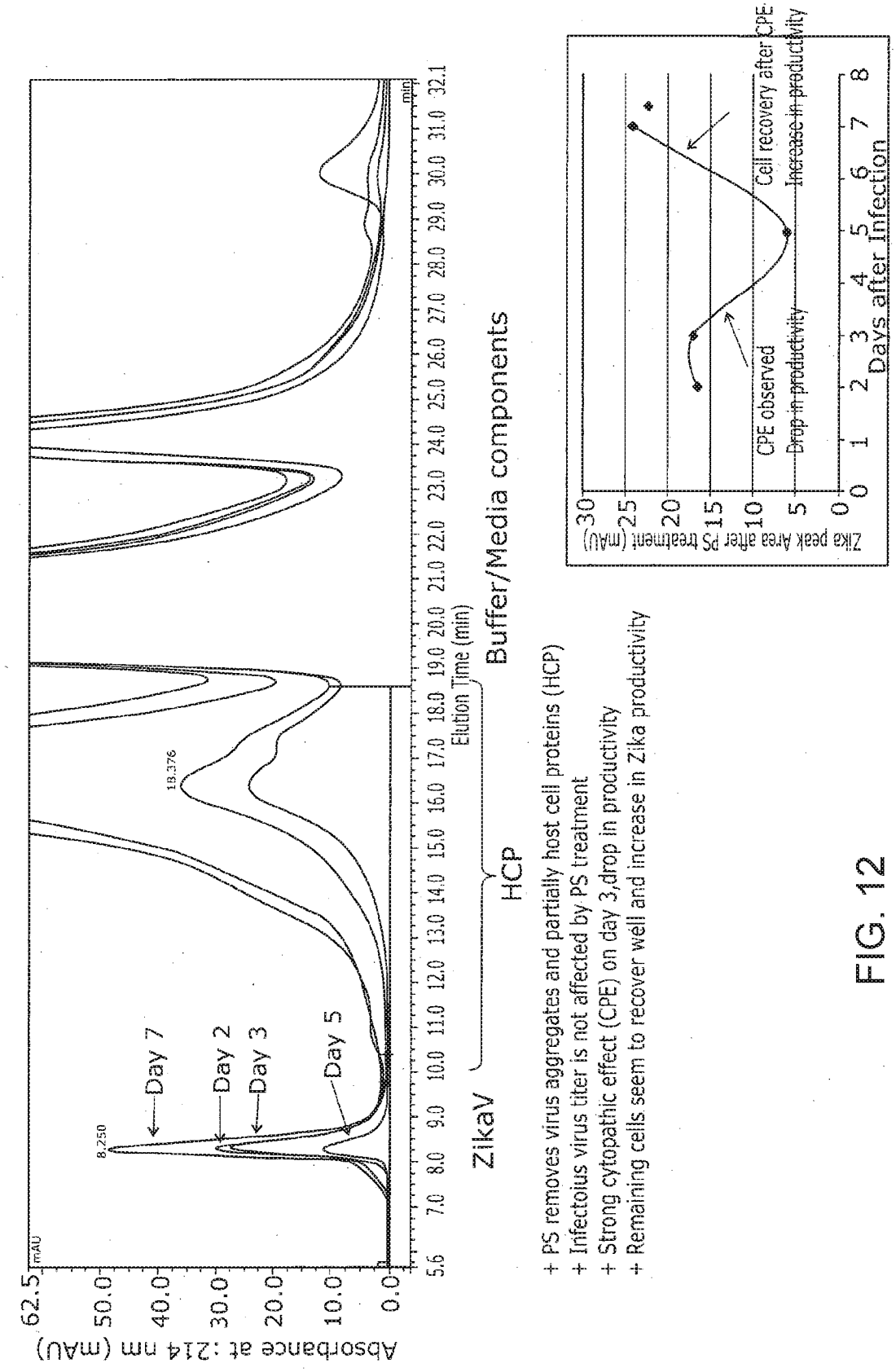

Absorbance at :214 nm (mAU)
mAU
62.5
50.0
40.0
30.0
20.0
10.0
0.0
min
5.6 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0 17.0 18.0 19.0 20.0 21.0 22.0 23.0 24.0 25.0 26.0 27.0 28.0 29.0 30.0 31.0 32.1
Elution Time (min)
8.250
18.376
Day 7
Day 2
Day 3
Day 5
ZikaV
HCP
Buffer/Media components
+ PS removes virus aggregates and partially host cell proteins (HCP)
+ Infectoius virus titer is not affected by PS treatment
+ Strong cytopathic effect (CPE) on day 3,drop in productivity
+ Remaining cells seem to recover well and increase in Zika productivity
Zika peak Area after PS treatment (mAU)
30
25
20
15
10
5
0
CPE observed
Drop in productivity
Cell recovery after CPE
Increase in productivity
0 1 2 3 4 5 6 7 8
Days after Infection

FIG. 12

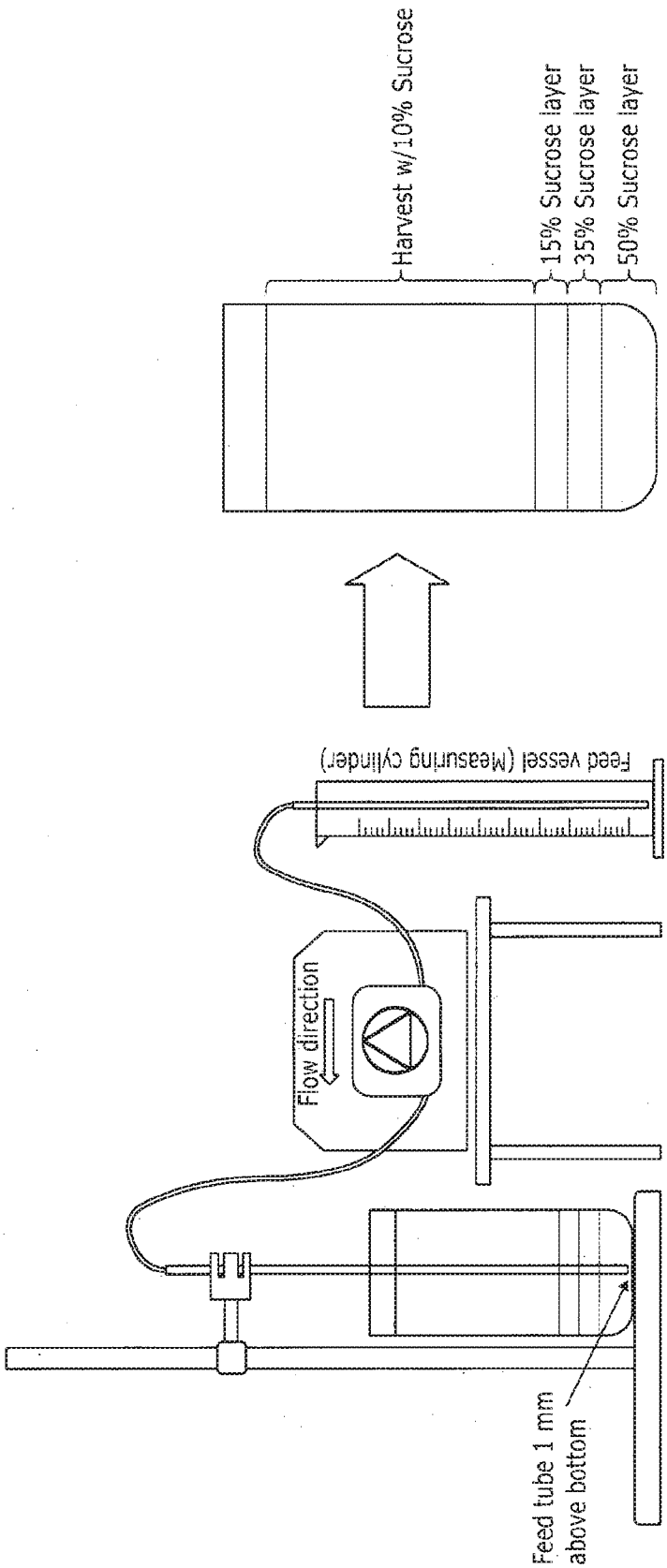

Harvest w/10% Sucrose
15% Sucrose layer
35% Sucrose layer
50% Sucrose layer
Feed vessel (Measuring cylinder)
Flow direction
Feed tube 1 mm above bottom

FIG. 13

Lane	Sample
1	Molecular weight Marker
2	JEV Sucrose Gradient Pool (Reference)
3	Zika Harvest pool (day 2,3,5,7)
4	Zika Harvest pool (day 2,3,5,7)concentrated 300x
5	Zika Harvest pool (day 2,3,5,7)concentrated 300x + Protamine sulfate
6	Ultrafiltration Permeate
7	SGC Bottle 1, Fraction 4
8	SGC Bottle 1, Fraction 5
9	SGC Bottle 1, Fraction 6
10	SGC Bottle 1, Fraction 7
11	SGC Bottle 1, Fraction 8
12	SGC Bottle 1, Fraction 9
13	SGC Bottle 1, Fraction 10
14	SGC Bottle 1, Fraction 11
15	SGC Bottle 1, Fraction 12
16	SGC Bottle 1, Fraction 13
17	SGC Bottle 1, Fraction 14
18	SGC Bottle 1, Fraction 15
19	SGC Bottle 1, Fraction 16
20	SGC Bottle 1, Fraction 17
21	SGC Bottle 1, Fraction 18
22	SGC Bottle 1, Fraction 19
23	SGC Bottle 1, Fraction 20
24	SGC Bottle 1, Fraction 21
25	SGC Bottle 1, Fraction 22
26	SGC Bottle 1, Fraction 23

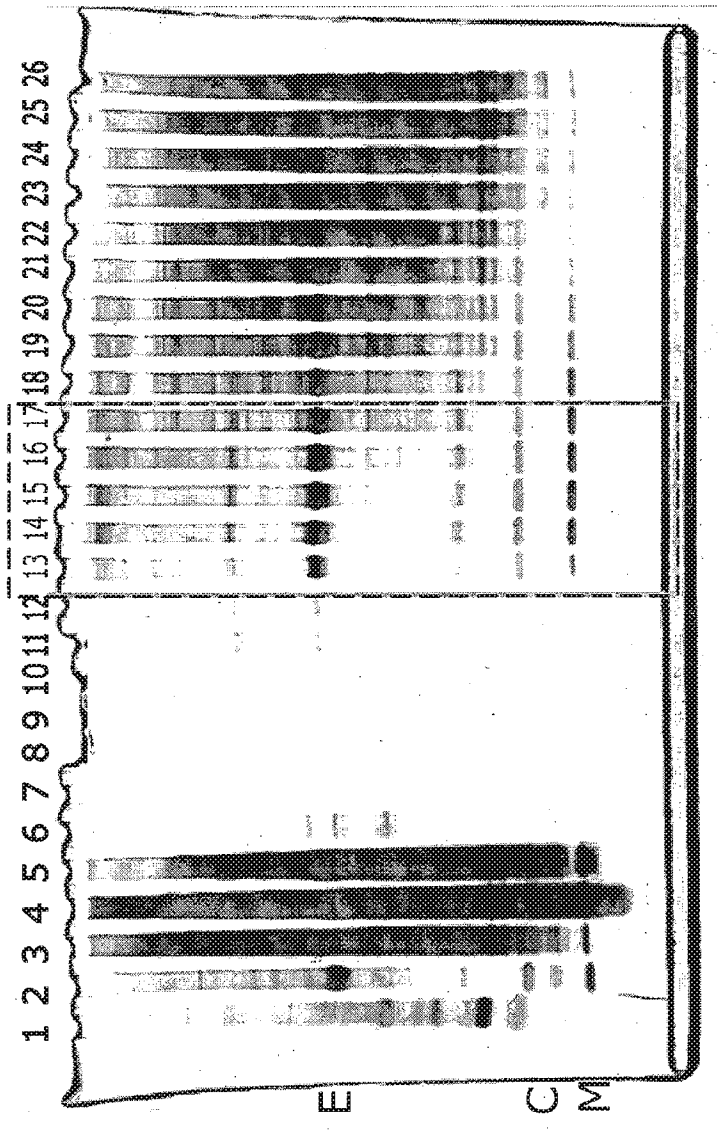


FIG. 14

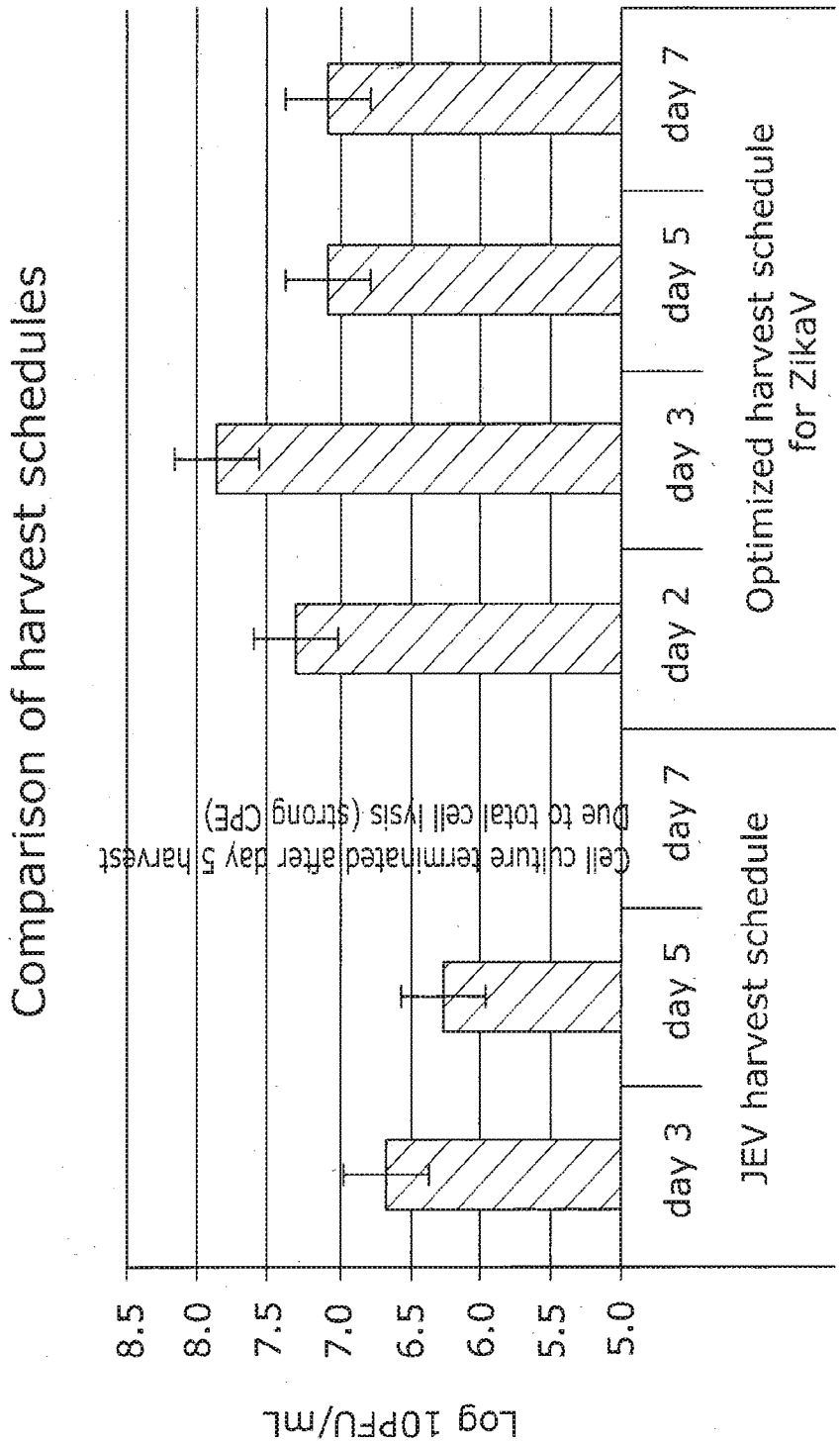

Comparison of harvest schedules
Log 10PFU/mL
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
JEV harvest schedule
day 3
day 5
day 7
Optimized harvest schedule for ZikaV
day 2
day 3
day 5
day 7
(Cell culture terminated after day 5 harvest
Due to total cell lysis (strong CPE)

FIG. 15

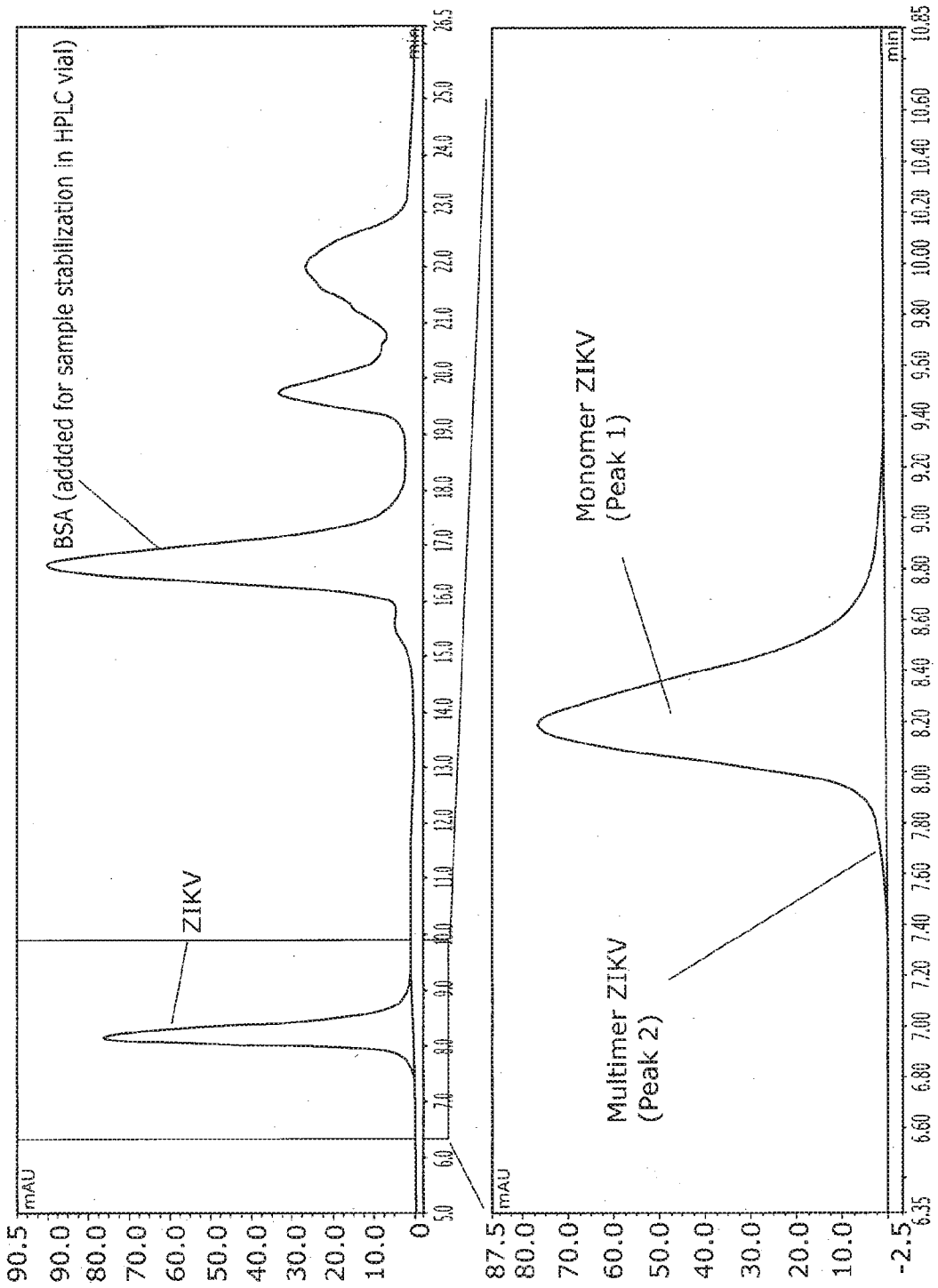

BSA (addded for sample stabilization in HPLC vial)
ZIKV
mAU
min
Monomer ZIKV
(Peak 1)
Multimer ZIKV
(Peak 2)

FIG. 16

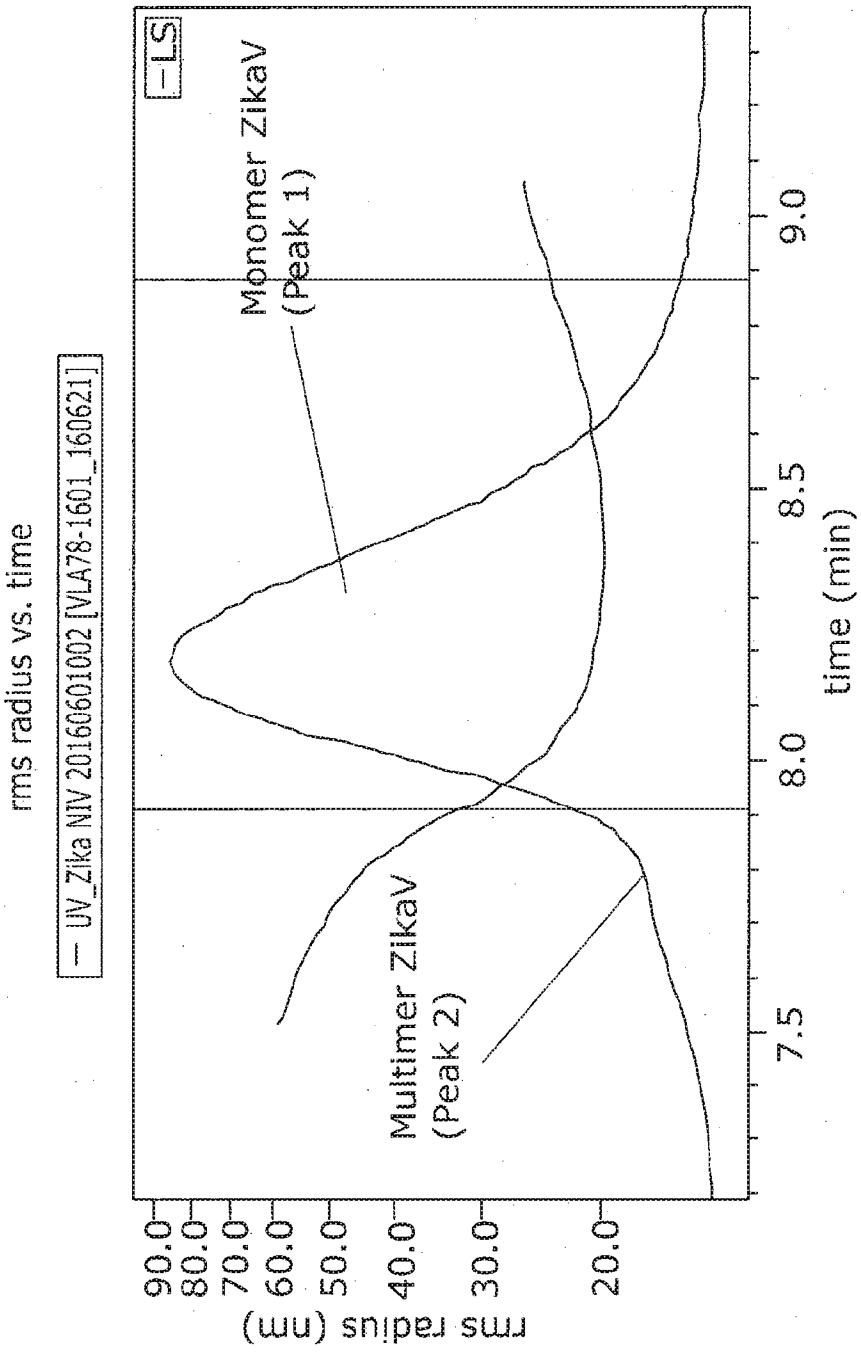

rms radius vs. time
UV_Zika NIV 20160601002 [VLA78-1601_160621]
LS
Monomer ZikaV
(Peak 1)
Multimer ZikaV
(Peak 2)
rms radius (nm)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
7.5
8.0
8.5
9.0
time (min)

FIG. 17

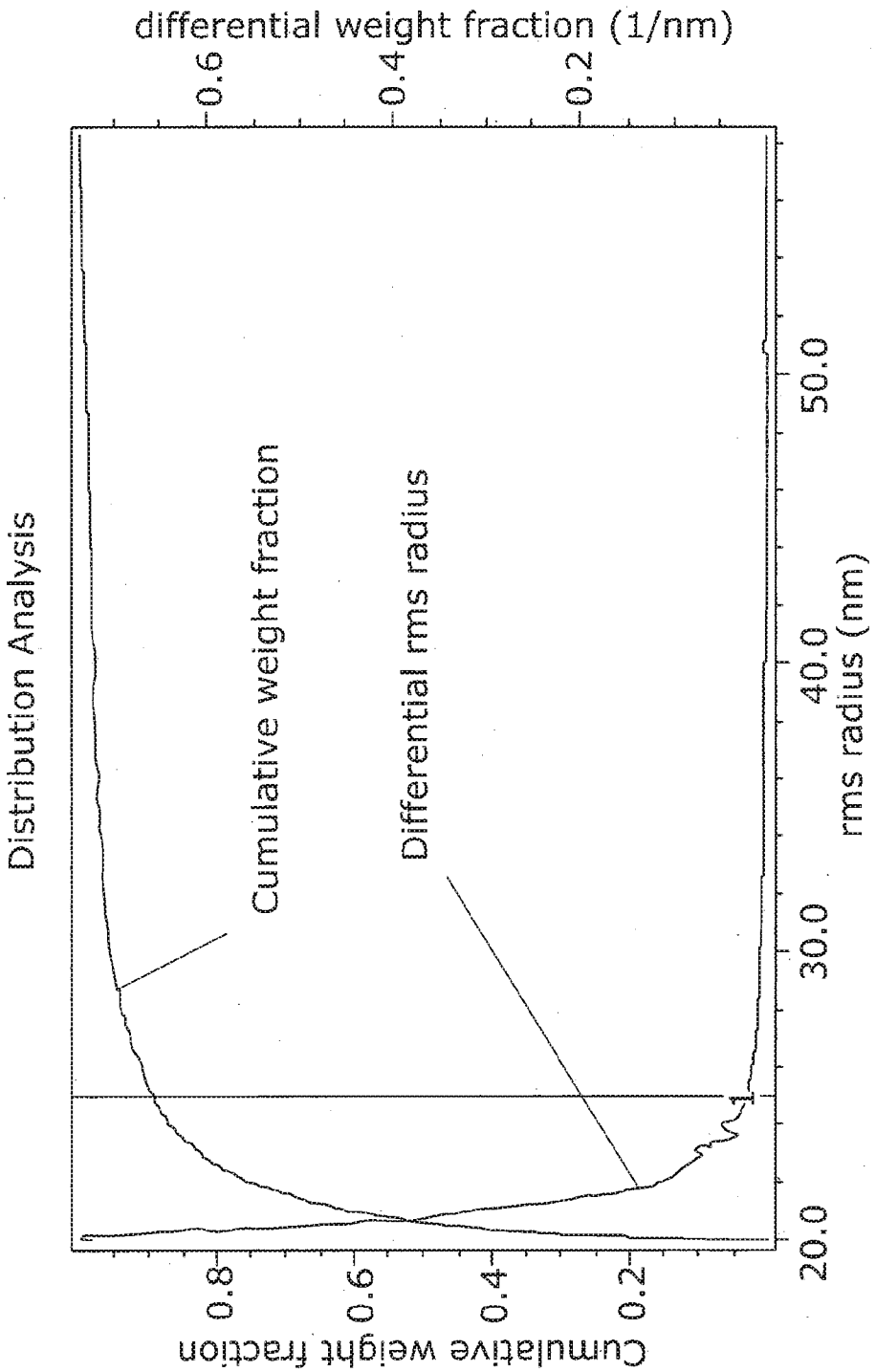
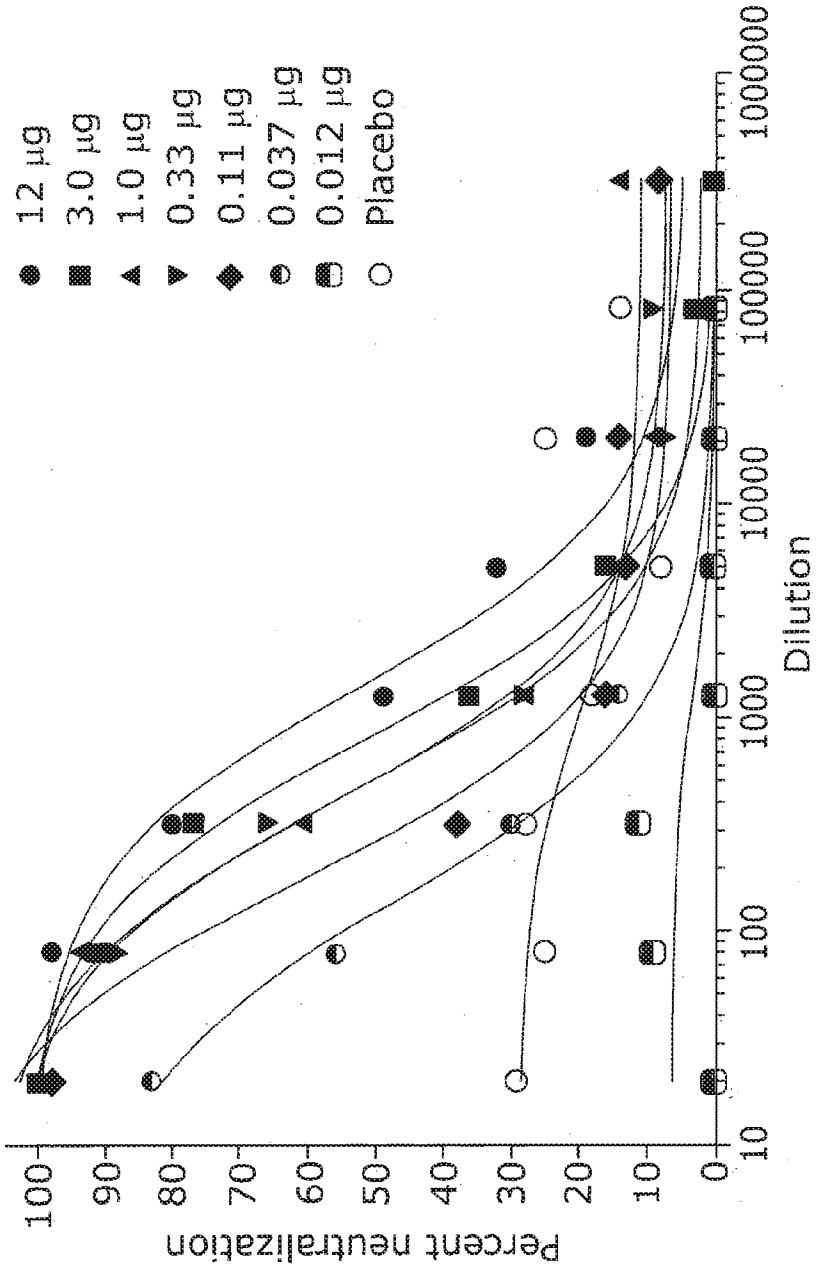
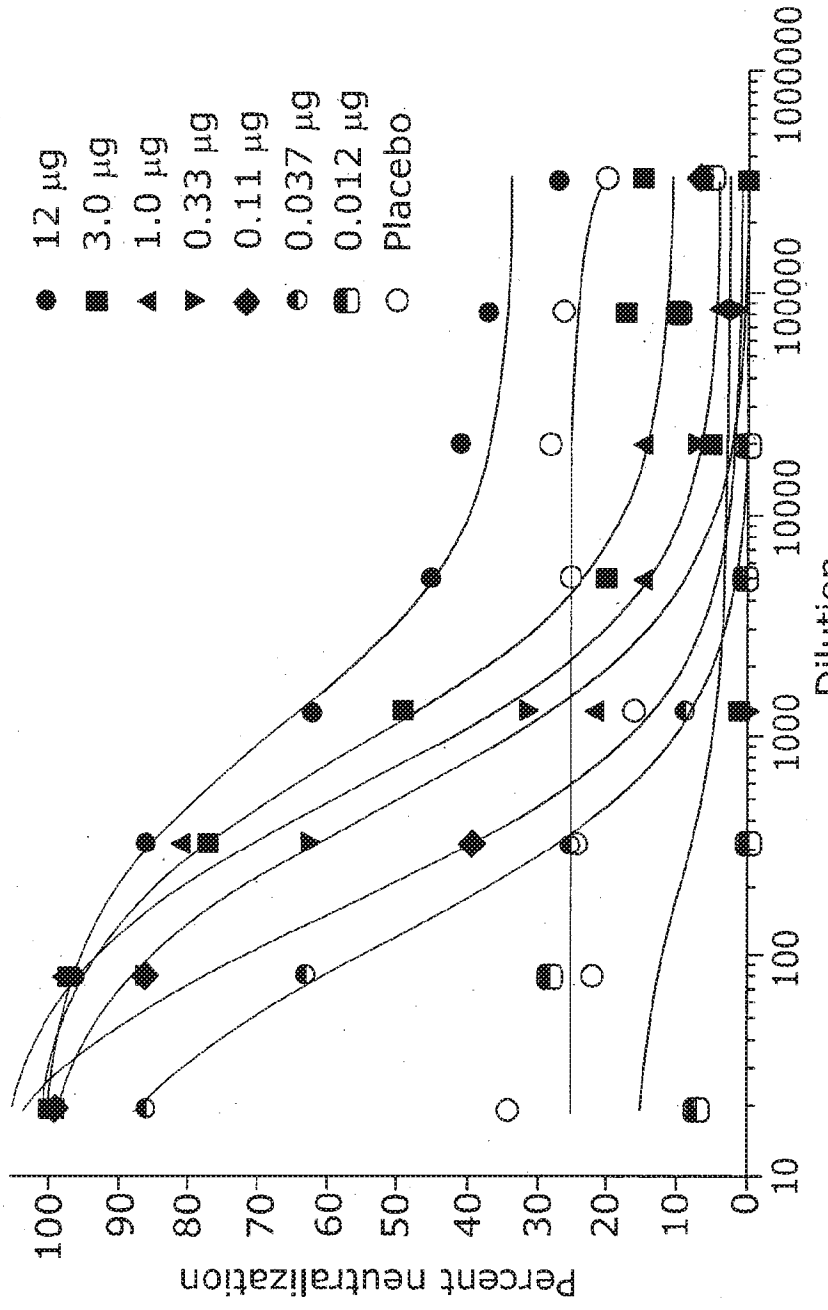

Distribution Analysis
Cumulative weight fraction
Differential rms radius
Cumulative weight fraction
0.2
0.4
0.6
0.8
rms radius (nm)
20.0
30.0
40.0
50.0
differential weight fraction (1/nm)
0.2
0.4
0.6

FIG. 18



	12 µg	3.0 µg	1.0 µg	0.33 µg	0.11 µg	0.037 µg
EC50	1371	807.6	380.7	456.1	179.9	142.0

FIG. 19



	12 µg	3.0 µg	1.0 µg	0.33 µg	0.11 µg	0.037 µg
EC50	1029	819.1	556.9	477.3	158.1	118.0

FIG. 20

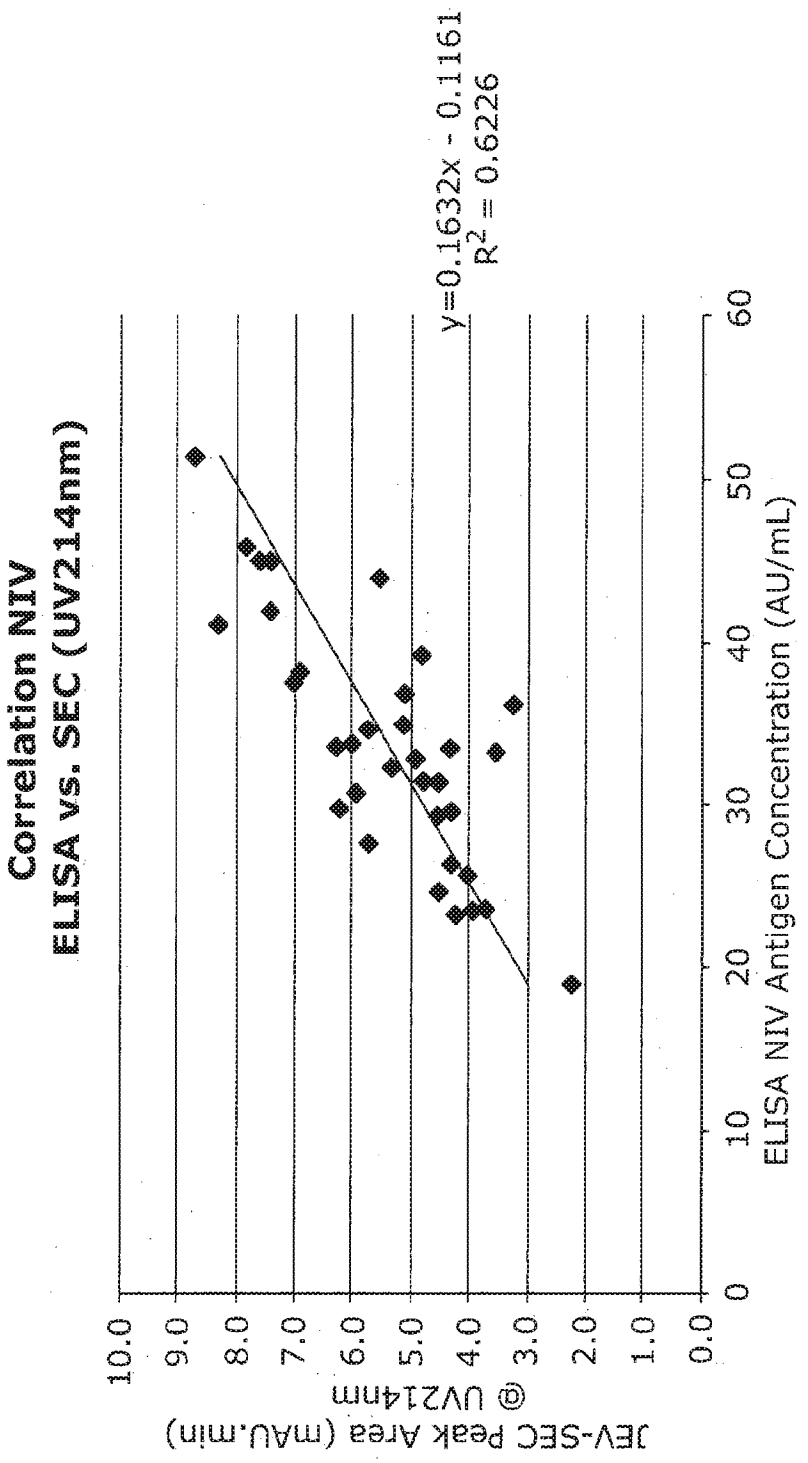

Correlation NIV
ELISA vs. SEC (UV214nm)
y=0.1632x - 0.1161
R2 = 0.6226
JEV-SEC Peak Area (mAU.min)
@ UV214nm
ELISA NIV Antigen Concentration (AU/mL)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
0
10
20
30
40
50
60

FIG. 21

Doc Code: PA.
Document Description: Power of Attorney

PTO/AIA/82B (07-13)
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I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Valneva Austria GmbH

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

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SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	28 Jun 2014
Name	Adrian Spillmann, Ph.D., LL.M.		
Title	Head of Corporate Intellectual Property – Valneva Austria GmbH		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

[ ] Total of 1 forms are submitted.

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		Filing Date	March 10, 2020
		First Named Inventor	Jana Barbero Calzado
		Art Unit	Not Yet Assigned
		Examiner Name	Not Yet Assigned
Total Number of Pages in This Submission		Attorney Docket Number	I0422.70125US01

ENCLOSURES (*Check all that apply*)

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[ ] After Final	[ ] Petition to Convert to a Provisional Application	[ ] Proprietary Information
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SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	July 22, 2020	Reg. No.	75,713

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Dated: July 22, 2020 Electronic Signature for Taryn Bollers: /Taryn Bollers/

ZIKA VIRUS VACCINE

RELATED APPLICATIONS

This Application is a continuation of U.S. Application Serial No. 16/063,007, filed June 15, 2018, which is a national stage filing under 35 U.S.C. § 371 of International Patent Application Serial No. PCT/EP2016/082664, filed December 23, 2016, the contents of each of which is herein incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 15, 2020, is named I042270125US01-SUBSEQ-CEW and is 455 kilobytes in size.

FIELD OF INVENTION

The disclosure relates to methods for the purification of Zika viruses for use in vaccines and in particular relates to an improved sucrose gradient process step allowing the separation of impurities such as protamine sulphate. The disclosure also relates to Zika virus vaccines and compositions and methods for producing said vaccines and administering the vaccines to subjects for the generation of an anti-Zika virus immune response.

BACKGROUND OF THE INVENTION

Adverse responses to protamine sulfate have been known for many years. Previous exposure to protamine can induce a humoral immune response and predispose susceptible individuals to the development of untoward reactions from the subsequent use of this drug. Patients exposed to protamine through the use of protamine-containing insulin or during heparin neutralization may experience life-threatening reactions and fatal anaphylaxis upon receiving large doses of protamine intravenously. Severe reactions to intravenous protamine can occur in the absence of local or systemic allergic reactions to subcutaneous injection of protamine-containing insulin. Although there is no clear evidence for hypersensitivity reactions of protamine sulphate linked to vaccination, vaccines containing protamine impurities have a precaution and contraindication warning in their

labels stating that a serious allergic reaction after a previous dose of such a protamine containing vaccine (e.g. IXIARO®, see CDC site www.cdc.gov/japaneseencephalitis/vaccine/) is a contraindication to further doses. Thus elimination of said impurity is a medical request for an improved safety profile. On the other hand protamine sulphate is an excellent tool (and often better than other tools such as benzonase) to purify crude harvests of viruses grown on cell substrates.

In 2007, Zika virus was detected for the first time outside of the endemic regions of Asia and Africa since its discovery in a Rhesus monkey in Uganda in 1947. Since then, the virus has caused a large epidemic in French Polynesia, spreading through islands in the Pacific and into South and Central America by 2015 (WHO "Zika Situation Report" February 5, 2016). Evidence suggests that in addition to being transmitted by *Aedes* species mosquitos, other vectors may exist, and the virus may be transmitted by blood transfusion, transplacentally, and through sexual transmission (WHO Zika Virus Fact Sheet, Feb. 2016). Though the symptoms of Zika virus infection include mild fever, rash, and conjunctivitis, there is a likely correlation between infection and neurological disorders, including Guillain-Barré syndrome and microcephaly in fetuses/neonates subsequent to infection during pregnancy. There is currently no specific treatment or vaccine for Zika virus and the only preventative measures involve control of the mosquito vector. Zika virus presents a substantial public health threat due to the wide circulation of the *Aedes* mosquito, multiple routes of transmission, and potentially severe neurological effects of infection.

A preventative vaccine against Zika virus is a pressing medical need in endemic areas and in geographical areas where the vector is spreading. Furthermore, as Zika infection has dire consequences on embryonic and fetal development, a safe and effective vaccine for women of child-bearing potential or pregnant women is needed. Vaccines administered during pregnancy must be very safe for both the mother and the developing fetus. While live attenuated viral vaccines are highly effective, they are often not considered safe enough for administration to pregnant women. In this regard, inactivated viral vaccines, which lack the ability to propagate in the vaccinated subject, are considered much safer. Development of an inactivated Zika virus vaccine for administration to at-risk patients would fill this need.

SUMMARY OF THE INVENTION

During the course of virus purification, it was observed that addition of protamine sulfate to a virus harvest produced on a cell substrate removed not only contaminating DNA derived from host cells, as expected, but surprisingly also virtually eliminated immature and otherwise non-infectious virus

particles from the preparation. This finding provided a streamlined, gentle, reproducible and broadly-applicable process for obtaining highly-purified infectious virus particles for applications such as vaccine preparation. In addition, it was surprisingly found that said protamine sulfate can be very efficiently separated from the virus fraction allowing for a safer vaccine produced at high yields.

Disclosed herein are virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering said Zika virus vaccines for the prevention of Zika virus infection and/or for the production of an anti-virus immune response in subjects, for example subjects at risk of being exposed to Zika virus. In particular, the invention is directed to a virus vaccine comprising an optimally inactivated Zika virus particle, wherein the Zika virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability. Another advantage of the invention is that related methods of producing said vaccines and compositions are very efficient and provide pure compositions largely devoid of impurities, in particular protamine sulphate, allowing for high volume production of vaccines. Detail experimental examples to the above are provided for Zika virus.

The herein disclosed in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was unexpectedly higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, Nature doi:10.1038/nature18952.). Inactivated viruses are among the safest vaccines and especially preferred for delivery to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing, alignments were performed with the multi alignment package Jalview (Waterhouse et al., 2009, Bioinformatics 25 (9) 1189-1191). In the drawings:

FIG.~~Figure~~ 1: Average distance tree (by % identity, nt), complete genomes.

FIG.~~Figure~~ 2: Neighbor joining tree (by % identity, nt), complete genomes.

FIG.~~Figure~~ 3: Pairwise alignment-Jalview (% identity, nt), complete genomes.

FIG.~~Figure~~ 4: Average distance tree (by % identity, aa), E-protein.

FIG.~~Figure~~ 5: Neighbor joining tree (by % identity. aa), E-protein.

FIG.~~Figure~~ 6: Pairwise alignment-Jalview (% identity, aa), E-protein.

FIGs.~~Figures~~ 7A-7C: Alignment (shading: % identity, aa), E-protein.

FIG.~~Figure~~ 8: An example of virus particle maturation in the host cell. As observed in flaviviruses, full maturation of the particles requires proteolytic cleavage of the precursor membrane glycoprotein (prM) by the host protease furin. Not all prM molecules are cleaved, resulting in the release of mature, mosaic or immature-like conformations from the cells. Mosaic and immature forms are generally not infectious—only mature virions are infective and have hemagglutinin (HA) / TCID50 activity. (Figure adapted from Plevka, et al., Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres, EMBO reports (2011) 12, 602–606).

FIGs.~~Figure~~ 9A-9B: An exemplary downstream virus purification process from the crude harvest to formulation of the drug substance (vaccine), a preferred embodiment of the process of the invention (**FIG. 9A**). A flow-chart of an exemplary virus inactivation process is shown in (**FIG. 9B**).

FIG.~~Figure~~ 10: PS treatment results in selective removal of Zika virus aggregates and Vero HCP and LMW impurities (SEC-HPLC of 30x concentrated Zika Virus harvest day 5).

FIG.~~Figure~~ 11: SEC-HPLC of individual 30x concentrated Zika harvest prior to PS treatment at different time points.

FIG.~~Figure~~ 12: SEC-HPLC of individual 30x concentrated Zika harvest post PS treatment at different time points. The smaller graph indicates the observed cytopathic effect (CPE) over time.

FIG.~~Figure~~ 13: Preparation of the sucrose gradient.

FIG.~~Figure~~ 14: Representative SDS-PAGE from the sucrose gradient harvest of a Zika purification is shown.

FIG.~~Figure~~ 15: Comparison of JEV and ZikaV harvest schedules/yields.

FIG.~~Figure~~ 16: SEC-HPLC elution profile of ZikaV NIV. Data were processed on Dionex Ultimate 3000 / Superose 6 Increase column. Both panels are from the same chromatogram. The upper graph is the complete elution profile; the lower graph is an enlargement of the ZIKAV elution peak.

FIG.~~Figure~~ 17: SEC-MALLS analysis of inactivated ZikaV.

FIG.~~Figure~~ 18: Cumulative particle size distribution of Zika NIV.

FIG.~~Figure~~ 19: Graphical representation of the neutralization of the Zika virus H/PF/2013 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated using the 3-parameter method.

FIG.~~Figure~~ 20: Graphical representation of the neutralization of the Zika virus MR766 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated using the 3-parameter method.

FIG.~~Figure~~ 21: Correlation between JEV antigen content in neutralized inactivated virus (NIV) analysed by ELISA and SEC-HPLC (Dionex Ultimate 3000, Superose 6 column).

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering said virus vaccines for the prevention of virus infection and/or for the production of an anti-virus immune response in subjects, for example subjects at risk of being exposed to virus. In particular, the invention is directed to a virus vaccine comprising an optimally inactivated virus particle, wherein the virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability. Another advantage of the invention is that related methods of producing said vaccines and compositions are very efficient and provide pure compositions largely devoid of impurities, in particular protamine sulphate, allowing for high volume production of vaccines. Examples to the above are provided for Zika virus.

Disclosed herein are downstream processes for purifying Zika virus particles from a crude preparation. The downstream process can be applied to either a virus which has not adapted for propagation on a particular cell substrate or for a partially / fully cell substrate adapted Zika virus particle.

Aspects of the invention provide processes for the purification of infectious Zika virus particles comprising the steps of (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate; (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, to obtain a virus preparation (b); and further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation to obtain a virus preparation (c) comprising the infectious virus particles.

In some embodiments, the concentration of protamine sulphate in step (b) is about 1 to 10 mg/ml, more preferably about 1 to 5 mg/ml, more preferably about 1 to 2 mg/ml. In one embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/mL. In one embodiment, the concentration of protamine sulphate is 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml. In a preferred embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/ml.

In some embodiments, the residual host cell DNA of the virus preparation (c) is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL. In a preferred embodiment, the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL. In some embodiments, the residual host cell protein of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the residual host cell protein of the virus preparation (c) is less than 100 ng/mL. In some embodiments, the residual non-infectious virus particles of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the content of residual non-infectious virus particles of the virus preparation (c) is less than 100 ng/mL.

In some embodiments, the residual protamine is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL, more preferably is below the detection limit of HPLC, in particular below the detection limit in the final drug substance. In some embodiments, the PS content is tested by HPLC or size exclusion chromatography (SEC). For example, HPLC is validated for PS determination in JEV sucrose gradient pool samples as a routine release assay and is very sensitive (i.e., LOQ 3 µg/mL; LOD 1 µg/mL). In the current invention, PS content in in Zika virus DS samples was <LOD. In one embodiment, the HPLC assessment of PS content can be performed on a Superdex Peptide 10/300GL column (GE: 17-5176-01) using 30% Acetonitrile, 0,1% Trifluoroacetic acid as solvent with a flow rate of 0,6 ml/min at 25°C and detection at 214 nm. A more sensitive method of measurement for residual protamine in a purified virus preparation is mass spectrometry (MS). In some embodiments, the residual PS levels in a Zika virus preparation are tested by MS or other such highly sensitive method, e.g. nuclear magnetic resonance (NMR). With this method, residual PS, as well as fragments and/or break-down products of PS, can be detected at trace amounts, such as levels as low as, for example, 10^6, 10^7 or 10^8 molecules per typical sample load. In some embodiments, the PS levels are tested in the sucrose gradient pool. In some embodiments, the PS levels are tested in the drug product. In some embodiments, the PS levels are tested in the drug substance.

In some embodiments, the crude harvest (a) comprising the virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b). In some embodiments, the one or more pre-purification step(s) comprises digesting host cell genomic DNA in the crude harvest (a) comprising the virus particles and impurities by enzymatic treatment. In some embodiments, the one or more pre-purification step(s) comprises filtration, ultrafiltration, concentration, buffer exchange and/or diafiltration. In some embodiments, the one or more pre-purification steps is filtration using a filter

having a pore size equal to or less than 1 µm. In some embodiments, the filter has a pore size equal to or less than 0.2 µm. In a preferred embodiment, the filter has a pore size of 0.2 µm. In some embodiments, the concentration and/or ultra/diafiltration and/or buffer exchange is performed by tangential flow filtration (TFF). In some embodiments, ultra/diafiltration of the crude harvest (a) comprising the virus particles and impurities is performed using a hollow fiber membrane having a cut-off of equal to or less than 300 kDa. In a preferred embodiment, the hollow fiber membrane has a cut-off of about 100 kDa.

The process according to the current invention may also comprise the use of a sucrose gradient, preferably an optimized sucrose gradient. The sucrose gradient is preferably optimized for the removal of protamine sulfate, also for the removal of immature viral particles or other viral particles which are non-infectious or host cell proteins or nucleic acids (DNA, RNA, mRNA, etc) or other host cell debris. In the current invention the optimized sucrose gradient comprises at least two, at least three, at least four layers of sucrose solutions with different densities. In one embodiment, the virus preparation to be purified is provided in a sucrose solution which has a density of about 8%, about 9%, about 10%, about 11%, about 12% sucrose (w/w), preferably about 10%. In one embodiment, one sucrose solution in the gradient has a density of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55% sucrose (w/w), preferably about 50%. In one embodiment, one sucrose solution in the gradient has a density of about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40% sucrose (w/w), preferably about 35%. In one embodiment, one sucrose solution in the gradient has a density of about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20% sucrose (w/w), preferably about 15% sucrose. In a preferred embodiment, the sucrose gradient comprises three layers of sucrose solutions of about 50%, about 35% and about 15% (w/w) sucrose and the virus composition to be purified is contained in about 10% (w/w) sucrose. Because the invention provided for means to not only test for host cell DNA but also immature viral particles, the skilled person in the art is able to more precisely optimize the sucrose gradient for most efficient purification and include additional tools such as PRNT assay to monitor purification success.

In some embodiments, the virus particle is a live virus, a chimeric virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In a further step, the virus particles of the invention may be optionally inactivated. In some embodiments, the virus particle is an attenuated form of the virus particle. For example, the virus may have reduced infectivity, virulence, and/or replication in a host, as compared to a wild-type virus. In some embodiments, the virus is a mutated or modified virus, for example the nucleic acid of the virus may contain at least one mutation relative to the wild-

type virus. In some embodiments, the virus is a recombinant live virus, meaning a virus that is generated recombinantly and may contain nucleic acid from different sources.

In some embodiments, the Zika virus particle is a live virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In preferred embodiments, the Zika virus is a Zika virus from the Asian lineage.

In some embodiments, the relative reduction of impurity of the final virus preparation relative to the liquid medium (a) comprising the virus particles and impurities is in a range from 60 to 95%. In some embodiments, the residual impurity of the final virus preparation is less than 1%.

In some embodiments, the Zika virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line. In some embodiments, said cell line is a duck cell line. In some embodiments, said cell line is a diploid avian cell line. In some embodiments, said cell line is an EB66 cell line. In a preferred embodiment, said cell line is a Vero cell line.

Aspects of the invention provide a use of any of the processes described herein for manufacturing a composition for immunization against a viral infection. In a preferred embodiment, the composition or vaccine is directed against Zika virus such as e.g. a Zika virus of the Asian lineage.

Other aspects provide compositions comprising the Zika virus particles obtainable by any of the processes described herein for treating and/or preventing (i.e. protecting from) a viral infection. In a preferred embodiment, the viral infection is caused by Zika virus such as e.g. a Zika virus of the Asian lineage.

Furthermore, disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering the Zika virus vaccines for the prevention of Zika virus infection and/or for the production of an anti-Zika virus immune response in subjects, for example subjects at risk of being exposed to Zika virus.

Zika virus is a flavivirus closely related to Dengue virus and is similarly transmitted by the *Aedes* species mosquito, although other arthropod vectors for Zika virus are possible. Since it was first isolated from a Rhesus monkey in the Zika forest of Uganda in 1947, there were very few reported incidents of human infection, especially outside of the endemic regions of Africa and Asia until a large outbreak in French Polynesia in 2007 (Haddow *et al. PLoS Neglected Tropical Diseases* (2012) 6(2), Malone *et al. PLoS Neglected Tropical Diseases* (2016) 10(3),). The virus has since spread through islands of the Pacific, including Oceania, and into South and Central America (WHO "Zika Situation Report" February 5, 2016).

In addition to being spread by the bite of an infected mosquito, evidence also suggests transmission may occur between individuals, such as from the blood of an infected individual, *in utero*/transplacental transmission from an infected mother to the fetus, sexual transmission between sexual partners, and possibly by other local transmission routes. There is a possible association between Zika virus infection during pregnancy and microcephaly in the fetus/neonate. Microcephaly is a rare condition in which a baby's head circumference is significantly less than expected based on the average for their age, sex, and ethnicity. This is a result of the brain failing to undergo proper embryonic development, and in 90% of cases is associated with mental retardation (Rocha *et al.* (2016) *Bull World Health Organ* 8 Feb. 2016).

There is a probable association between individuals having had a prior Zika virus infection and the incidence of Guillain-Barré syndrome, a neurological disorder in which the individual's immune system destroys the myelin sheath surrounding axons of the peripheral nervous system (WHO "Zika Situation Report" February 5, 2016).

No specific treatments or vaccines for Zika virus currently exist, and the only measures at this time to prevent infection are through vector control and avoiding travel to regions experiencing outbreaks.

Described herein are Zika virus vaccines and compositions comprising inactivated Zika virus that provide a safe method for generating an immune response to Zika virus, including virus-neutralizing antibodies, that may help prevent against Zika virus infection.

Any strain of Zika virus may be used in the methods and compositions described herein. In some embodiments, the Zika virus is an isolate from an infected subject during a Zika virus outbreak. In some embodiments, the Zika virus is a strain isolated from Africa or from the African virus lineage. In some embodiments, the Zika virus is a strain isolated from Asia or from the Asian lineage (includes

also strains from French Polynesia). In some embodiments, the Zika virus is a strain isolated from the Americas (South America, Central America, or North America), such as a Suriname Zika virus strain. In some embodiments, the Zika virus has an RNA genome corresponding (but not limited) to the DNA sequence provided by GenBank Accession No. AY632535.2, KU321639.1, KU497555.1, KU501215.1, KU509998.1, KU527068.1, KU681081.3, KU681082.3, KU707826.1, KU744693.1, or LC002520.1 or RNA genome disclosed partially or fully herein (SEQ ID NO: 2 to 69).

In some embodiments, the process of the invention results in an enrichment of infectious Zika virus particles from the crude harvest comprising infectious Zika virus particles and non-infectious virus particles and other virus products such that the enrichment of the infectious virus particles is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, preferably at least 80%, especially at least 85% relative to the total virus particle content of the crude harvest (a) comprising the virus particles and impurities.

In some embodiments, the residual impurity of the final virus preparation with respect to all impurities in the crude harvest is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, preferably less than 5% as determined by SEC-HPLC (Size Exclusion Chromatography – HPLC).

A unique aspect of the current invention is the realization that know-how related to the vaccine design and purification approach used for the Japanese Encephalitis Vaccine (JEV) IXIARO® (see Srivastava A.K. et al., 2001, Vaccine 19, 4557-4565, WO99/11762) may be employed and improved upon in order to expedite the development of a Zika virus vaccine and provide it to the subjects in need as soon as possible. The industrial process as disclosed for IXIARO®, providing a very effective vaccine against JEV, was complemented by further significant improvements disclosed herein in order to provide a more efficient (higher yield) and safer (less or no protamine sulphate with its allergic potential) Zika vaccine compared to the available JEV vaccine. A particular innovation of the herein disclosed vaccines is their greatly reduced protamine salt (SEQ ID NO: 1) content in the final drug substance facilitated by the development of an improved sucrose gradient. Said sucrose gradient not only allowed the separation of protamine sulphate but also allowed for a very effective inactivation by formaldehyde and resulted in the case of Zika with over 90% yield with the improved process disclosed herein vs about 35% yield with the published JEV process, see experimental part for comparison).

Aspects of the disclosure relate to methods of producing a virus in Vero tissue culture cells. Vero cells are a commonly used tissue culture cell line derived from the kidney of an African green

monkey. The Vero cells used in the methods described herein are the Vero (WHO) cell line, obtained from the Health Protection Agency general cell collection under catalogue number 88020401.

Vero cells can be grown to confluent monolayers, for example in tissue culture flasks; in suspension (on microcarriers), for example in roller bottles; or in any other cell culture system for viral production. In some embodiments, the Vero cells are grown in a bioreactor for viral production. For plaque assays or the plaque reduction neutralization test (PRNT), Vero cells are grown in monolayers in tissue culture flasks, dishes, or wells of a plate. To infect the Vero cells with the virus, the culture medium is inoculated with virus and the cells are incubated with the virus for a period of time. The cells may be washed after inoculation to remove any virus that did not adsorb to the cells in a given amount of time.

The methods provided herein involve passaging the virus in Vero cells. As used herein, the terms "passage" or "passaging" refer to infecting a population of Vero cells with virus and subsequently inoculating a second population of Vero cells with virus produced by infection of the first Vero cell population. In some embodiments, a portion of the culture medium from the infected Vero cells (containing virus that was released from the infected cells) is used to inoculate a second population of Vero cells. This is referred to as one passage or one round of passaging. The passaging may be performed serially, for example, a portion of the culture medium from the infected second population of Vero cells is used to inoculate a third population of Vero cells, and so on. In some embodiments, virus obtained from a single plaque is used to inoculate another population of cells.

In some embodiments, the virus is passaged in Vero cells several times, such as at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times. In some embodiments, the virus is passaged in Vero cells at least 4 times or 5 times. In some embodiments, the virus is passaged in Vero cells at least 30 times. It is important that the virus population, i.e. the virus sequences, stays as much as possible constant over said passaging. If adaption of the virus occurs (i.e. appearance of mutated viruses in the original virus population), it is preferred that said passages are not used in the context of manufacturing of said virus, e.g. for Zika it was found that up to passage 3 and culturing to day 7 can be used without major shifts in virus population, i.e. introduction of virus population with mutations. However this observation needs to be done for each virus strain and may be different.

In some embodiments, the Vero cells are incubated for at least 2 days after inoculation with the virus at e.g. a typical 0.01 MOI (multiplicity of infection) to allow for viral production prior to passaging.

In some embodiments, the Vero cells are incubated for at least 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 days e.g. at least 7 days after inoculation with the virus prior to passaging. The number of days the Vero cells are incubated after viral inoculation may depend on factors such as the multiplicity of infection used to inoculate the cells and the viral titer desired in the culture medium. Serial passaging of the virus in Vero cells may result in generation of a Vero cell adapted virus strain.

The culture medium from the infected Vero cells may be harvested (collected) to obtain the virus. In some embodiments, the culture medium is harvested from infected Vero cells and is replaced with fresh culture medium, which is then harvested after another period of time. In some embodiments, the culture medium harvested from infected Vero cells is pooled from independent Vero cell cultures and/or from independent days. Harvesting can be repeated up to 4 times by 7 or 9 days post infection, for example, and result in a high yield of virus per unit cell culture. In order to minimize the adaption of Zika virus strain to Vero cells, it was found that Vero cells could be incubated for at least 7 days, more preferably 5 days, prior to passaging and subsequently supernatants could be harvested at days 2, 3, 5 and 7 or 2, 3, and 5 (see also experimental part). The harvested culture medium can be stored at +4°C prior to purification of the virus from the culture medium up to 2 weeks.

In some embodiments, debris from infected and lysed Vero cells may be removed from the harvested culture medium, referred to as a "clarification" of the culture medium. The harvested culture medium may be clarified by common methods known in the art, such as low-speed centrifugation, for example, at 1500 *g* for 10 min, and/or by filtration through a filter of pore size of 0.45 µm. The harvested culture medium can be stored at +4°C prior to concentration.

To concentrate the titer of the Zika virus in the harvested culture medium, it may be subjected to concentration by any method known in the art. For example, the harvested culture medium may be concentrated by methods including, without limitation, ultrafiltration, ultracentrifugation, centrifugal concentrator, vacuum centrifugation, and lyophilization. In some embodiments, the harvested culture medium is concentrated by ultrafiltration and the retentate containing the Zika virus is collected. In some embodiments, the harvested culture medium is concentrated by precipitation in which polyethylene glycol (PEG) 8000 is dissolved in the culture medium (up to 10%) and the precipitate is dissolved in a buffer, for example phosphate-buffered saline (PBS, pH 7.0).

The harvested culture medium may be precipitated to produce a virus supernatant. In some embodiments, the harvested culture medium is precipitated to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. In some

embodiments, the harvested culture medium is concentrated prior to precipitation. In some embodiments, the harvested culture medium is precipitated by adding protamine sulfate (e.g. SEQ ID NO: 1) to the harvested culture medium and incubating the mixture, for example at +4°C or on ice. In some embodiments, the harvested culture medium is treated with benzonase to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. However, it was found that the treatment with protamine sulfate is preferred (see experimental part). In some embodiments, the precipitated culture medium is centrifuged to collect precipitated material and the supernatant containing the virus, referred to as a "virus supernatant", is collected.

The virus supernatant may be further purified after precipitation, for example density gradient ultracentrifugation. In some embodiments, the virus supernatant is further purified by sucrose gradient. Fractions may be collected from the sucrose gradients and assayed for presence of the virus. Methods for assaying for virus positive fractions include plaque assay, hemagglutination assay, polyacrylamide gel electrophoresis, and antigen assays such as Western blotting and ELISA. The fractions containing virus may be pooled based on titer of the virus and level of other impurities. The level or amount of impurities present in the virus supernatant can be estimated by testing for Vero cell DNA, virus aggregates and/or Vero cell protein (see experimental part). A particular embodiment of the invention is the improved sucrose gradient that allows for an efficient protamine separation as shown in the experimental part. It was surprisingly found that the addition of a virus-containing fraction with 10% (w/w) sucrose to a simple three layer sucrose density gradient (e.g. a gradient comprising a 15% (w/w) sucrose solution, a 35% (w/w) sucrose solution, and a 50% (w/w) sucrose solution) resulted in efficient separation of protamine sulphate without much loss of virus. Thus a particularly preferred embodiment of the invention is the use of a sucrose density gradient that is able to efficiently separate protamine sulphate, wherein said sucrose density gradient is used in the purification of virus such as the viruses described herein, i.e. a Zika virus.

To achieve a safe vaccine or composition for the administration to subjects, the virus supernatant may be inactivated (see experimental part for Zika virus). As used herein, the terms "inactivated" and "optimally inactivated" may be used interchangeably and refer to a process (or its result) by which the virus is rendered unable to infect a host cell (non-infectious), but that does not affect or substantially affect the antigenicity of the virus, for example, the immunogenic antigens exposed on the surface of the virus are able to stimulate an immune response in a subject (*e.g.*, antigen-specific antibodies). By "does not affect or substantially affect the antigenicity of the virus" is meant that the inactivated virus retains at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even essentially 100% of the antigenicity of a virus that is not subjected to inactivation.

A variety of methods are known in the art for inactivating viruses. In some embodiments, the virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

In some embodiments, the inactivating is by chemical inactivation and involves contacting the virus with one or more chemical inactivation agents for a period of time under conditions such that the virus is inactivated but the antigenic epitopes are substantially intact. In some embodiments, the virus is inactivated for a period of time that is longer than is required to completely inactivate the virus. In some embodiments, the virus supernatant is inactivated for the number of days required to inactivate the virus plus at least one additional day. Samples of the virus supernatant may be taken at one or more times throughout the inactivation process and assessed for viral viability (infectivity) by any method known in the art, such as by infecting a monolayer of host cells (*i.e.*, plaque assay). Using such a procedure, the period of time that is required to completely inactivate the virus can be determined, and a longer period of time is selected to ensure complete inactivation.

In some embodiments, the virus is contacted with a chemical inactivation agent for between 1 day and 50 days, between 2 days and 40 days, between 2 days and 30 days, between 2 days and 20 days, between 2 days and 10 days, between 3 days and 9 days, between 4 days and 8 days, between 5 days and 7 days, between 2 days and 5 days, or between 5 and 10 days. In some embodiments, the virus is contacted with one or more chemical inactivation agents for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days, 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, 42 days, 43 days, 44 days, 45 days, 46 days, 47 days, 48 days, 49 days, or at least 50 days.

In some embodiments, the chemical inactivation is performed at about +5°C, +10°C, +15°C, +20°C, +25°C, +30°C, +35°C, +40°C, or about +45°C. In some embodiments, the chemical inactivation is performed at about +4°C. In some embodiments, the chemical inactivation is performed at about +22°C.

Any chemical inactivation agent known in the art may be suitable for inactivating the virus in the methods described herein. It will be appreciated by one of skill in the art that factors such as the

chemical inactivation agent and the temperature at which inactivation is performed may affect the length of time (number of days) required to completely inactivate the virus. Examples of chemical inactivation agents include, without limitation, formaldehyde, enzymes, β-propiolactone, ethanol, trifluroacetic acid, acetonitrile, bleach, urea, guanidine hydrochloride, tri-n-butyl phosphate, ethylene-imine or a derivatives thereof, and organic solvents such as Tween, Triton, sodium deoxycholate, and sulphobetaine. A preferred inactivation is inactivation with formaldehyde at 22°C +/-2°C for about 10 days.

In some embodiments, the inactivating agent is neutralized after chemical inactivation of the virus. In some embodiments, the inactivating agent is formaldehyde and is neutralized after chemical inactivation using sodium thiosulphate or sodium metabisulfite.

In some embodiments, the virus is inactivated by thermal inactivation. In some embodiments, the thermal inactivation involves exposing the virus to heat, such as dry heat or vapor heat, for a period of time. In some embodiments, the thermal inactivation involves exposing the virus to temperatures of about +40°C, +45°C, +50°C, +55°C, +60°C, +65°C, +70°C, +75°C, +80°C, +85°C, +90°C,+ 95°C, or about +100°C. In some embodiments, the virus is exposed to heat for at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, about 96 hours, or longer. A preferred thermal inactivation involves exposing the virus to temperatures of about +56°C for 60 minutes.

In some embodiments, the virus is inactivated by exposing the virus to acidic or alkaline conditions for a period of time such that the virus is completely inactivated. The pH of a virus preparation may be adjusted to a desired pH, for example by the addition of an acid, a base, or a buffer with a particular pH to the virus preparation. In some embodiments, the virus is inactivated at an acidic pH of about 2, 2.5, 3, 3.5, 4, 4.5, 5 or about 5.5. In other embodiments, the virus is inactivated at an alkaline pH of about 8, 8.5, 9, 9.5, 10, or about 10.5.

In some embodiments, the virus is inactivated using UV inactivation. UV inactivation involves exposing the virus to energy-rich radiation, such as UV-A, UV-B, or UV-C light for a period of time. It will be appreciated that any two or more methods of inactivation may be combined and performed concurrently or serially.

The inactivated virus may be subsequently dialyzed to remove any undesired material, including the inactivating agent and any neutralizing agent, and/or to replace the buffer with a buffer that is pharmaceutically acceptable for administration to subjects. In some embodiments, the inactivated virus is dialyzed with PBS. In addition or alternatively, the inactivated virus may be filtered, such as sterile filtered, through a 0.22 μm filter.

Any of the methods or uses described herein may be for the prevention of a Zika virus infection in a subject. As used herein, the terms "prevent," "preventing" and "protection from" include the administration of a virus vaccine or composition to a subject to reduce, or delay the onset of the manifestation of clinical or subclinical symptoms, complications, pathologies or biochemical indicia of a disease or infection, or to reduce or inhibit the spread/transmission of the Zika virus. As used herein, antigen(s), such as an inactivated Zika virus, that is administered to a subject prophylactically (e.g., prior to infection) may be referred to as a vaccine.

Zika Vaccine

As described herein Zika virus may cause any of a variety of symptoms upon infection of a subject, and is generally characterized by mild fever; rash (exanthema) on face, neck, trunk, upper arms; headache; sensitivity to light; non-inflammatory joint pain; conjunctivitis; lack of appetite; diarrhea; abdominal pain; and/or dizziness. Zika virus infection during pregnancy is associated with microcephaly in the fetus/neonate. There is also a probable association between the onset of Guillain-Barré syndrome or symptoms thereof. Diagnosis of Zika virus infection in subjects exposed to Zika virus or suspected of being exposed to Zika virus involves detecting the presence of virus-specific antibodies and/or molecular testing, such as PCR or real-time PCR detection of Zika virus.

Provided herein are methods for administering a dose of a therapeutically effective amount of a Zika virus vaccine to a subject in need thereof. In some embodiments, the subject is a mammalian subject, such as a human, non-human primate, rodent, rabbit, sheep, dog, cat, horse, or cow. In some embodiments, the subject is a mouse. In some embodiments, the subject is a human subject, such as a child, an adult, or an elderly adult. In some embodiments, the subject is a female subject. In some embodiments, the subject is pregnant or planning on becoming pregnant. In some embodiments, the subject is at risk of being exposed to Zika virus. In some embodiments, the subject is living in or traveling to an area where Zika virus is present or is thought to be present. In some embodiments, the subject has been previously infected with or vaccinated against Dengue virus; i.e., at risk for antibody-dependent enhancement of disease. In some embodiments, the subject is living in or

traveling to an area that is experiencing a Zika virus infection outbreak. In some embodiments, the subject is living in or traveling to an area where an arthropod vector capable of transmitting the Zika virus vector is present or is thought to be present.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject in a therapeutically effective amount or a dose of a therapeutically effective amount. As used herein, a "therapeutically effective amount" of vaccine is any amount that results in a desired response or outcome in a subject, such as those described herein, including but not limited to prevention of infection, an immune response or an enhanced immune response to Zika virus, or prevention or reduction of symptoms associated with Zika disease.

In some embodiments, the therapeutically effective amount of a Zika virus vaccine or composition described herein is an amount sufficient to generate antigen-specific antibodies (*e.g.*, anti-Zika virus antibodies). In some embodiments, the therapeutically effective amount is sufficient to provide seroprotection in a subject; i.e., to generate sufficient antigen-specific antibodies to prevent/protect from infection. In some embodiments, seroprotection is conferred on at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects. In some embodiments, seroprotection is defined by a reduction in the number of Zika virus plaques by 50% or more in a plaque reduction neutralization test (PRNT) by a 1:10 or higher dilution of sera from a vaccinated subject. In some embodiments, an effective amount of the Zika vaccine is sufficient to seroconvert a subject with at least 70% probability. In some embodiments, the therapeutically effective amount is sufficient to seroconvert a subject with at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98%, or at least 99% probability. Whether a subject has been seroconverted can be assessed by any method known in the art, such as obtaining a serum sample from the subject and performing an assay to detect anti-Zika virus antibodies. In some embodiments, a subject is seroconverted if a serum sample from the subject contains an amount of anti-Zika virus antibodies that surpasses a threshold or predetermined baseline. A subject is generally considered seroconverted if there is at least a 4-fold increase in anti-Zika virus antibodies (*i.e.,* anti-Zika E protein IgG antibodies) in a serum sample from the subject as compared to a serum sample previously taken from the same subject.

In some embodiments, seroconversion of a subject is assessed by performing a plaque reduction neutralization test (PRNT). Briefly, PRNT is used to determine the serum titer required to reduce the number of Zika virus plaques by 50% (PRNT50) as compared to a control serum/antibody. The PRNT50 may be carried out using monolayers of Vero cells or any other cell type/line that can be infected with Zika virus. Sera from subjects are diluted and incubated with live, non-inactivated Zika

virus. The serum/virus mixture may be applied to the Vero cells and incubated for a period of time. Plaques formed on the Vero cell monolayers are counted and compared to the number of plaques formed by the Zika virus in the absence of serum or a control antibody. A threshold of neutralizing antibodies of 1:10 dilution of serum in a PRNT50 is generally accepted as evidence of protection (Hombach *et. al.* Vaccine (2005) 23:5205-5211).

In some embodiments, the Zika virus may be formulated for administration in a composition, such as a pharmaceutical composition. The term "pharmaceutical composition" as used herein means a product that results from the mixing or combining of at least one active ingredient, such as an inactivated Zika virus, and one or more inactive ingredients, which may include one or more pharmaceutically acceptable excipient.

Pharmaceutical compositions of the invention, including vaccines, can be prepared in accordance with methods well known and routinely practiced in the art (see *e.g.*, Remington: The Science and Practice of Pharmacy, Mack Publishing Co. 20th ed. 2000; and Ingredients of Vaccines – Fact Sheet from the Centers for Disease Control and Prevention, e.g., adjuvants and enhancers such as alum to help the vaccine improve its work, preservatives and stabilizers to help the vaccine remain unchanged (e.g., albumin, phenols, glycine)). Pharmaceutical compositions are preferably manufactured under GMP conditions. Typically a therapeutically effective dose of the inactivated Zika virus preparation is employed in the pharmaceutical composition of the invention. The inactivated Zika virus is formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, the prophylactic response).

Dosages of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired pharmaceutical response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors.

A physician, veterinarian or other trained practitioner, can start doses of the inactivated Zika virus vaccine employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect (*e.g.*, production of anti-Zika virus antibodies) is achieved. In general, effective doses of the compositions of the present invention, for the prophylactic treatment of groups of people as described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and the titer of anti-Zika virus antibodies desired. Dosages need to be titrated to optimize safety and efficacy. In some embodiments, the dosing regimen entails subcutaneous or intramuscular administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 7. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 14. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 28. In some embodiments, the inactivated Zika virus is administered to the subject once.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject with, prior to, or after administration of one or more adjuvants. An adjuvant is a molecule that enhances a response in a subject, such as an immune response, to an antigen or other molecule. In some embodiments, an adjuvant may stabilize an antigen or other molecule. Determining whether a Zika virus vaccine or compositions thereof are administered with an adjuvant depends on various factors (e.g., type and extent of response desired) and will be evident to one of skill in the art. In some embodiments, administering any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may enhance the production of virus neutralizing (anti-Zika virus) antibodies. In some embodiments, a subject that is administered any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may only require a single administration of the Zika virus vaccine or composition to be seroconverted (produce a level of anti-Zika virus antibodies). Examples of adjuvants may include, without limitation, aluminium salt (aluminium hydroxide or aluminium phosphate), calcium phosphate hydroxide, paraffin oil, killed bacteria, bacterial toxins, toxoids, subunits of bacteria, squalene, thimerosal, detergents, IL-1, IL-2, IL-12, 2-component adjuvants, such as 2-component adjuvants containing an antibacterial peptide and a TLR9 agonist (*e.g.*, IC31®), and combinations such as Freund's complete adjuvant and Freund's incomplete adjuvant. In some embodiments, the Zika virus vaccines or compositions is administered with aluminium hydroxide. In some embodiments, the inactivated Zika virus vaccine or composition is administered with aluminium phosphate salt. A preferred aluminium salt is the aluminium hydroxide with reduced Cu content, e.g. lower than

1,25ppb based on the weight of the Zika composition, an adjuvant described in detail in WO 2013/083726 or Schlegl et al., Vaccine 33 (2015) 5989-5996.

In some embodiments, the adjuvant is comprised of two components. In some embodiments, the 2-component adjuvant comprises an antibacterial peptide and a TLR9 agonist. In some embodiments, the antibacterial peptide is provided by the amino acid sequence $KLKL_5KLK$ (SEQ ID NO: 71). In some embodiments, the TLR9 agonist is a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN). In some embodiments, the I-ODN comprises the nucleic acid sequence $(dIdC)_{13}$ (SEQ ID NO: 70). In some embodiments, the adjuvant is IC31®. In some embodiments, the adjuvant is in nanoparticle form (See, *e.g.*, US Patent No. 8,765,148 B2, incorporated by reference in its entirety). In some embodiments, the adjuvant is IC31®, i.e. $KLKL_5KLK$ (SEQ ID NO: 71) and the nucleic acid sequence $(dIdC)_{13}$ (SEQ ID NO: 70), in combination with an aluminium salt such as aluminium hydroxide.

The Zika virus vaccines or compositions described herein may be administered to a subject concomitantly with one or more vaccines to another infectious agent, such as another infectious agent is that present or thought to be present in the same geographic area as Zika virus. In some embodiments, the other infectious agent is one that the subject is also at risk of being in contact with. In some embodiments, the other infectious agent is transmitted by the same arthropod vector as Zika virus. In some embodiments, the other infectious agent is Japanese Encephalitis virus, Yellow Fever virus, Dengue virus and/or Chikungunya virus.

Also within the scope of the present disclosure are kits for use in prophylactically administering to a subject, for example to prevent or reduce the severity of Zika virus infection. Such kits can include one or more containers comprising a composition containing inactivated Zika virus, such as an inactivated Zika virus vaccine. In some embodiments, the kit may further include one or more additional containing comprising a second composition, such as a second vaccine. In some embodiments, the second vaccine is a vaccine for another arbovirus. In some embodiments, the second vaccine is a Dengue virus vaccine and/or a Chikungunya virus vaccine.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the composition containing inactivated Zika virus to prevent, delay the onset, or reduce the severity of Zika virus infection. The kit may further comprise a description of selecting a subject suitable for administration based on identifying whether that subject is at risk for exposure to Zika virus or

contracting a Zika virus infection. In still other embodiments, the instructions comprise a description of administering a composition containing inactivated Zika virus to a subject at risk of exposure to Zika virus or contracting Zika virus infection.

The instructions relating to the use of the composition containing inactivated Zika virus generally include information as to the dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine readable instructions are also acceptable.

The kits of the present disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as a syringe or an infusion device. The container may have a sterile access port, for example the container may be a vial having a stopper pierceable by a hypodermic injection needle. At least one active agent in the composition is an inactivated Zika virus, as described herein.

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including", "comprising", or "having,", "containing", "involving", and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, virology, cell or tissue culture, genetics and protein and nucleic chemistry described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art

and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated.

The present invention is further illustrated by the following examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove. However, the citation of any reference is not intended to be an admission that the reference is prior art.

Table 1. Overview of process buffers and stock solutions.

Buffer	Composition	Final pH	Final conductivity [mS/cm]
A	0.5 M NaOH		n.a.
B	0.1 M NaOH		n.a.
C	25 mM Tris, 150 mM NaCl	7.4±0.2	16.5
D	1 M Tris	7.4±0.2	n.a.
E	4.5 M NaCl	n.a.	n.a.
F	1 M NaCl	n.a.	n.a.
G	1 % SDS	n.a.	n.a.
H	50 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
I	35 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
J	15 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
K	10 x PBS	7.4±0.2	n.a.
L	50 mg/mL Protamine sulphate	7.4±0.2	n.a.
M	Drug substance formulation buffer (10mM Tris(hydroxymethyl)-aminomethan, 5 % Sucrose, 1% (10 mg/mL) rHSA)	7.4±0.2	1.3

Table 2. Abbreviations.

°Bx	Degrees Brix = sugar content (w/w) of an aqueous solution*
BSA	Bovine serum albumin
CC700	Capto™ Core 700
CPE	Cytopathic effect
EtOH	Ethanol
EU	Endotoxin units
DS	Drug Substance
DP	Drug Product
DSP	Downstream Process
HCP	Host cell protein
hcDNA	Host cell DNA
hpi	Hours post infection
HPLC	High Performance Liquid Chromatography
ID	Inner diameter
JEV	Japanese Encephalitis virus
LAL	Limulus amebocyte lysate
LDS buffer	Lithium dodecyl sulfate sample loading buffer

LOD	Limit of detection
LOQ	Limit of quantitation
MALLS	Multiangle light scattering
mAU	Milli absorbance units
MS	Mass spectroscopy
NIV	Neutralized inactivated virus
PBS	Phosphate buffered saline
PD	Process development
PFU	Plaque forming units
p.i.	Post-infection
PS	Protamine sulphate or protamine sulfate
rcf	Relative centrifugal force
rHSA	Recombinant human serum albumin
Rms radius	Root mean square radius
rMSB	Research master seed bank
RSD	Relative standard deviation
SEC	Size exclusion chromatography
SGC	Sucrose gradient centrifugation
SGP	Sucrose gradient purified
SDS	Sodium dodecyl sulphate
TBS	Tris buffered saline
TFF	Tangential flow filtration
TCID50	Tissue culture infectious dose 50%
UF/DF	Ultrafiltration/diafiltration
WFI	Water for injection
ZikaV	Zika virus

*Degrees Brix (°Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. °Bx corresponds to the sucrose content in percent (w/w), e.g., 45 °Bx equals 45 % (w/w) sucrose.

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Table A. Primers for Zika virus sequencing: lower case letters indicate bases not included in ZIKA but containing restriction sites for later cloning when needed (therefore, two Tms provided).

Primer Pair	**Oligoname**	**Primer sequence (5'-3') restriction sites (lower case)**	**Tm (gene-specific)**	**Tm (entire primer)**	**Amplicon size [bp]**
1	9320_Zika_PF_1F	SEQ ID NO: 74 ttaggatccGTTGTTGATCTGTGTGAAT	69.9	74.6	707
	9321_Zika_PF_1R	SEQ ID NO: 75 taactcgagCGTACACAACCCAAGTT	69.3	75.6	
2	9322_Zika_PF_2F	SEQ ID NO: 76 ttaggatccTCACTAGACGTGGGAGTG	70	73.9	704
	9323_Zika_PF_2R	SEQ ID NO: 77 taactcgagAAGCCATGTCYGATATTGAT	69.8	73.7	
3	9324_Zika_PF_3F	SEQ ID NO: 78 ttaggatccGCATACAGCATCAGGTG	72.3	74.5	712
	9325_Zika_PF_3R	SEQ ID NO: 79 taactcgagTGTGGAGTTCCGGTGTCT	72	76.4	
4	9326_Zika_PF_4F	SEQ ID NO: 80 ttaggatccGAATAGAGCGAARGTTGAGATA	70.9	74	712
	9327_Zika_PF_4R	SEQ ID NO: 81 taactcgAGTGGTGGGTGATCTTCTTCT	70.5	73.7	
5	9328_Zika_PF_5F	SEQ ID NO: 82	70.3	75	704

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
		ttaggatcCAGTCACAGTGGAGGTACAGTAC			
	9329_Zika_PF_5R	SEQ ID NO: 83 taactcgagCRCAGATACCATCTTCCC	71.5	77.3	
6	9330_Zika_PF_6F	SEQ ID NO: 84 ttaggatCCCTTATGTGCTTGGCCTTAG	70.7	72.7	698
	9331_Zika_PF_6R	SEQ ID NO: 85 taactcgagTCTTCAGCCTCCATGTG	70.4	76.9	
7	9332_Zika_PF_7F	SEQ ID NO: 86 ttaggatccAATGCCCACTCAAACATAGA	71.9	75	716
	9333_Zika_PF_7R	SEQ ID NO: 87 taactcgagTCATTCTCTTCTTCAGCCCTT	71	74	
8	9334_Zika_PF_8F	SEQ ID NO: 88 ttaggatccAAGGGTGATCGAGGAAT	70.9	75.2	703
	9335_Zika_PF_8R	SEQ ID NO: 89 taactcgagTTCCCTTCAGAGAGAGGAGC	71.9	73.4	
9	9336_Zika_PF_9F	SEQ ID NO: 90 ttaggatccTCTTTTGCAAACTGCGATC	71.9	75	699
	9337_Zika_PF_9R	SEQ ID NO: 91 taactcgagTCCAGCTGCAAAGGGTAT	71	74.9	
10	9338_Zika_PF_10F	SEQ ID NO: 92 ttaggatccGTGTGGACATGTACATTGA	71.4	75.8	706
	9339_Zika_PF_10R	SEQ ID NO: 93 taactcgagCCCATTGCCATAAAGTC	70.4	75.8	
11	9340_Zika_PF_11F	SEQ ID NO: 94 ttaggatccTCATACTGTGGTCCATGGA	71.6	78.1	692
	9341_Zika_PF_11R	SEQ ID NO: 95 taactcgagGCCCATCTCAACCCTTG	74	78	
12	9342_Zika_PF_12F	SEQ ID NO: 96 ttaggatccTAGAGGGCTTCCAGTGC	70.9	74	707
	9343_Zika_PF_12R	SEQ ID NO: 97 taactcgAGATACTCATCTCCAGGTTTGTTG	70.2	72.2	
13	9344_Zika_PF_13F	SEQ ID NO: 98 ttaggatccGAAAACAAAACATCAAGAGTG	70.6	75.4	726
	9345_Zika_PF_13R	SEQ ID NO: 99 taactcgagGAATCTCTCTGTCATGTGTCCT	71.9	75.6	
14	9346_Zika_PF_14F	SEQ ID NO: 100 ttaggatccTTGATGGCACGACCAAC	73.1	75.6	715
	9347_Zika_PF_14R	SEQ ID NO: 101 ttaggatccGTTGTTGATCTGTGTGAAT	70.8	77.9	
15	9348_Zika_PF_15F	SEQ ID NO: 102 taactcgagCAGGTCAATGTCCATTG	71.9	75.4	719
	9349_Zika_PF_15R	SEQ ID NO: 103 ttaggatccTGTTGTGTTCCTATTGCTGGT	73.9	77.2	
16	9350_Zika_PF_16F	SEQ ID NO: 104 taactcgaGTGATCAGRGCCCCAGC	72.3	75.4	703
	9351_Zika_PF_16R	SEQ ID NO: 105 ttaggatccTGCTGCCCAGAAGAGAA	72	76.3	
17	9352_Zika_PF_17F	SEQ ID NO: 106 taactcgaGCACCAACAYGGGTTCTT	73.6	76	705
	9353_Zika_PF_17R	SEQ ID NO: 107 ttaggatcCTCAAGGACGGTGTGGC	72	75.5	
18	9354_Zika_PF_18F	SEQ ID NO: 108 taactcgagCAATGATCTTCATGTTGGG	71.7	75.8	699
	9355_Zika_PF_18R	SEQ ID NO: 109 ttaggatccTATGGGGGAGGACTGGT	71	74.1	
19	9356_Zika_PF_19F	SEQ ID NO: 110 taactcGAGCCCAGAACCTTGGATC	73.3	75.5	711
	9357_Zika_PF_19R	SEQ ID NO: 111 ttaggatcCAGACCCCCAAGAAGGC	71.3	76.9	
20	9358_Zika_PF_20F	SEQ ID NO: 112 taactcgagCCCCTTTGGTCTTGTCT	71.7	75	706
	9359_Zika_PF_20R	SEQ ID NO: 113	71.9	73.9	

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
		ttaggatccAGGAAGGATGTATGCAGATG			
21	9360_Zika_PF_21F	SEQ ID NO: 114 taactcgagACATTTGCGCATATGATTTTG	70.4	75.7	709
	9361_Zika_PF_21R	SEQ ID NO: 115 ttaggatccAGGAAGGACACACAAGAGT	71.8	75	
22	9362_Zika_PF_22F	SEQ ID NO: 116 taactcgagACAGGCTGCACAGCTTT	70	79.1	581
	9363_Zika_PF_22R	SEQ ID NO: 117 ttaggatccTCTCTCATAGGGCACAGAC	74.8	81.1	

SEQUENCES

SEQ ID NO: 1
A typical form of protamine
PRRRRSSSRP VRRRRRPRVS RRRRRRGGRR RR

Provided below are examples of nucleic acid sequences of the genomes of Zika viruses that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 2
KU321639.1 Zika virus strain ZikaSPH2015, Brazil, complete genome
GTTGTTACTGTTGCTGACTCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTGG
ATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACG
CGGAGTAGCCCGTGTGAGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGTCATGGGCCCATCAGG
ATGGTCTTGGCAATTCTAGCCTTTTTGAGATTCACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTG
GGGAAAAAAGAGGCTATGGAAATAATAAAGAAGTTCAAGAAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGA
AGGAGAAGAAGAGACGGGGCGCAGATACTAGTGTCGGAATTGTTGGCCTCCTGCTGACCACAGCTATGGCAGCGGAGG
TCACTAGACGTGGGAGTGCATACTATATGTACTTGGACAGAAACGATGCTGGGGAGGCCATATCTTTTCCAACCACATTG
GGGATGAATAAGTGTTATATACAGATCATGGATCTTGGACACATGTGTGATGCCACCATGAGCTATGAATGCCCTATGCT
GGATGAGGGGGTGGAACCAGATGACGTCGATTGTTGGTGCAACACGACGTCAACTTGGGTTGTGTACGGAACCTGCCAT
CACAAAAAAGGTGAAGCACGGAGATCTAGAAGAGCTGTGACGCTCCCCTCCCATTCCACTAGGAAGCTGCAAACGCGGT
CGCAAACCTGGTTGGAATCAAGAGAATACACAAAGCACTTGATTAGAGTCGAAAATTGGATATTCAGGAACCCTGGCTTC
GCGTTAGCAGCAGCTGCCATCGCTTGGCTTTTGGGAAGCTCAACGAGCCAAAAAGTCATATACTTGGTCATGATACTGCT
GATTGCCCCGGCATACAGCATCAGGTGCATAGGAGTCAGCAATAGGGACTTTGTGGAAGGTATGTCAGGTGGGACTTGG
GTTGATATTGTCTTGGAACATGGAGGTTGTGTCACCGTAATGGCACAGGACAAACCGACTGTCGACATAGAGCTGGTTAC
AACAACAGTCAGCAACATGGCGGAGGTAAGATCCTACTGCTATGAGGCATCAATATCAGACATGGCTTCGGACAGCCGCT
GCCCAACACAAGGTGAAGCCTACCTTGACAAGCAATCAGACACTCAATATGTCTGCAAAAGAACGTTAGTGGACAGAGGC
TGGGGAAATGGATGTGGACTTTTTGGCAAAGGGAGTCTGGTGACATGCGCTAAGTTTGCATGCTCCAAGAAAATGACCG
GGAAGAGCATCCAGCCAGAGAATCTGGAGTACCGGATAATGCTGTCAGTTCATGGCTCCCAGCACAGTGGGATGATCGT
TAATGACACAGGACATGAAACTGATGAGAATAGAGCGAAGGTTGAGATAACGCCCAATTCACCAAGAGCCGAAGCCACC
CTGGGGGGTTTTGGAAGCCTAGGACTTGATTGTGAACCGAGGACAGGCCTTGACTTTTCAGATTTGTATTACTTGACTATG
AATAACAAGCACTGGTTGGTTCACAAGGAGTGGTTCCACGACATTCCATTACCTTGGCACGCTGGGGCAGACACCGGAAC
TCCACACTGGAACAACAAAGAAGCACTGGTAGAGTTCAAGGACGCACATGCCAAAAGGCAAACTGTCGTGGTTCTAGGG
AGTCAAGAAGGAGCAGTTCACACGGCCCTTGCTGGAGCTCTGGAGGCTGAGATGGATGGTGCAAAGGGAAGGCTGTCCT
CTGGCCACTTGAAATGTCGCCTGAAAATGGATAAACTTAGATTGAAGGGCGTGTCATACTCCTTGTGTACCGCAGCGTTCA
CATTCACCAAGATCCCGGCTGAAACACTGCACGGGACAGTCACAGTGGAGGTACAGTACGCAGGGACAGATGGACCTTG

CAAGGTTCCAGCTCAGATGGCGGTGGACATGCAAACTCTGACCCCAGTTGGGAGGTTGATAACCGCTAACCCCGTAATCA
CTGAAAGCACTGAGAACTCTAAGATGATGCTGGAACTTGATCCACCATTTGGGGACTCTTACATTGTCATAGGAGTCGGG
GAGAAGAAGATCACCCACCACTGGCACAGGAGTGGCAGCACCATTGGAAAAGCATTTGAAGCCACTGTGAGAGGTGCCA
AGAGAATGGCAGTCTTGGGAGACACAGCCTGGGACTTTGGATCAGTTGGAGGCGCTCTCAACTCATTGGGCAAGGGCAT
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ATGTGGTTGGGTCTGAACACAAAGAATGGATCTATTTCCCTTATGTGCTTGGCCTTAGGGGGAGTGTTGATCTTCTTATCC
ACAGCCGTCTCTGCTGATGTGGGGTGCTCGGTGGACTTCTCAAAGAAGGAGACGAGATGCGGTACAGGGGTGTTCGTCT
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AGCCTGGGAAGATGGTATCTGCGGGATCTCCTCTGTTTCAAGAATGGAAAACATCATGTGGAGATCAGTAGAAGGGGAG
CTCAACGCAATCCTGGAAGAGAATGGAGTTCAACTGACGGTCGTTGTGGGATCTGTAAAAAACCCCATGTGGAGAGGTC
CACAGAGATTGCCCGTGCCTGTGAACGAGCTGCCCCACGGCTGGAAGGCTTGGGGGAAATCGCACTTCGTCAGAGCAGC
AAAGACAAATAACAGCTTTGTCGTGGATGGTGACACACTGAAGGAATGCCCACTCAAACATAGAGCATGGAACAGCTTTC
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ATGACACATGGAGGCTGAAGAGGGCCCATCTGATCGAGATGAAAACATGTGAATGGCCAAAGTCCCACACATTGTGGAC
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AAAGAACCAGAAAGCAACTTAGTAAGGTCAATGGTGACTGCAGGATCAACTGATCACATGGATCACTTCTCCCTTGGAGT
GCTTGTGATTCTGCTCATGGTGCAGGAAGGGCTGAAGAAGAGAATGACCACAAAGATCATCATAAGCACATCAATGGCA
GTGCTGGTAGCTATGATCCTGGGAGGATTTTCAATGAGTGACCTGGCTAAGCTTGCAATTTTGATGGGTGCCACCTTCGC
GGAAATGAACACTGGAGGAGATGTAGCTCATCTGGCGCTGATAGCGGCATTCAAAGTCAGACCAGCGTTGCTGGTATCTT
TCATCTTCAGAGCTAATTGGACACCCCGTGAAAGCATGCTGCTGGCCTTGGCCTCGTGTCTTTTGCAAACTGCGATCTCCG
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CTGATAACATCACCTTGGCAATCCTGGCTGCTCTGACACCACTGGCCCGGGGCACACTGCTTGTGGCGTGGAGAGCAGGC
CTTGCTACTTGCGGGGGGTTTATGCTCCTCTCTCTGAAGGGAAAAGGCAGTGTGAAGAAGAACTTACCATTTGTCATGGC
CCTGGGACTAACCGCTGTGAGGCTGGTCGACCCCATCAACGTGGTGGGGCTGCTGTTGCTCACAAGGAGTGGGAAGCGG
AGCTGGCCCCCTAGCGAAGTACTCACAGCTGTTGGCCTGATATGCGCATTGGCTGGAGGGTTCGCCAAGGCAGATATAGA
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ACTGCTAGGTTCAACACAAGTTGGAGTGGGAGTTATGCAAGAGGGGGTCTTTCACACTATGTGGCACGTCACAAAAGGA
TCCGCGCTGAGAAGCGGTGAAGGGAGACTTGATCCATACTGGGGAGATGTCAAGCAGGATCTGGTGTCATACTGTGGTC
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TTCACGTCTACTACAGCCAATCAGAGTCCCCAACTATAATCTGTATATTATGGATGAGGCCCACTTCACAGATCCCTCAAGT
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CAGGAAGCCATTGACAACCTCGCTGTGCTCATGCGGGCAGAGACTGGAAGCAGGCCTTACAAAGCCGCGGCGGCCCAAT
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GGTGTGGCAACGGGAGGCCATGCTGTGTCCCGAGGAAGTGCAAAGCTGAGATGGTTGGTGGAGCGGGGATACCTGCAG
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ATCCGCAGTGAGCACGCGGAAACGTGGTTCTTTGACGAGAACCACCCATATAGGACATGGGCTTACCATGGAAGCTATGA
GGCCCCCACACAAGGGTCAGCGTCCTCTCTAATAAACGGGGTTGTCAGGCTCCTGTCAAAACCCTGGGATGTGGTGACTG
GAGTCACAGGAATAGCCATGACCGACACCACACCGTATGGTCAGCAAAGAGTTTTCAAGGAAAAAGTGGACACTAGGGT
GCCAGACCCCCAAGAAGGTACTCGTCAGGTTATGAGCATGGTCTCTTCCTGGTTGTGGAAAGAGCTAGGCAAACACAAAC
GGCCACGAGTCTGTACCAAAGAAGAGTTCATCAACAAGGTTCGTAGCAATGCAGCATTAGGGGCAATATTTGAAGAGGA
AAAAGAGTGGAAGACTGCAGTGGAAGCTGTGAACGATCCAAGGTTCTGGGCTCTAGTGGACAAGGAAAGAGAGCACCA
CCTGAGAGGAGAGTGCCAGAGTTGTGTGTACAACATGATGGGAAAAAGAGAAAAGAAACAAGGGGAATTTGGAAAGGC
CAAGGGCAGCCGCGCCATCTGGTATATGTGGCTAGGGGCTAGATTTCTAGAGTTCGAAGCCCTTGGATTCTTGAACGAGG
ATCACTGGATGGGGAGAGAGAACTCAGGAGGTGGTGTTGAAGGGCTGGGATTACAAAGACTCGGATATGTCCTAGAAG
AGATGAGTCGCATACCAGGAGGAAGGATGTATGCAGATGACACTGCTGGCTGGGACACCCGCATCAGCAGGTTTGATCT
GGAGAATGAAGCTCTAATCACCAACCAAATGGAGAAAGGGCACAGGGCCTTGGCATTGGCCATAATCAAGTACACATAC
CAAAACAAAGTGGTAAAGGTCCTTAGACCAGCTGAAAAAGGGAAAACAGTTATGGACATTATTTCGAGACAAGACCAAA
GGGGGAGCGGACAAGTTGTCACTTACGCTCTTAACACATTTACCAACCTAGTGGTGCAACTCATTCGGAATATGGAGGCT
GAGGAAGTCCTAGAGATGCAAGACTTGTGGCTGCTGCGGAGGTCAGAGAAAGTGACCAACTGGTTGCAGAGCAACGGA
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SEQ ID NO: 3
KU497555.1 Zika virus isolate Brazil-ZKV2015, Brazil, complete genome

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SEQ ID NO: 4
KU501215.1 Zika virus strain PRVABC59, Puerto Rico, complete genome
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SEQ ID NO: 5
KU509998.1 Zika virus strain Haiti/1225/2014, Haiti, complete genome
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SEQ ID NO: 6
KU527068.1 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, complete genome
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SEQ ID NO: 7

KU681081.3 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, complete genome

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SEQ ID NO: 8
KU681082.3 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, complete genome
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SEQ ID NO: 9

KU707826.1 Zika virus isolate SSABR1, Brazil, complete genome

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SEQ ID NO: 11
LC002520.1 Zika virus genomic RNA, strain: MR766-NIID, Uganda, complete genome
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SEQ ID NO: 12
AY632535.2 NC_012532.1 Zika virus strain MR 766, Uganda, complete genome
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AACTCATTCGGAATATGGAGGCTGAGGAAGTTCTAGAGATGCAAGACTTGTGGCTGCTGCGGAGGTCAGAGAAAGTGAC
CAACTGGTTGCAGAGCAACGGATGGGATAGGCTCAAACGAATGGCAGTCAGTGGAGATGATTGCGTTGTGAAGCCAATT
GATGATAGGTTTGCACATGCCCTCAGGTTCTTGAATGATATGGGAAAAGTTAGGAAGGACACACAAGAGTGGAAACCCT
CAACTGGATGGGACAACTGGGAAGAAGTTCCGTTTTGCTCCCACCACTTCAACAAGCTCCATCTCAAGGACGGGAGGTCC
ATTGTGGTTCCCTGCCGCCACCAAGATGAACTGATTGGCCGGGCCCGCGTCTCTCCAGGGGCGGGATGGAGCATCCGGG
AGACTGCTTGCCTAGCAAAATCATATGCGCAAATGTGGCAGCTCCTTTATTTCCACAGAAGGGACCTCCGACTGATGGCCA
ATGCCATTTGTTCATCTGTGCCAGTTGACTGGGTTCCAACTGGGAGAACTACCTGGTCAATCCATGGAAAGGGAGAATGG
ATGACCACTGAAGACATGCTTGTGGTGTGGAACAGAGTGTGGATTGAGGAGAACGACCACATGGAAGACAAGACCCCA
GTTACGAAATGGACAGACATTCCCTATTTGGGAAAAAGGGAAGACTTGTGGTGTGGATCTCTCATAGGGCACAGACCGC
GCACCACCTGGGCTGAGAACATTAAAAACACAGTCAACATGGTGCGCAGGATCATAGGTGATGAAGAAAAGTACATGGA
CTACCTATCCACCCAAGTTCGCTACTTGGGTGAAGAAGGGTCTACACCTGGAGTGCTGTAAGCACCAATCTTAGTGTTGTC
AGGCCTGCTAGTCAGCCACAGCTTGGGGAAAGCTGTGCAGCCTGTGACCCCCCCAGGAGAAGCTGGGAAACCAAGCCTA
TAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCTGTGAGCCCCTCAGAGGACACTGAGTCAAAAAACC
CCACGCGCTTGGAGGCGCAGGATGGGAAAAGAAGGTGGCGACCTTCCCCACCCTTCAATCTGGGGCCTGAACTGGAGAT
CAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAG

In some embodiments, the Zika virus has a RNA genome corresponding to the DNA sequence provided by the nucleic acid sequence of any one of SEQ ID NOs: 2-13 or 72. In some embodiments, the Zika virus has a variant genome that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%. 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-13 or 72.

Provided below are amino acid sequences of the E-proteins of Zika strains that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 14

isol-ARB15076.AHF49784.1.Central_African_Republic/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
TDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 15
isol-IbH_30656.AEN75265.1.Nigeria/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHSGADTETPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
RDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSIIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 16
ArB1362.AHL43500.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXXXXXXXNR
AEVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 17
ArD128000.AHL43502.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMXXXXXGHETDEN
RAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHRLVRKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWLKKGSSIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 18
ArD158095.AHL43505.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 19
ArD158084.AHL43504.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV

EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 20
isol-ARB13565.AHF49783.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 21
isol-ARB7701.AHF49785.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 22
isol-ArD_41519.AEN75266.1.Senegal/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVE
FKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVT
VEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKA
FEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 23
MR766-NIID.BAP47441.1.Uganda/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 24
LC002520.1/326-829 Zika virus genomic RNA, strain: MR766-NIID, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 25

isol-MR_766.AEN75263.1.Uganda/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 26
ArD7117.AHL43501.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAVCTAAKVPAETLHGTVT
VEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKA
FEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 27
AY632535.2/326-825 NC_012532.1 Zika virus strain MR 766, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 28
MR_766.AAV34151.1.Uganda/291-790 Flavivirus envelope glycoprotein E. |Q32ZE1|Q32ZE1_9FL
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 29
MR_766.YP_009227198.1.Uganda/1-500 envelope protein E [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 30
KU681081.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHTGADTGTPHWNNKEALVEF

KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVLNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 31
isol-Zika_virus%H.sapiens-tc%THA%2014%SV0127-_14.AMD61710.1.Thailand/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHTGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVLNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 32
CK-ISL_2014.AIC06934.1.Cook_Islands/1-504 Flavivirus envelope glycoprotein E. (Fragment) OS=Zika virus GN=E PE=4 SV=1
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 33
Natal_RGN.AMB18850.1.Brazil:_Rio_Grande_do_Norte,_Natal/291-794 Flavivirus envelope glycoprotein E.]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 34
isol-Si323.AMC37200.1.Colombia/1-504 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 35
KU707826.1/317-820 Zika virus isolate SSABR1, Brazil, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF

EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 36
KU509998.1/326-829 Zika virus strain Haiti/1225/2014, Haiti, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 37
isol-GDZ16001.AML82110.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 38
BeH819015.AMA12085.1.Brazil/291-794 Flavivirus envelope glycoprotein E.]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 39
MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 40
KU501215.1/308-811 Zika virus strain PRVABC59, Puerto Rico, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 41
Haiti%1225%2014.AMB37295.1.Haiti/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 42
KU527068.1/308-811 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 43
isol-Z1106027.ALX35662.1.Suriname/5-508 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 44
isol-FLR.AMM39804.1.Colombia:_Barranquilla/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 45
PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.Canada/254-757 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 46
BeH818995.AMA12084.1.Brazil/291-794 Flavivirus envelope glycoprotein E. [Zika virus].
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV

EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 47
H/PF/2013.AHZ13508.1.French_Polynesia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 48
PRVABC59.AMC13911.1.Puerto_Rico/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 49
KU321639.1/326-829 Zika virus strain ZikaSPH2015, Brazil, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 50
ZikaSPH2015.ALU33341.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 51
103344.AMC13912.1.Guatemala/291-794 polyprotein [Zika virus]. 103344.AMC13912.1.Guatemala Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEIRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 52
isol-Brazil-ZKV2015.AMD16557.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 53
KU497555.1/308-811 Zika virus isolate Brazil-ZKV2015, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 54
isol-ZJ03.AMM39806.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGARRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 55
isol-FSS13025.AFD30972.1.Cambodia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 56
isol-Z1106032.ALX35660.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 57
isol-Z1106033.ALX35659.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF

KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 58
isol-BeH828305.AMK49165.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDTQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFE
ATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGV
LIFLSTAVSA

SEQ ID NO: 59
isol-GD01.AMK79468.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNGTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 60
isol-Z1106031.ALX35661.1.Suriname/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVLAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 61
ACD75819.1.Micronesia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 62
KU681082.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 63
isol-Zika_virus%H.sapiens-tc%PHL%2012%CPC-0740.AMD61711.1.Philippines/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 64
isol-BeH823339.AMK49164.2.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVSTTVSNMAEVRSYCYEATISDIASDSRCPTQGEAYLD
KQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENR
AKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFK
DAHAKRQTAVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVE
VQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFE
ATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGV
LIFLSTAVSA

SEQ ID NO: 65
isol-P6-740.AEN75264.1.Malaysia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWXRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 66
KU744693.1/326-829 Zika virus isolate VE_Ganxian, China, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMLVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETVDGTVTV
EGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSG

SEQ ID NO: 67
isol-VE_Ganxian.AMK79469.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMLVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETVDGTVTV
EGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSG

SEQ ID NO: 68
ArD157995.AHL43503.1.-/291-794 Flavivirus envelope glycoprotein E.

ISCIGVSNRDLVEGMSGGTWVDVVLEHGGCVTEMAQDKPTVDIELVTMTVSNMAEVRSYCYEASLSDMASASRCPTQGEPSL
DKQSDTQSVCKRTLGDRGWGNGCGIFGKGSLVTCSKFTCCKKMPGKSIQPENLEYRIMLPVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQSAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 69
MR_766.ABI54475.1.Uganda/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
TDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 70
5′-(dIdC)$_{13}$-3′
dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC

SEQ ID NO: 71
KLK peptide
KLKLLLLLKLK

SEQ ID NO: 72
ZIKV Sequence H/PF/2013 as sequenced
CAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTTGGATTTGGAAACGAGAGTT
TCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACGCGGAGTAGCCCGTGTG
AGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGTCATGGGCCCATCAGGATGGTCTTGGCGATTCT
AGCCTTTTTGAGATTCACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTGGGGAAAAAAGAGGCTA
TGGAAATAATAAAGAAGTTCAAGAAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGAAGGAGAAGAAGAGAC
GAGGCGCAGATACTAGTGTCGGAATTGTTGGCCTCCTGCTGACCACAGCTATGGCAGCGGAGGTCACTAGACGTGGGAG
TGCATACTATATGTACTTGGACAGAAACGACGCTGGGGAGGCCATATCTTTTCCAACCACATTGGGGATGAATAAGTGTT
ATATACAGATCATGGATCTTGGACACATGTGTGATGCCACCATGAGCTATGAATGCCCTATGCTGGATGAGGGGGTGGAA
CCAGATGACGTCGATTGTTGGTGCAACACGACGTCAACTTGGGTTGTGTACGGAACCTGCCATCACAAAAAAGGTGAAGC
ACGGAGATCTAGAAGAGCTGTGACGCTCCCCTCCCATTCCACTAGGAAGCTGCAAACGCGGTCGCAAACCTGGTTGGAAT
CAAGAGAATACACAAAGCACTTGATTAGAGTCGAAAATTGGATATTCAGGAACCCTGGCTTCGCGTTAGCAGCAGCTGCC
ATCGCTTGGCTTTTGGGAAGCTCAACGAGCCAAAAAGTCATATACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGC
ATCAGGTGCATAGGAGTCAGCAATAGGGACTTTGTGGAAGGTATGTCAGGTGGGACTTGGGTTGATGTTGTCTTGGAAC
ATGGAGGTTGTGTCACCGTAATGGCACAGGACAAACCGACTGTCGACATAGAGCTGGTTACAACAACAGTCAGCAACAT
GGCGGAGGTAAGATCCTACTGCTATGAGGCATCAATATCGGACATGGCTTCGGACAGCCGCTGCCCAACACAAGGTGAA
GCCTACCTTGACAAGCAATCAGACACTCAATATGTCTGCAAAAGAACGTTAGTGGACAGAGGCTGGGGAAATGGATGTG
GACTTTTTGGCAAAGGGAGCCTGGTGACATGCGCTAAGTTTGCATGCTCCAAGAAAATGACCGGGAAGAGCATCCAGCC
AGAGAATCTGGAGTACCGGATAATGCTGTCAGTTCATGGCTCCCAGCACAGTGGGATGATCGTTAATGACACAGGACATG
AAACTGATGAGAATAGAGCGAAGGTTGAGATAACGCCCAATTCACCAAGAGCCGAAGCCACCCTGGGGGGTTTTGGAAG
CCTAGGACTTGATTGTGAACCGAGGACAGGCCTTGACTTTTCAGATTTGTATTACTTGACTATGAATAACAAGCACTGGTT
GGTTCACAAGGAGTGGTTCCACGACATTCCATTACCTTGGCACGCTGGGGCAGACACCGGAACTCCACACTGGAACAACA
AAGAAGCACTGGTAGAGTTCAAGGACGCACATGCCAAAAGGCAAACTGTCGTGGTTCTAGGGAGTCAAGAAGGAGCAG
TTCACACGGCCCTTGCTGGAGCTCTGGAGGCTGAGATGGATGGTGCAAAGGGAAGGCTGTCCTCTGGCCACTTGAAATG
TCGCCTGAAAATGGATAAACTTAGATTGAAGGGCGTGTCATACTCCTTGTGTACCGCAGCGTTCACATTCACCAAGATCCC
GGCTGAAACACTGCACGGGACAGTCACAGTGGAGGTACAGTACGCAGGGACAGATGGACCTTGCAAGGTTCCAGCTCAG
ATGGCGGTGGACATGCAAACTCTGACCCCAGTTGGGAGGTTGATAACCGCTAACCCCGTAATCACTGAAAGCACTGAGAA
CTCTAAGATGATGCTGGAACTTGATCCACCATTTGGGGACTCTTACATTGTCATAGGAGTCGGGGAGAAGAAGATCACCC

ACCACTGGCACAGGAGTGGCAGCACCATTGGAAAAGCATTTGAAGCCACTGTGAGAGGTGCCAAGAGAATGGCAGTCTT
GGGAGACACAGCCTGGGACTTTGGATCAGTTGGAGGCGCTCTCAACTCATTGGGCAAGGGCATCCATCAAATTTTTGGAG
CAGCTTTCAAATCATTGTTTGGAGGAATGTCCTGGTTCTCACAAATTCTCATTGGAACGTTGCTGATGTGGTTGGGTCTGA
ACACAAAGAATGGATCTATTTCCCTTATGTGCTTGGCCTTAGGGGGAGTGTTGATCTTCTTATCCACAGCTGTCTCTGCTGA
TGTGGGGTGCTCGGTGGACTTCTCAAAGAAGGAGACGAGATGCGGTACAGGGGTGTTCGTCTATAACGACGTTGAAGCC
TGGAGGGACAGGTACAAGTACCATCCTGACTCCCCCCGTAGATTGGCAGCAGCAGTCAAGCAAGCCTGGGAAGATGGTA
TCTGTGGGATCTCCTCTGTTTCAAGAATGGAAAACATCATGTGGAGATCAGTAGAAGGGGAGCTCAACGCAATCCTGGAA
GAGAATGGAGTTCAACTGACGGTCGTTGTGGGATCTGTAAAAAACCCCATGTGGAGAGGTCCACAGAGATTGCCCGTGC
CTGTGAACGAGCTGCCCCACGGCTGGAAGGCTTGGGGGAAATCGTACTTCGTCAGAGCAGCAAAGACAAATAACAGCTT
TGTCGTGGATGGTGACACACTGAAGGAATGCCCACTCAAACATAGAGCATGGAACAGCTTTCTTGTGGAGGATCATGGGT
TCGGGGTATTTCACACTAGTGTCTGGCTCAAGGTTAGAGAAGATTATTCATTAGAGTGTGATCCAGCCGTTATTGGAACAG
CTGTTAAGGGAAAGGAGGCTGTACACAGTGATCTAGGCTACTGGATTGAGAGTGAGAAGAATGACACATGGAGGCTGA
AGAGGGCCCATCTGATCGAGATGAAAACATGTGAATGGCCAAAGTCCCACACATTGTGGACAGATGGAATAGAAGAGAG
TGATCTGATCATACCCAAGTCTTTAGCTGGGCCACTCAGCCATCACAATACCAGAGAGGGCTACAGGACCCAAATGAAAG
GGCCATGGCACAGTGAAGAGCTTGAAATTCGGTTTGAGGAATGCCCAGGCACTAAGGTCCACGTGGAGGAAACATGTGG
AACAAGAGGACCATCTCTGAGATCAACCACTGCAAGCGGAAGGGTGATCGAGGAATGGTGCTGCAGGGAGTGCACAAT
GCCCCCACTGTCGTTCCGGGCTAAAGATGGCTGTTGGTATGGAATGGAGATAAGGCCCAGGAAAGAACCAGAAAGTAAC
TTAGTAAGGTCAATGGTGACTGCAGGATCAACTGATCACATGGATCACTTCTCCCTTGGAGTGCTTGTGATTCTGCTCATG
GTGCAGGAAGGGCTGAAGAAGAGAATGACCACAAAGATCATCATAAGCACATCGATGGCAGTGCTGGTAGCTATGATCC
TGGGAGGATTTTCAATGAGTGACCTGGCTAAGCTTGCAATTTTGATGGGTGCCACCTTCGCGGAAATGAACACTGGAGGA
GATGTAGCTCATCTGGCGCTGATAGCGGCATTCAAAGTCAGACCAGCGTTGCTGGTATCTTTCATCTTCAGAGCTAATTGG
ACACCCCGTGAAAGCATGCTGCTGGCCTTGGCCTCGTGTCTTTTGCAAACTGCGATCTCCGCCTTGGAAGGCGACCTGATG
GTTCTCATCAATGGTTTTGCTTTGGCCTGGTTGGCAATACGAGCGATGGTTGTTCCACGCACTGATAACATCACCTTGGCA
ATCCTGGCTGCTCTGACACCACTGGCCCGGGGCACACTGCTTGTGGCGTGGAGAGCAGGCCTTGCTACTTGCGGGGGGTT
TATGCTCCTCTCTCTGAAGGGAAAAGGCAGTGTGAAGAAGAACTTACCATTTGTCATGGCCCTGGGACTAACCGCTGTGA
GGCTGGTCGACCCCATCAACGTGGTGGGACTGCTGTTGCTCACAAGGAGTGGGAAGCGGAGCTGGCCCCCTAGCGAAGT
ACTCACAGCTGTTGGCCTGATATGCGCATTGGCTGGAGGGTTCGCCAAGGCAGATATAGAGATGGCTGGGCCCATGGCC
GCGGTCGGTCTGCTAATTGTCAGTTACGTGGTCTCAGGAAAGAGTGTGGACATGTACATTGAAAGAGCAGGTGACATCAC
ATGGGAAAAAGATGCGGAAGTCACTGGAAACAGTCCCCGGCTCGATGTGGCGCTAGATGAGAGTGGTGATTTCTCCCTG
GTGGAGGATGACGGTCCCCCCATGAGAGAGATCATACTCAAGGTGGTCCTGATGACCATCTGTGGCATGAACCCAATAGC
CATACCCTTTGCAGCTGGAGCGTGGTACGTATACGTGAAGACTGGAAAAAGGAGTGGTGCTCTATGGGATGTGCCTGCTC
CCAAGGAAGTAAAAAAGGGGGAGACCACAGATGGAGTGTACAGAGTAATGACTCGTAGACTGCTAGGTTCAACACAAGT
TGGAGTGGGAGTTATGCAAGAGGGGGTCTTTCACACTATGTGGCACGTCACAAAAGGATCCGCGCTGAGAAGCGGTGAA
GGGAGACTTGATCCATACTGGGGAGATGTCAAGCAGGATCTGGTGTCATACTGTGGTCCATGGAAGCTAGATGCCGCCT
GGGACGGGCACAGCGAGGTGCAGCTCTTGGCCGTGCCCCCCGGAGAGAGAGCGAGGAACATCCAGACTCTGCCCGGAA
TATTTAAGACAAAGGATGGGGACATTGGAGCGGTTGCGCTGGATTACCCAGCAGGAACTTCAGGATCTCCAATCCTAGAC
AAGTGTGGGAGAGTGATAGGACTTTATGGCAATGGGGTCGTGATCAAAAATGGGAGTTATGTTAGTGCCATCACCCAAG
GGAGGAGGGAGGAAGAGACTCCTGTTGAGTGCTTCGAGCCTTCGATGCTGAAGAAGAAGCAGCTAACTGTCTTAGACTT
GCATCCTGGAGCTGGGAAAACCAGGAGAGTTCTTCCTGAAATAGTCCGTGAAGCCATAAAAACAAGACTCCGTACTGTGA
TCTTAGCTCCAACCAGGGTTGTCGCTGCTGAAATGGAGGAAGCCCTTAGAGGGCTTCCAGTGCGTTATATGACAACAGCA
GTCAATGTCACCCACTCTGGAACAGAAATCGTCGACTTAATGTGCCATGCCACCTTCACTTCACGTCTACTACAGCCAATCA
GAGTCCCCAACTATAATCTGTATATTATGGATGAGGCCCACTTCACAGATCCCTCAAGTATAGCAGCAAGAGGATACATTT
CAACAAGGGTTGAGATGGGCGAGGCGGCTGCCATCTTCATGACCGCCACGCCACCAGGAACCCGTGACGCATTTCCGGA
CTCCAACTCACCAATTATGGACACCGAAGTGGAAGTCCCAGAGAGAGCCTGGAGCTCAGGCTTTGATTGGGTGACGGATC
ATTCTGGAAAAACAGTTTGGTTTGTTCCAAGCGTGAGGAACGGCAATGAGATCGCAGCTTGTCTGACAAAGGCTGGAAA
ACGGGTCATACAGCTCAGCAGAAAGACTTTTGAGACAGAGTTCCAGAAAACAAAACATCAAGAGTGGGACTTTGTCGTG
ACAACTGACATTTCAGAGATGGGCGCCAACTTTAAAGCTGACCGTGTCATAGATTCCAGGAGATGCCTAAAGCCGGTCAT
ACTTGATGGCGAGAGAGTCATTCTGGCTGGACCCATGCCTGTCACACATGCCAGCGCTGCCCAGAGGAGGGGGCGCATA
GGCAGGAATCCCAACAAACCTGGAGATGAGTATCTGTATGGAGGTGGGTGCGCAGAGACTGACGAAGACCATGCACACT
GGCTTGAAGCAAGAATGCTCCTTGACAATATTTACCTCCAAGATGGCCTCATAGCCTCGCTCTATCGACCTGAGGCCGACA
AAGTAGCAGCCATTGAGGGAGAGTTCAAGCTTAGGACGGAGCAAAGGAAGACCTTTGTGGAACTCATGAAAAGAGGAG
ATCTTCCTGTTTGGCTGGCCTATCAGGTTGCATCTGCCGGAATAACCTACACAGATAGAAGATGGTGCTTTGATGGCACGA
CCAACAACACCATAATGGAAGACAGTGTGCCGGCAGAGGTGTGGACCAGACACGGAGAGAAAAGAGTGCTCAAACCGA
GGTGGATGGACGCCAGAGTTTGTTCAGATCATGCGGCCCTGAAGTCATTCAAGGAGTTTGCCGCTGGGAAAAGAGGAGC
GGCTTTTGGAGTGATGGAAGCCCTGGGAACACTGCCAGGACACATGACAGAGAGATTCCAGGAAGCCATTGACAACCTC

GCTGTGCTCATGCGGGCAGAGACTGGAAGCAGGCCTTACAAAGCCGCGGCGGCCCAATTGCCGGAGACCCTAGAGACCA
TTATGCTTTTGGGGTTGCTGGGAACAGTCTCGCTGGGAATCTTTTTCGTCTTGATGAGGAACAAGGGCATAGGGAAGATG
GGCTTTGGAATGGTGACTCTTGGGGCCAGCGCATGGCTCATGTGGCTCTCGGAAATTGAGCCAGCCAGAATTGCATGTGT
CCTCATTGTTGTGTTCCTATTGCTGGTGGTGCTCATACCTGAGCCAGAAAAGCAAAGATCTCCCCAGGACAACCAAATGGC
AATCATCATCATGGTAGCAGTAGGTCTTCTGGGCTTGATTACCGCCAATGAACTCGGATGGTTGGAGAGAACAAAGAGTG
ACCTAAGCCATCTAATGGGAAGGAGAGAGGAGGGGGCAACCATAGGATTCTCAATGGACATTGACCTGCGGCCAGCCTC
AGCTTGGGCCATCTATGCTGCCTTGACAACTTTCATTACCCCAGCCGTCCAACATGCAGTGACCACTTCATACAACAACTAC
TCCTTAATGGCGATGGCCACGCAAGCTGGAGTGTTGTTTGGTATGGGCAAAGGGATGCCATTCTACGCATGGGACTTTGG
AGTCCCGCTGCTAATGATAGGTTGCTACTCACAATTAACACCCCTGACCCTAATAGTGGCCATCATTTTGCTCGTGGCGCAC
TACATGTACTTGATCCCAGGGCTGCAGGCAGCAGCTGCGCGTGCTGCCCAGAAGAGAACGGCAGCTGGCATCATGAAGA
ACCCTGTTGTGGATGGAATAGTGGTGACTGACATTGACACAATGACAATTGACCCCCAAGTGGAGAAAAAGATGGGACA
GGTGCTACTCATAGCAGTAGCCGTCTCCAGCGCCATACTGTCGCGGACCGCCTGGGGGTGGGGGGAGGCTGGGGCCCTG
ATCACAGCGGCAACTTCCACTTTGTGGGAAGGCTCTCCGAACAAGTACTGGAACTCCTCTACAGCCACTTCACTGTGTAAC
ATTTTTAGGGGAAGTTACTTGGCTGGAGCTTCTCTAATCTACACAGTAACAAGAAACGCTGGCTTGGTCAAGAGACGTGG
GGGTGGAACAGGAGAGACCCTGGGAGAGAAATGGAAGGCCCGCTTGAACCAGATGTCGGCCCTGGAGTTCTACTCCTAC
AAAAAGTCAGGCATCACCGAGGTGTGCAGAGAAGAGGCCCGCCGCGCCCTCAAGGACGGTGTGGCAACGGGAGGCCAT
GCTGTGTCCCGAGGAAGTGCAAAGCTGAGATGGTTGGTGGAGCGGGGATACCTGCAGCCCTATGGAAAGGTCATTGATC
TTGGATGTGGCAGAGGGGGCTGGAGTTACTACGCCGCCACCATCCGCAAAGTTCAAGAAGTGAAAGGATACACAAAAGG
AGGCCCTGGTCATGAAGAACCCATGTTGGTGCAAAGCTATGGGTGGAACATAGTCCGTCTTAAGAGTGGGGTGGACGTC
TTTCATATGGCGGCTGAGCCGTGTGACACGTTGCTGTGTGACATAGGTGAGTCATCATCTAGTCCTGAAGTGGAAGAAGC
ACGGACGCTCAGAGTCCTCTCCATGGTGGGGGATTGGCTTGAAAAAAGACCAGGAGCCTTTTGTATAAAAGTGTTGTGCC
CATACACCAGCACTATGATGGAAACCCTGGAGCGACTGCAGCGTAGGTATGGGGGAGGACTGGTCAGAGTGCCACTCTC
CCGCAACTCTACACATGAGATGTACTGGGTCTCTGGAGCGAAAAGCAACACCATAAAAAGTGTGTCCACCACGAGCCAGC
TCCTCTTGGGGCGCATGGACGGGCCCAGGAGGCCAGTGAAATATGAGGAGGATGTGAATCTCGGCTCTGGCACGCGGG
CTGTGGTAAGCTGCGCTGAAGCTCCCAACATGAAGATCATTGGTAACCGCATTGAAAGGATCCGCAGTGAGCACGCGGA
AACGTGGTTCTTTGACGAGAACCACCCATATAGGACATGGGCTTACCATGGAAGCTATGAGGCCCCCACACAAGGGTCAG
CGTCCTCTCTAATAAACGGGGTTGTCAGGCTCCTGTCAAAACCCTGGGATGTGGTGACTGGAGTCACAGGAATAGCCATG
ACCGACACCACACCGTATGGTCAGCAAAGAGTTTTCAAGGAAAAAGTGGACACTAGGGTGCCAGACCCCCAAGAAGGCA
CTCGTCAGGTTATGAGCATGGTCTCTTCCTGGTTGTGGAAAGAGCTAGGCAAACACAAACGGCCACGAGTCTGTACCAAA
GAAGAGTTCATCAACAAGGTTCGTAGCAATGCAGCATTAGGGGCAATATTTGAAGAGGAAAAAGAGTGGAAGACTGCAG
TGGAAGCTGTGAACGATCCAAGGTTCTGGGCTCTAGTGGACAAGGAAAGAGAGCACCACCTGAGAGGAGAGTGCCAGA
GTTGTGTGTACAACATGATGGGAAAAAGAGAAAAGAAACAAGGGGAATTTGGAAAGGCCAAGGGCAGCCGCGCCATCT
GGTATATGTGGCTAGGGGCTAGATTTCTAGAGTTCGAAGCCCTTGGATTCTTGAACGAGGATCACTGGATGGGGAGAGA
GAACTCAGGAGGTGGTGTTGAAGGGCTGGGATTACAAAGACTCGGATATGTCCTAGAAGAGATGAGTCGCATACCAGGA
GGAAGGATGTATGCAGATGACACTGCTGGCTGGGACACCCGCATCAGCAGGTTTGATCTGGAGAATGAAGCTCTAATCA
CCAACCAAATGGAGAAAGGGCACAGGGCCTTGGCATTGGCCATAATCAAGTACACATACCAAAACAAAGTGGTAAAGGT
CCTTAGACCAGCTGAAAAAGGGAAGACAGTTATGGACATTATTTCGAGACAAGACCAAAGGGGGAGCGGACAAGTTGTC
ACTTACGCTCTTAACACATTTACCAACCTAGTGGTGCAACTCATTCGGAATATGGAGGCTGAGGAAGTTCTAGAGATGCAA
GACTTGTGGCTGCTGCGGAGGTCAGAGAAAGTGACCAACTGGTTGCAGAGCAACGGATGGGATAGGCTCAAACGAATG
GCAGTCAGTGGAGATGATTGCGTTGTGAAGCCAATTGATGATAGGTTTGCACATGCCCTCAGGTTCTTGAATGATATGGG
AAAAGTTAGGAAGGACACACAAGAGTGGAAACCCTCAACTGGATGGGACAACTGGGAAGAAGTTCCGTTTTGCTCCCAC
CACTTCAACAAGCTCCATCTCAAGGACGGGAGGTCCATTGTGGTTCCCTGCCGCCACCAAGATGAACTGATTGGCCGGGC
CCGCGTCTCTCCAGGGGCGGGATGGAGCATCCGGGAGACTGCTTGCCTAGCAAAATCATATGCGCAAATGTGGCAGCTC
CTTTATTTCCACAGAAGGGACCTCCGACTGATGGCCAATGCCATTTGTTCATCTGTGCCAGTTGACTGGGTTCCAACTGGG
AGAACTACCTGGTCAATCCATGGAAAGGGAGAATGGATGACCACTGAAGACATGCTTGTGGTGTGGAACAGAGTGTGGA
TTGAGGAGAACGACCACATGGAAGACAAGACCCCAGTTACGAAATGGACAGACATTCCCTATTTGGGAAAAAGGGAAGA
CTTGTGGTGTGGATCTCTCATAGGGCACAGACCGCGCACCACCTGGGCTGAGAACATTAAAAACACAGTCAACATGGTGC
GCAGGATCATAGGTGATGAAGAAAAGTACATGGACTACCTATCCACCCAAGTTCGCTACTTGGGTGAAGAAGGGTCTACA
CCTGGAGTGCTGTAAGCACCAATCTTAGTGTTGTCAGGCCTGCTAGTCAGCCACAGCTTGGGGAAAGCTGTGCAGCCTGT
GACCCCCCCAGGAGAAGCTGGGAAACCAAGCCTATAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCT
GTGAGCCCCTCAGAGGACACTGAGTCAAAAAACCCCACGCGCTTGGAGGCGCAGGATGGGAAAAGAAGGTGGCGACCT
TCCCCACCCTTCAATCTGGGGCCTGAACTGGAGATCAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAGACCC
CCCGGAAAACGCAAAACAGCATATTGACGCTGGGAAAGACCAGAGACTCCATGAGTTTCCACCACGCTGGCCGCCAGGC
ACAGATCGCCGAATAGCGGCGGCCGGTGTGGGG

SEQ ID NO: 73
AHZ13508.1, Zika virus polyprotein from Polynesian outbreak (H/PF/2013)
MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIKPSLGLINRWGSVGKKEAMEIIKKFKK
DLAAMLRIINARKEKKRRGADTSVGIVGLLLTTAMAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDA
TMSYECPMLDEGVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREYTKHLIRVENWI
FRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIEL
VTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTG
KSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKH
WLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLK
CRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKM
MLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLF
GGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSADVGCSVDFSKKETRCGTGVFVYNDVEAWRDRYKYHP
DSPRRLAAAVKQAWEDGICGISSVSRMENIMWRSVEGELNAILEENGVQLTVVVGSVKNPMWRGPQRLPVPVNELPHGWK
AWGKSYFVRAAKTNNSFVVDGDTLKECPLKHRAWNSFLVEDHGFGVFHTSVWLKVREDYSLECDPAVIGTAVKGKEAVHSDL
GYWIESEKNDTWRLKRAHLIEMKTCEWPKSHTLWTDGIEESDLIIPKSLAGPLSHHNTREGYRTQMKGPWHSEELEIRFEECPG
TKVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGCWYGMEIRPRKEPESNLVRSMVTAGSTDHMDHFSLG
VLVILLMVQEGLKKRMTTKIIISTSMAVLVAMILGGFSMSDLAKLAILMGATFAEMNTGGDVAHLALIAAFKVRPALLVSFIFRA
NWTPRESMLLALASCLLQTAISALEGDLMVLINGFALAWLAIRAMVVPRTDNITLAILAALTPLARGTLLVAWRAGLATCGGFM
LLSLKGKGSVKKNLPFVMALGLTAVRLVDPINVVGLLLLTRSGKRSWPPSEVLTAVGLICALAGGFAKADIEMAGPMAAVGLLIV
SYVVSGKSVDMYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEDDGPPMREIILKVVLMTICGMNPIAIPFAAGAWYVY
VKTGKRSGALWDVPAPKEVKKGETTDGVYRVMTRRLLGSTQVGVGVMQEGVFHTMWHVTKGSALRSGEGRLDPYWGDVK
QDLVSYCGPWKLDAAWDGHSEVQLLAVPPGERARNIQTLPGIFKTKDGDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGVVIK
NGSYVSAITQGRREEETPVECFEPSMLKKKQLTVLDLHPGAGKTRRVLPEIVREAIKTRLRTVILAPTRVVAAEMEEALRGLPVRY
MTTAVNVTHSGTEIVDLMCHATFTSRLLQPIRVPNYNLYIMDEAHFTDPSSIAARGYISTRVEMGEAAAIFMTATPPGTRDAFP
DSNSPIMDTEVEVPERAWSSGFDWVTDHSGKTVWFVPSVRNGNEIAACLTKAGKRVIQLSRKTFETEFQKTKHQEWDFVVTT
DISEMGANFKADRVIDSRRCLKPVILDGERVILAGPMPVTHASAAQRRGRIGRNPNKPGDEYLYGGGCAETDEDHAHWLEAR
MLLDNIYLQDGLIASLYRPEADKVAAIEGEFKLRTEQRKTFVELMKRGDLPVWLAYQVASAGITYTDRRWCFDGTTNNTIMEDS
VPAEVWTRHGEKRVLKPRWMDARVCSDHAALKSFKEFAAGKRGAAFGVMEALGTLPGHMTERFQEAIDNLAVLMRAETGS
RPYKAAAAQLPETLETIMLLGLLGTVSLGIFFVLMRNKGIGKMGFGMVTLGASAWLMWLSEIEPARIACVLIVVFLLLVVLIPEPE
KQRSPQDNQMAIIIMVAVGLLGLITANELGWLERTKSDLSHLMGRREEGATIGFSMDIDLRPASAWAIYAALTTFITPAVQHAV
TTSYNNYSLMAMATQAGVLFGMGKGMPFYAWDFGVPLLMIGCYSQLTPLTLIVAIILLVAHYMYLIPGLQAAAARAAQKRTAA
GIMKNPVVDGIVVTDIDTMTIDPQVEKKMGQVLLIAVAVSSAILSRTAWGWGEAGALITAATSTLWEGSPNKYWNSSTATSLC
NIFRGSYLAGASLIYTVTRNAGLVKRRGGGTGETLGEKWKARLNQMSALEFYSYKKSGITEVCREEARRALKDGVATGGHAVSR
GSAKLRWLVERGYLQPYGKVIDLGCGRGGWSYYAATIRKVQEVKGYTKGGPGHEEPMLVQSYGWNIVRLKSGVDVFHMAAE
PCDTLLCDIGESSSSPEVEEARTLRVLSMVGDWLEKRPGAFCIKVLCPYTSTMMETLERLQRRYGGGLVRVPLSRNSTHEMYWV
SGAKSNTIKSVSTTSQLLLGRMDGPRRPVKYEEDVNLGSGTRAVVSCAEAPNMKIIGNRIERIRSEHAETWFFDENHPYRTWAY
HGSYEAPTQGSASSLINGVVRLLSKPWDVVTGVTGIAMTDTTPYGQQRVFKEKVDTRVPDPQEGTRQVMSMVSSWLWKELG
KHKRPRVCTKEEFINKVRSNAALGAIFEEEKEWKTAVEAVNDPRFWALVDKEREHHLRGECQSCVYNMMGKREKKQGEFGKA
KGSRAIWYMWLGARFLEFEALGFLNEDHWMGRENSGGGVEGLGLQRLGYVLEEMSRIPGGRMYADDTAGWDTRISRFDLE
NEALITNQMEKGHRALALAIIKYTYQNKVVKVLRPAEKGKTVMDIISRQDQRGSGQVVTYALNTFTNLVVQLIRNMEAEEVLEM
QDLWLLRRSEKVTNWLQSNGWDRLKRMAVSGDDCVVKPIDDRFAHALRFLNDMGKVRKDTQEWKPSTGWDNWEEVPFC
SHHFNKLHLKDGRSIVVPCRHQDELIGRARVSPGAGWSIRETACLAKSYAQMWQLLYFHRRDLRLMANAICSSVPVDWVPTG
RTTWSIHGKGEWMTTEDMLVVWNRVWIEENDHMEDKTPVTKWTDIPYLGKREDLWCGSLIGHRPRTTWAENIKNTVNMV
RRIIGDEEKYMDYLSTQVRYLGEEGSTPGVL

SEQ ID NO: 74
9320_Zika_PF_1F
ttaggatccGTTGTTGATCTGTGTGAAT

SEQ ID NO: 75
9321_Zika_PF_1R
taactcgagCGTACACAACCCAAGTT

SEQ ID NO: 76
9322_Zika_PF_2F

ttaggatccTCACTAGACGTGGGAGTG

SEQ ID NO: 77
9323_Zika_PF_2R
taactcgagAAGCCATGTCYGATATTGAT

SEQ ID NO: 78
9324_Zika_PF_3F
ttaggatccGCATACAGCATCAGGTG

SEQ ID NO: 79
9325_Zika_PF_3R
taactcgagTGTGGAGTTCCGGTGTCT

SEQ ID NO: 80
9326_Zika_PF_4F
ttaggatccGAATAGAGCGAARGTTGAGATA

SEQ ID NO: 81
9327_Zika_PF_4R
taactcgAGTGGTGGGTGATCTTCTTCT

SEQ ID NO: 82
9328_Zika_PF_5F
ttaggatcCAGTCACAGTGGAGGTACAGTAC

SEQ ID NO: 83
9329_Zika_PF_5R
taactcgagCRCAGATACCATCTTCCC

SEQ ID NO: 84
9330_Zika_PF_6F
ttaggatCCCTTATGTGCTTGGCCTTAG

SEQ ID NO: 85
9331_Zika_PF_6R
taactcgagTCTTCAGCCTCCATGTG

SEQ ID NO: 86
9332_Zika_PF_7F
ttaggatccAATGCCCACTCAAACATAGA

SEQ ID NO: 87
9333_Zika_PF_7R
taactcgagTCATTCTCTTCTTCAGCCCTT

SEQ ID NO: 88
9334_Zika_PF_8F
ttaggatccAAGGGTGATCGAGGAAT

SEQ ID NO: 89
9335_Zika_PF_8R
taactcgagTTCCCTTCAGAGAGAGGAGC

SEQ ID NO: 90

9336_Zika_PF_9F
ttaggatccTCTTTTGCAAACTGCGATC

SEQ ID NO: 91
9337_Zika_PF_9R
taactcgagTCCAGCTGCAAAGGGTAT

SEQ ID NO: 92
9338_Zika_PF_10F
ttaggatccGTGTGGACATGTACATTGA

SEQ ID NO: 93
9339_Zika_PF_10R
taactcgagCCCATTGCCATAAAGTC

SEQ ID NO: 94
9340_Zika_PF_11F
ttaggatccTCATACTGTGGTCCATGGA

SEQ ID NO: 95
9341_Zika_PF_11R
taactcgagGCCCATCTCAACCCTTG

SEQ ID NO: 96
9342_Zika_PF_12F
ttaggatccTAGAGGGCTTCCAGTGC

SEQ ID NO: 97
9343_Zika_PF_12R
taactcgAGATACTCATCTCCAGGTTTGTTG

SEQ ID NO: 98
9344_Zika_PF_13F
ttaggatccGAAAACAAAACATCAAGAGTG

SEQ ID NO: 99
9345_Zika_PF_13R
taactcgagGAATCTCTCTGTCATGTGTCCT

SEQ ID NO: 100
9346_Zika_PF_14F
ttaggatccTTGATGGCACGACCAAC

SEQ ID NO: 101
9347_Zika_PF_14R
ttaggatccGTTGTTGATCTGTGTGAAT

SEQ ID NO: 102
9348_Zika_PF_15F
taactcgagCAGGTCAATGTCCATTG

SEQ ID NO: 103
9349_Zika_PF_15R
ttaggatccTGTTGTGTTCCTATTGCTGGT

SEQ ID NO: 104
9350_Zika_PF_16F
taactcgaGTGATCAGRGCCCCAGC

SEQ ID NO: 105
9351_Zika_PF_16R
ttaggatccTGCTGCCCAGAAGAGAA

SEQ ID NO: 106
9352_Zika_PF_17F
taactcgaGCACCAACAYGGGTTCTT

SEQ ID NO: 107
9353_Zika_PF_17R
ttaggatcCTCAAGGACGGTGTGGC

SEQ ID NO: 108
9354_Zika_PF_18F
taactcgagCAATGATCTTCATGTTGGG

SEQ ID NO: 109
9355_Zika_PF_18R
ttaggatccTATGGGGGAGGACTGGT

SEQ ID NO: 110
9356_Zika_PF_19F
taactcGAGCCCAGAACCTTGGATC

SEQ ID NO: 111
9357_Zika_PF_19R
ttaggatcCAGACCCCCAAGAAGGC

SEQ ID NO: 112
9358_Zika_PF_20F
taactcgagCCCCTTTGGTCTTGTCT

SEQ ID NO: 113
9359_Zika_PF_20R
ttaggatccAGGAAGGATGTATGCAGATG

SEQ ID NO: 114
9360_Zika_PF_21F
taactcgagACATTTGCGCATATGATTTTG

SEQ ID NO: 115
9361_Zika_PF_21R
ttaggatccAGGAAGGACACACAAGAGT

SEQ ID NO: 116
9362_Zika_PF_22F
taactcgagACAGGCTGCACAGCTTT

SEQ ID NO: 117
9363_Zika_PF_22R
ttaggatccTCTCTCATAGGGCACAGAC

In some embodiments, the Zika virus has polyprotein, including an envelope (E) protein, with an amino acid sequence provided by any one of SEQ ID NOs: 14-69 or 72. In some embodiments, the polyprotein or E protein sequence is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%. 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-69 or 72.

The terms "identical" or percent "identity" in the context of two or more nucleic acids or amino acid sequences refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*e.g.*, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) over a specified region or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length. In some embodiments, the identity exists over the length of a protein, such as the E protein.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. Methods of alignment of sequences for comparison are well known in the art. See, *e.g.,* by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, J. MoI. Biol. 48:443, 1970, by the search for similarity method of Pearson and Lipman. Proc. Natl. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, Jalview and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group. 575 Science Dr., Madison. WI), by multi sequence alignment implementation using e.g. CLUSTALW (Larkin et al., (2007). Bioinformatics, 23, 2947-2948.) or MAFFT (Katoh & Toh 2008 Briefings in Bioinformatics 9:286-298), or by manual alignment and visual inspection (see. *e.g.,* Brent et al., Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (Ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977 and Altschul et al., J. MoI. Biol. 215:403-410, 1990, respectively.

EXAMPLES

Example 1: Production of a Zika drug substance suitable for application as a vaccine in humans and animals

Materials and Methods:

For the production of ZikaV the JEV process platform (Srivastava et al., Vaccine 19 (2001) 4557-4565; US 6,309,650B1) was used as a basis. Small changes of certain process steps were adapted to ZikaV properties and to improve purity. A short summary of the process steps is outlined below (see also Figure 9A and B). Briefly, the unexpected and novel purification properties of protamine sulphate (PS) were evaluated in purification processes for Zika Virus. As shown in Figure 10, non-infectious virus particle aggregates, HCP and other LMW impurities were removed by PS precipitation as shown by removal of aggregate shoulder in SEC-HPLC and no loss of infectious virus titer by PS treatment. Further optimization of the Zika purification protocol is provided below.

Upstream:

- Roller Bottle based Vero cell expansion (25x850cm2 CellBind):
- 5% CO_2, 35°C, MEM+2mM L-Glutamine + 10% FBS
- Infection with ZikaV research Master Seed Bank (rMSB) at MOI 0.01
- Virus Production without serum
- 5% CO_2, 35°C, MEM+2mM L-Glutamine
- Multiple harvests (days 2, 3, 5 and 7) with re-feed
- Sterile filtration of harvests and storage at 2-8°C until further processing

Downstream:

- Pooling of harvests and concentration by ultrafiltration (100kDa)
- Stabilization of concentrated harvest (Tris/10% sucrose) for storage if required (-80°C)
- Removal of hcDNA by Protamine Sulphate (2 mg/mL)
- Sucrose Gradient Purification (optimized three layered gradient)
- Formaldehyde Inactivation (0.02%, 22°C, 10 days), neutralization with Na-metabisulfite
- Dilution to DS antigen target content and formulation with Aluminium hydroxide (0.5mg Al/mL)

Zika Virus Strain H/PF/2013 was originally isolated from a 51-year-old woman (accession number KJ776791.1, also SEQ ID NO: 13 herein) from French Polynesia. A sample was obtained from the European Virus Archive (EVAg; Ref-SKU: 001v-EVA1545). Based on this material, a research

master seed bank (rMSB) was prepared on Vero cells as the cell substrate and the genomic sequence was checked by sequencing. Because the genomic sequence at the 5´and 3´flanking sequences of Zika virus strain H/PF/2013 was unknown, primers for sequencing were designed in those regions based on other Zika virus strains whereas the internal primers were designed from the published sequence (SEQ ID NOs: 74 to 117, see also Table A). The sequence obtained from the rMSB by use of these primers is provided by SEQ ID NO: 72. There was 100% overlap of the sequence with the published sequence of Zika Virus Strain H/PF/2013 (SEQ ID NO: 13). However, we sequenced additional regions 5´ (an additional 40 bp) and 3' (an additional 160 bp) represented in SEQ ID NO: 72. In a preferred embodiment, the Zika virus of the invention comprises SEQ ID NO: 72. The genomic RNA is somewhat longer than the sequence according to SEQ ID NO: 72 (perhaps an additional 200 bp). Additionally, a Zika virus adapted to a host cell such as e.g. Vero cells may be expected to contain one or more mutations. For these reasons, the Zika virus of the current invention comprises the sequence of SEQ ID NO: 72 or, preferably, a sequence with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 72. Furthermore, because the viral genome is likely to contain even further flanking regions to SEQ ID NO: 72; in one embodiment, the Zika virus of the invention contains the sequence of SEQ ID NO: 72 and optionally further comprises extensions at the 5' and/or 3' ends of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120 or at least 130 nucleotides. In a preferred embodiment, the Zika virus comprises at least the coding sequence for the entire polyprotein of Zika Virus Strain H/PF/2013 of the invention i.e. the amino acid sequence of SEQ ID NO: 73 or a polyprotein with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 73. Furthermore, the Zika virus comprises at least the coding sequence for the E-protein of Zika Virus Strain H/PF/2013 of the invention SEQ ID NO: 47 or an E-protein thereof with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 47.

Virus growth on Vero Cells

Vero cells were grown in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS). Roller bottle cultures of Vero cell monolayers were infected with Zika Virus Strain H/PF/2013 at a multiplicity of infection (moi) of 0.01 plaque forming units (pfu) per cell. After 2 hours of virus adsorption, the cultures were washed 3 times with PBS and fed with EMEM without FBS and incubated at +35°C with 5% CO_2. Infected Vero cell cultures were incubated until the virus titer reaches a desired level.

The culture medium was harvested at days 2, 3, 5 and 7 and were pooled from those harvest days and then centrifuged in a standard centrifuge. The supernatants were then filtered. Virus culture supernatants were concentrated by TFF ultrafiltration to remove cell culture media components and to reduce batch volume.

Evaluation of harvest Procedure

The current JEV harvest process has scheduled harvests on days 3, 5, 7 and 9 post infection. To mimic the JEV process roller bottles were infected with ZIKV bank P4-FBS at an MOI of 0.01 in infection medium (MEM with 2% FBS + 2mM L-glutamine) for 2 hours. After removing the inoculum the cells were washed twice with PBS and 200 mL production medium (MEM+ 2mM L-glutamine) was added.

After taking a sample on day 2 the first virus harvest was conducted on day 3 after infection. At this point significantly higher CPE could be observed compared to cells where virus was removed on day 2. Plaque assay analysis showed that the viral titers on day 2 were in the same range as for the standard harvesting schedule. However, starting with the day 3 harvest, the observed titers were significantly lower correlating with the increased CPE observed compared to the standard harvest schedule. On day 5 post infection no more living cells could be observed at all and the experiment was terminated with a final day 5 harvest.

Table 3: The calculated titers per plaque assay are summarized in the list below.

	Log 10 PFU/mL
sample day2	7.02
harvest day 3	6.66
harvest day 5	6.26

This finding led to an optimized harvest schedule to better control of CPE and allow additional harvest day 5 and 7, see Figure 15. For both harvest days the optimized ZikaV protocol yield significant higher virus titers compared to the modified protocol showing that the time of the first harvest is crucial for production yields. Additionally first harvesting at day 3 results in maximum 2 harvest points whereas first harvesting at day 2 allows for 4 harvest points further increasing the yield gain.

Downstream Purification of Zika virus

The purification process was carried out at room temperature (18-22°C) unless stated otherwise. Virus purification started with concentration of filtered combined harvest using 100 kDa cut-off TFF

ultrafiltration modules to remove cell culture media components and reduce batch volume. After concentration, the pooled filtered harvest material was adjusted to a final concentration of 25 mM Tris pH 7.5 and 10% sucrose (w/w) using stock solution of both components (see Figure 11 for SEC-HPLC of different harvests prior to PS treatment). This allowed for freezing the concentrated harvest at <-65°C if required.

Host cell DNA and protein reduction as well reduction of non-infectious virus aggregates in the concentrated material was achieved by precipitation with protamine sulphate (2 mg/mL) followed by sucrose density centrifugation (2-8°C) as final polishing step (see Figure 20 for SEC-HPLC of different harvests post PS treatment). The purification process was designed to be completed within 2 working days with SGC starting on end of day 1 followed by fractionation and SDS-PAGE analysis on day 2. The sucrose gradient fractions were stored at 2-8 °C during the SDS-PAGE analysis (Silver staining) to identify the pure fractions containing ZikaV (see Figure 21). After pooling the relevant fractions, the pool was diluted and inactivated by Formalin. After pooling the relevant fractions of sucrose gradient centrifugation, the pool was diluted 1:3 in PBS and inactivated by Formalin (0.02% v/v, 200ppm). Fractions were subjected to analysis by SDS-PAGE.

Effect of PS treatment on virus recovery

Samples of individual 30x concentrated harvests days 2, 3, 5 and 7 were analysed before (Figure 11) and after PS (Figure 12) treatment by SEC-HPLC and plaque assay. SEC-HPLC was used for determination of relative total ZikaV content (active + inactive) expressed as peak area, whereas the rel. ZikaV peak purity is given as relative content of virus monomer population to total virus peak. Plaque assay states the content of total active virus particles in each sample. Experimental results are summarized in Table 4. The virus peak recovery by SEC-HPLC was only between 12 to 36% with peak purity after PS treatment in the range of >90% (no virus aggregates detected). The recovery of active virus particles by plaque assay was all > 100% (130-700%, range within the variability of the assay) showing that no active virus particles were lost during PS treatment. These results show that during PS treatment only non-infective (immature and/or aggregated virus) particles were removed.

Table 4: ZikaV recovery by SEC-HPLC and plaque assay before and after PS treatment.

SEC-HPLC

	Peak area mAU*min			
Harvest day	30x conc	30x + PS	SEC Recovery (%)	rel. virus monomer content after PS (%)
Day 2	101.36	18.63	18	89%
Day 3	144.51	17.48	12	90%

Day 5	19.97	5.92	30	96%
Day 7	68.80	24.43	36	99%

Plaque Assay

	PFU/mL		
Harvest day	30x conc	30x + PS	Plaque Recovery (%)
Day 2	3E+08	5E+08	179
Day 3	2E+08	4E+08	193
Day 5	1E+08	9E+08	700
Day 7	3E+08	4E+08	132

Sucrose gradient centrifugation

The PS treated harvest was split in two parts and loaded on two centrifuge bottles. Sucrose density gradient centrifugation (SGC) was used for final concentration and polishing of the ZikaV material. The ZikaV PS treated concentrated harvest was loaded on top of a solution consisting of three layers of sucrose with different densities. The three sucrose layers were selected based on a preliminary study which showed the formation of a linear sucrose gradient and complete separation of the virus particles from residual contaminants as demonstrated for ChikV (Fig. 15D). The optimal volumes of the sucrose solutions were determined empirically. The volumes of individual layers for a centrifugation in 100 mL bottle scale are shown in Table 5.

Table 5: Individual layers/volumes for a centrifugation in bottle.

Solution	**Volume (mL)**
PS treated harvest in 10% sucrose (L)	40
15% sucrose (J)	15
35% sucrose (I)	15
50% sucrose (H)	20
Total volume	90

The sucrose gradient bottles were prepared by stratifying the individual sucrose layers. A plastic tube was attached to peristaltic pump tubing. The plastic tube was mounted on a laboratory stand using a clamp and placed into the centrifuge bottle. The nozzle of the plastic tube was touching the bottom of the bottle. Using a peristaltic pump the ZikaV material and the sucrose solutions were pumped into the cylinder. A measuring cylinder was used as feed vessel. The first solution pumped was the ZikaV material as it represented the solution of lowest density (10 % sucrose (w/w)). After the ZikaV material the sucrose solutions were pumped in ascending order starting with the 15 % (w/w) solution

J, followed by 35 % sucrose solution I and finishing with the highest density sucrose solution H (50 % (w/w)). The described setup is shown in Figure 14. After all sucrose solutions were transferred the plastic tubing was carefully removed in order not to disturb the layers.

Prior to centrifugation the centrifuge was pre-cooled to 4°C. The prepared SG bottles were carefully transferred into the pre-cooled rotor. (Note: Sudden movement of the bottles during transfer to the rotor must be avoided in order not to disturb the sucrose layers.) The bottles were centrifuged at ~11.000 RCF max at 4 °C for at least 20 hours, no brake/deceleration activated. In case a different centrifuge system with a different rotor is used the necessary speed and centrifugation times need to be calculated based on the k-factor in order to achieve comparable centrifugation efficiency.

Harvesting of the sucrose gradient was done manually using a peristaltic pump. A plastic tube attached to peristaltic pump tubing was used for harvesting the sucrose gradient. The bottle containing the gradient was mounted onto a laboratory stand in a tilted position (~12°) using a clamp. The plastic tubing was then placed into the bottle touching the bottom edge of the bottle and was fastened in position using a clamp. This resulted in a small gap of 1-2 mm between the tubing inlet and the bottom of the bottle (see Figure 14).

Using a peristaltic pump set to a flow rate of 30 mL per minute the gradient was harvested and manually split into 2 mL fractions. A total number of 32 fractions per bottle were harvested (~ 64 mL) and the remaining volume was discarded. The fractions were immediately tested by SDS-PAGE / silver stain to identify the virus containing fractions with sufficient high purity. Representative SDS-PAGE is shown in Figure 14. Fraction 10-14 were pooled and further processed.

The purified viral solution was inactivated by incubation with 0.02% formaldehyde over a period of ten days in a 22°C controlled-temperature incubator. The formaldehyde is neutralized by addition of sodium metabisulphite on the tenth day.

The sucrose gradient pool (~17mL after sampling) was further diluted 3-fold with PBS to a final volume of 51 mL in a PETG container. A volume of 1% formaldehyde (10,000 ppm) solution equivalent to 1/50 of the final volume of the pre-formaldehyde pool was added to this pool resulting in an effective concentration of 200 ppm. The formaldehyde-treated solution was mixed on a magnetic stirrer for 10 minutes. After sampling, the formaldehyde-treated viral solution was placed within a cooled incubator at 22°C ± 2°C. On Day 5 post addition of formaldehyde, the formaldehyde-treated viral solution was filtered through a 0.2 µm filter and then placed in the incubator at 22°C ±

2°C again. On Day 10, after removing the 10-Day inactivation final sample, a volume of 1 % (of the weight of the final formaldehyde-treated viral solution) of 200 mM-sodium metabisulphite solution (2mM final concentration) was aseptically transferred into the PETG container containing the formaldehyde-treated viral solution. After mixing for 5 minutes on a magnetic stirrer, the neutralized inactivated viral solution is held at room temperature (20 to 25°C) for a minimum of 30 minutes. After sampling, the neutralized inactivated viral solution is stored at 5°C ± 3°C until further processing.

Inactivation by formaldehyde

Critical parameters for this step are final formalin concentration, temperature, mixing and transfer into a new container. A preliminary acceptance criterion for maximum pfu/mL (determined by plaque assay) has been set on the diluted pool pre formaldehyde treatment.

The quality of the neutralized inactivated viral solution was monitored by the following parameters: Plaque assay on Day 10, SEC-HPLC, SDS-PAGE/Western Blot.

Interestingly, SEC-HPLC analysis of samples taken during the inactivation period followed by neutralization with bisulfite showed more or less constant peak area throughout the inactivation period. This is in contrast to JEV where losses of viral particles up to 60% are observed using the process disclosed by Srivastava et al. Vaccine 19 (2001) 4557-4565. In a scale-down model the viral losses were even much higher due to surface/area ratio at smaller scale and high losses due to unspecific adsorption. Differences of the ZikaV inactivation experiment and JEV inactivation were noticed as follows:

A) Much higher purity of ZikaV SGP pool with regard to residual PS (<2µg/mL) compared to JEV. The 3-fold ZikaV inactivated sample contained therefore <<1µg/mL of residual PS. Commercial JEV SGP pool contains on average ~120µg/mL (up to 152 µg/mL possible). The average dilution to inactivation solution of ~14-fold results in a residual PS content up to ~11 µg/mL. It may be that higher amount of residual PS could cause virus precipitation due to cross-linking / reaction with formalin.

B) ZikaV inactivation sample contained ~10% sucrose (3-fold dilution of SGP pool containing ~30-35% sucrose). Sucrose might have stabilizing effect of viral ZikaV particles during treatment with formalin.

Dilution to DS and Formulation with Aluminium hydroxide (DP)

For preparation of ZikaV drug substance used in mouse potency assay an antigen content (expressed as total viral particles or SEC peak area) of 5 times higher compared to Ixiaro was targeted. The basis for determination of antigen content was SEC-HPLC. Briefly, a Superose 6 10/300 Increase column

(GE Healthcare) equilibrated with PBS + 250 mM NaCl, pH 7.4 at 1 ml/min and 25°C, was used to detect ZikaV at 214 nm detection wavelength in harvest samples and throughout the downstream process. In the current JEV process the antigen content in NIV is determined by a specific ELISA. A good correlation was observed between antigen content determined by ELISA and SEC-HPLC. On average, the antigen content in commercial NIV samples is in the range of 33 AU/mL corresponding to ~5.2mAU JEV peak area, see Figure 21.

ZikaV NIV day10 (Zika peak ~36 mAU, analysed on Waters HPLC/Superose6 Increase column) was diluted with PBS to a target of 6.3 (~5.7x dilution). Aluminium hydroxide was added to a final concentration of 0.5 mg/mL Aluminium (1/20 v/v Alum 2% stock solution added) to prepare ZikaV Drug Product (DP). The DP was gently mixed for 5 min. An aliquot of the DP was removed, Alum sedimented by centrifugation and the clear supernatant analysed by SEC-HPLC. No ZikaV peak was detected in the supernatant indicating complete adsorption (estimated as >95%) of viral particles on the mineral adjuvant. Formulated ZikaV DP was stored at 2-8°C.

The impurity profile of the inactivated Zika virus DS is comparable to the profile of JEV DS with the exception of a lower PS content (Table 6).

Table 6: Determination of impurity profile in Zika and JEV DS samples:

	Specification (JEV DS)	**JEV**	**Zika**
HCP (ng/mL)	<100 LOQ 12 ng/mL	<LOQ	<LOQ
DNA (pg/mL)	<200 LOQ 40 pg/mL	<40	<40
Aggregates by SEC-MALLS (%)	Not specified, part of characterization LOQ 5%	<LOQ	<LOQ
PS (µg/mL)	Specification only at SGP pool to demonstrate consistent process performance (19-152µg/mL), *PS content in DS calculated based on PS content in SGP pool (~100 µg/mL) and average dilution factor (~28x) to DS; LOQ 2 µg/mL	~4*	<<LOQ

*Typical PS impurity in a JEV sample produced in accordance with protocol disclosed in Srivastava et al. Vaccine 19 (2001) 4557-4565.

SEC-MALLS Results

A representative SEC-HPLC elution profile of ZikaV NIV at 214 nm detection wave length is shown in Figure 16. Note that BSA (50µg/mL) was added to the sample to minimize losses in HPLC glass vial due to unspecific surface adsorption. ZikaV monomer content was estimated as ~98% with a multimer content of ~2%.

SEC-MALLS analysis (Figure 17) of the sample confirmed the radius Rz of the monomer ZikaV population peak 1 as 21.6 nm and ~49 nm for the multimer peak 2. Cumulative particle size distribution showed that 89% of all viral particles are within a radius range between 18 to 25nm (Figure 18).

Results confirm purity and homogeneity of ZikaV NIV.

Viral titer by plaque assay

Table 7: Active ZikaV pfus were quantified by plaque assay throughout the process.

Sample	**Pfu/mL**
Harvest day 2 (filtered)	$6.4x10^7$
Harvest day 3 (filtered)	$1.0x10^8$
Harvest day 5 (filtered)	$1.5x10^8$
Harvest day 7 (filtered)	$1.1x10^8$
PS treated harvest 300x concentrate (=SGP load)	$9.0x10^8$
SGP pool	$8.9x10^8$
Inactivation start (SGP pool 1:3 diluted)	$3.4x10^8$
Inactivation day 5	<LOD
Inactivation day 10	<LOD

Comparison of PS and Benzonase on process performance

A direct comparison of DNA removal method of concentrated ZikaV harvest pool was done. One aliquot was treated with PS (2 mg/mL, 15min at room temperature), the other aliquot was treated with Benzonase (50 U/mL, 2 mM MgCl2, 4h RT, 48h 2-8°C). Both samples were further purified by sucrose gradient as described in this report. Interestingly, the Benzonase treated samples did not yield any pure fractions after sucrose gradient centrifugation of the treated ZikaV harvest. In those fractions where the specific virus bands were detected, a high amount of host cell protein was detected throughout the collected fractions. The PS treated material resulted in pure ZikaV containing fractions as expected. This finding may suggest that PS is not only effective for DNA removal by precipitation; in addition it improves the recovery of virus particles in the gradient by disrupting interaction of DNA (fragments) and virus particles. Benzonase treatment does not remove DNA, it only results in its fragmentation. Residual DNA fragments might still interact with virus particles and residual HCPs resulting in cross-contamination and co-purification in the sucrose gradient. Pooled SGP fractions were also analysed by SEC-HPLC. Although a large peak was detected, SDS-PAGE confirmed that this sample was highly contaminated with HCPs. A large peak might be detected at UV214 and

280nm after SEC-HPLC analysis due to possible interaction of HCPs with large virus particles, changing the UV absorbance.

Immunogenicity of Vero grown Zika virus

Immunization of mice

Prior to immunization, groups of ten 6-week-old female CD1 mice were bled via vena facialis and pre-immune sera were prepared. One intraperitoneal immunizations of 200 µL were administered. A dose titration (12 µg, 3 µg, 1 µg, 0.33 µg, 0.11 µg, 0.037 µg and 0.012 µg, equivalent to the protein amount in IXIARO) of inactivated Zika virus formulated with aluminium hydroxide (Al(OH)3) at a final concentration of 0.7%. Three weeks after immunization, blood was collected and immune sera were prepared. All animal experiments were conducted in accordance with Austrian law (BGB1 Nr. 501/1989) and approved by "Magistratsabteilung 58".

Plaque reduction neutralization test (PRNT)

Twelve well plates were used for PRNT. Each well was seeded with 1 mL medium containing $4x10^5$ Vero cells and incubated 35°C with 5% CO_2 overnight. Pools of heat inactivated sera from each dose group were tested in triplicate. The target viruses (H/PF/2013 (SEQ ID NO: 13) or MR766 (SEQ ID NO: 11)) were diluted to 100 pfu/165 µL. Equal volumes of target virus and serum dilution were incubated at 35°C with 5% CO_2 for 1 hour. The cell culture medium was aspirated from the Vero cells and 330 µL of the mixture target virus/serum dilution were added to each well and the plates were rocked back and forth 5 times before incubating for 2 hours at 35°C with 5% CO_2. To each well 1 mL of a 2% methylcellulose solution containing EMEM and nutrients was added, the plates were then incubated for 5 days at 35°C with 5% CO_2 before staining the cells for 1 hour with crystal violet/5% formaldehyde and subsequently washed 3 times with deionized water. The plates were air dried and the numbers of plaques in each well were manually counted.

Results

Neutralization was observed with serum pools from mice immunized with inactivated Zika virus vaccine (H/PF/2013) down to 37 ng (dosing equivalent to the amount protein in IXIARO®) against Zika viruses of both the Asian (H/PF/2013) and African (MR766) lineages (Figures 19 and 20, respectively). Complete inhibition was seen at the 1:20 serum dilution with an immunization dose down to 110 ng (dosing equivalent to the amount protein in IXIARO®). The neutralization of both the Asian (H/PF/2013) and African (MR766) lineages of the Zika virus was equivalent, which indicates high cross-neutralization between different Zika virus strains of the inactivated Zika virus vaccine (H/PF/2013).

Another neutralization assay was performed using the microneutralization assay as described by Larocca, et al. (2016, Nature doi:10.1038/nature18952). It was found that the inactivated Zika virus of the current invention had an MN50 (microneutralization) titer of 90 at 1 µg of inactivated purified virus.

Further methods: The immunogenicity of inactivated Zika virus preparations is assessed using a mouse model of Zika infection. Groups of adult mice are immunized subcutaneously (s.c.) with 500, 50, or 5 ng of inactivated Zika virus with adjuvant (*e.g.* aluminium hydroxide with or without IC31®), or without adjuvant. An additional group of mice receive PBS as a negative control. Each group is administered the indicated inoculum at t=0 and in some cases also at three to four weeks later (t=3/4). Beginning approximately three weeks after administration of the last immunization, serum samples are obtained from each of the mice at regular intervals. The serum samples are tested for the presence of neutralizing antibodies using PRNT.

The *in vivo* protective efficacy of the inactivated Zika virus preparations is also assessed using a mouse model of Zika infection, *i.e.* IFN-alpha/beta receptor knock-out mice (A129) (see *e.g.* Dowall et al., 4. March 2016, dx.doi.org/10.1101/042358) or blocking of the IFN-alpha/beta receptor by administration of anti-IFN-alpha/beta receptor monoclonal antibodies to C57BL/6 or BALB/c mice (see e.g. Pinto et al., 7. December 2011, DOI: 10.1371/journal.ppat.1002407). For protection assays, groups of 10 three- to eight-weeks-old A129, C57BL/6 of BALB/c mice are inoculated subcutaneously in the hindquarters with inactivated Zika virus with adjuvant (aluminium hydroxide) or without adjuvant at t=0. Age-matched controls are inoculated with PBS or non-specific antigens in alum. Mice are optionally boosted with a second administration of the indicated inoculation three to four weeks later. The mice are then challenged subcutaneously at three to eight weeks post immunization by inoculation with a deadly dose of live Zika virus. One day prior to challenge of C57BL/6 and BALB/c mice, they are passively administered (intraperitoneally) anti-IFN-alpha/beta receptor monoclonal antibodies. Challenged mice are monitored daily for morbidity and mortality for up to twenty-one days. Another alternative is to challenge intracranially adult vaccinated/non-vaccinated adult mice and observe protection.

It is expected that the Zika virus produced by the process of the invention will provide very similar functional read-outs in *in vitro*, *in vivo* and finally human trials as the currently licensed JEV vaccine in the EU and US and elsewhere, IXIARO®. The dosage may alter but due to the very similar impurity profile and almost identical manufacture, a very similar efficacy and safety result will be

expected as was determined for the currently licensed JEV vaccine (licensed in the EU and US and elsewhere).

Discussion & Conclusion

The existing manufacturing platform for production of inactivated JEV vaccine IXIARO® was used as a basis for a manufacturing feasibility study of inactivated ZikaV vaccine candidate (Asian strain H/PF/2013). The virus was produced on Vero cells cultivated in roller bottles. The virus was purified by PS treatment followed by an optimized sucrose gradient. Inactivation was done by formalin treat (0.02 %, 10 days at 22 °C). For exploratory immunization studies in mice, a DP formulated with Alum was prepared with an estimated 5-fold higher virus particle content compared to IXIARO®, the commercial JEV Vaccine. The impurity profile of the DS met all criteria as defined in the specification for IXIARO®, the commercial JEV vaccine. The neutralization of both the Asian (H/PF/2013) and African (MR766) lineages of the Zika virus was equivalent, which indicates high cross-neutralization between different Zika virus strains of the inactivated Zika virus vaccine (H/PF/2013).

The in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, supra.). Inactivated viruses are among the safest vaccines and especially preferred for deliver to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Further more detailed aspects of the invention:

A1. A Zika virus vaccine comprising an inactivated Zika virus particle, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability.

A2. The Zika virus vaccine of A1, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability.

A3. The vaccine of A1 or A2, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13 or 72, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 or 72 and able to pack a virulent Zika virus.

A4. The vaccine of any one of A1-A3, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 and able to pack a virulent Zika virus.

A5. The vaccine of any one of A1-A4, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

A6. The vaccine of A5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent to completely inactivate the Zika virus as measured by plaque assay.

A7. The vaccine of A6, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

A8. The vaccine of A7, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

A9. The vaccine of any one of A5-A8, wherein the chemical activation is performed at about +4°C or about +22°C.

A10. The vaccine of any one of A1-A9, further comprising an adjuvant.

A11. The vaccine of A10, wherein the adjuvant is an aluminum salt adjuvant.

A12. The vaccine of A11, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

A13. The vaccine of any one of A10-A12, wherein the vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).

A14. The vaccine of A13, wherein the peptide comprises the sequence KLKL5KLK (SEQ ID NO: 71) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 70).

A15. The vaccine of any one of A1-A14, further comprising one or more pharmaceutically acceptable excipient.

A16. The vaccine of any one of A1-A15, wherein the vaccine contains protamine sulphate or fragments or break-down products of PS at amounts too low to detect by HPLC, i.e., below 1 μg/mL, especially below 100 ng/mL.

A17. The vaccine of A16, wherein said protamine sulphate or fragments or break-down products of PS can be detected by mass spectroscopy or another sensitive method.

B1. A kit comprising a Zika virus vaccine of any one of A1-A15.

B2. The kit of B1, further comprising a second vaccine.

B3. The kit of B2, wherein the second vaccine is a West Nile virus vaccine, a Japanese Encephalitis virus vaccine, a Yellow Fever virus vaccine, a Dengue virus vaccine or a Chikungunya virus vaccine.

C1. A method, comprising administering a first dose of a therapeutically effective amount of the Zika virus vaccine of any one of A1-A15 to a subject in need thereof.

C2. The method of C1, further comprising administering a second dose of a therapeutically effective amount of the Zika virus vaccine.

C3. The method of C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

C4. The method of C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

C5. The method of C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

C6. The method of any one of C1-C5, wherein the administering results in production of Zika virus neutralizing antibodies.

D1. A method of producing a Zika virus vaccine, comprising

(i) passaging a Zika virus on Vero cells, thereby producing a culture supernatant comprising the Zika virus;

(ii) harvesting the culture medium of (i);

(iii) precipitating the harvested culture medium of (ii), thereby producing a Zika virus supernatant; and

(iv) optimally inactivating the Zika virus in the Zika virus supernatant of (iii) thereby producing an inactivated Zika virus.

D2. The method of D1, further comprising concentrating the culture medium of (ii) prior to step (iii).

D3. The method of D1 or D2, wherein the precipitating of (iii) comprises contacting the culture medium of (ii) with protamine sulfate or benzoate.

D4. The method of any one of D1-D3, further comprising (v) dialyzing the inactivated Zika virus of (iv), thereby producing a dialyzed Zika virus.

D5. The method of D4, further comprising (vi) filtering the dialyzed Zika virus of (v).

D6. The method of any one of D1-D5, wherein the inactivating is by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

D7. The method of D6, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for at least 4 days.

D8. The method of D6 or D7, wherein the chemical inactivation agent comprises formaldehyde.

D9. The method of any one of D6-D8, wherein the chemical activation is performed at about +4°C or about +22°C.

D10. The method of D8 or D9, further comprising neutralizing the formaldehyde.

D11. The method of D10, wherein the neutralizing is performed with sodium metabisulfite.

E1. The use of the optimally inactivated Zika virus vaccine of any one of A1-A15 for the treatment and/or prevention of a Zika virus infection.

E2. The use of E1, wherein the inactivated Zika virus vaccine is administered in a first dose of a therapeutically effective amount to a subject in need thereof.

E3. The use of E2, wherein the inactivated Zika virus vaccine is administered in a second dose of a therapeutically effective amount to the subject.

E4. The use of E3, wherein the second dose of the inactivated Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

E5. The use of E3, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

E6. The use of E3, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

E7. The use of any one of E1-E6, wherein the vaccine administration results in production of Zika virus neutralizing antibodies.

F1. A pharmaceutical composition for use in the treatment or prevention of a Zika virus infection, wherein said pharmaceutical composition comprises the optimally inactivated Zika virus vaccine of any one of A1-A15.

F2. The pharmaceutical composition of F1, wherein the inactivated Zika virus vaccine is administered in a first dose of a therapeutically effective amount to a subject in need thereof.

F3. The use of F2, wherein the inactivated Zika virus vaccine is administered in a second dose of a therapeutically effective amount to the subject.

F4. The use of F3, wherein the second dose of the inactivated Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

F5. The use of F3, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

F6. The use of F3, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

F7. The use of any one of F1-F6, wherein the vaccine administration results in production of Zika virus neutralizing antibodies.

G1. Use of an optimized sucrose gradient centrifugation for removal of protamine sulphate from purified infectious Zika virus particles.

G2. The use according to G1, wherein said optimized sucrose gradient centrifugation comprises a virus comprising fraction in a 10% +/- 1% (w/w) sucrose solution and three further layers of sucrose solutions with different densities, i.e. a first sucrose solution with 15% +/- 1% (w/w) sucrose, a second sucrose solution with 35% +/- 1% (w/w) sucrose, and a third sucrose solution with a 50% +/- 1% (w/w) sucrose.

G3. A process of purification of infectious Zika virus particles, comprising the steps of:

a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);

c) further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation, wherein the optimized sucrose gradient is provided such that the protamine can be completely or almost completely separated from the virus fraction; and wherein the protamine concentration is reduced by this step to the extent that the protamine concentration in the final drug substance is below 1 µg/ml, preferably below 0.5 µg/mL, more preferably below 0.1 µg/mL, most preferably below 0.05µg/mL.

G4. The process of G3, wherein said optimized sucrose density gradient centrifugation comprises a virus comprising fraction in a 10% +/- 1% (w/w) sucrose solution and three layers of sucrose with different densities, i.e. a first sucrose solution with 15% +/- 1% (w/w) sucrose, a second sucrose solution with 35% +/- 1% (w/w) sucrose, and a third sucrose solution with a 50% +/- 1% (w/w) sucrose.

G5. The process of any one of G3 to G4, additionally comprising a further purification step of: (d) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles.

G6. The process of any of G3 to G5, wherein the residual host cell DNA content of the Zika virus preparation (c) is less than 10 ng/mL and the residual host cell protein content of the final virus preparation (c) is less than 100 ng/mL.

G7. The process of any of G3 to G6, wherein said crude harvest (a) comprising Zika virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

G8. The process of G7, wherein the one or more pre-purification step(s) comprises

a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or

b) digestion of host cell genomic DNA by enzymatic treatment; and/or

c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

G9. The process of any one of G3 to G8, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml or 2 mg/ml.

G10. The process of any one of G3 to G9, wherein the enrichment of infectious Zika virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

G11. The process of any one of G7 to G10, wherein the one or more pre-purification step(s) prior to step (b) of any of G8 to G11 is performed using a filter having a pore size equal to or less than 1 μm, preferably 0.2 μm.

G12. The process of any one of G3 to G11, wherein the residual impurity of the Zika virus preparation (c) is less than 10%.

G13. The process of any one of G3 to G12, wherein the Zika virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

G14. The process of G13, wherein said cell line is a Vero cell line.

G15. The process of any one of G3 to G14, wherein said infectious Zika virus particle is an infectious virus particle that is a live virus, a live attenuated virus, a chimeric virus, a modified live virus, or a recombinant live virus.

G16. The process of any one of G3 to G15, wherein said Zika virus is preferably a strain of the Asian lineage.

G17. The process of any one of G3 to G16, wherein said process resulting in final virus preparation (c) or (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

G18. Use of the process according to any one of G3 to G17 for manufacturing a composition for immunization against a virus infection.

G19. The use according to G18, wherein said virus infection is an infection caused by a Zika virus.

Q1. A process of purification of infectious Zika virus particles, comprising the steps of:
(a) providing a crude harvest (a) comprising Zika virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;
(b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);
(c) further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation, wherein the optimized sucrose gradient is provided such that the protamine can be completely or almost completely separated from the virus fraction; and wherein the protamine concentration is reduced by this step to the extent that the protamine concentration in the final drug substance is below 1 µg/ml, preferably below 0.5 µg/mL, more preferably below 0.1 µg/mL, most preferably below 0.05µg/mL.

Q2. The process of Q2, wherein the virus particles are from Zika virus.

Q3. The process of Q1 or Q2, additionally comprising the step of:
(d) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles.

Q4. The process of any of Q1 to 3, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.

Q5. The process of any of Q1 to 4, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

Q6. The process of Q5, wherein the one or more pre-purification step(s) comprises
(a) filtration using a filter having a pore size equal to or less than 0.2 µm; and/or

(b) digestion of host cell genomic DNA by enzymatic treatment; and/or

(c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

Q7. The process of any one of Q1 to 6, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml or 2 mg/ml.

Q8. The process of any one of Q1 to 7, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

Q9. The process of any one of Q5 to 8, wherein the one or more pre-purification step(s) prior to step (b) of any of Q5 to 8 is performed using a filter having a pore size equal to or less than 1 μm, preferably 0.2 μm.

Q10. The process of any one of Q1 to 9, wherein the residual impurity of the virus preparation (c) is less than 10%.

Q11. The process of any one of Q1 to 10, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

Q12. The process of Q11, wherein said cell line is a Vero cell line.

Q13. The process of any one of Q1 to 12, wherein the infectious virus particles is an infectious Zika virus particle that is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

Q14. The process of any one of Q1 to 13, wherein the Zika virus is a Zika virus strain of the Asian lineage or an immunogenic variant thereof.

Q15. The process of any one of Q1 to 14, wherein said process resulting in final virus preparation (c) or (d) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

Q16. Use of the process according to any one of Q1 to 15 for manufacturing a composition for immunization against a virus infection.

Q17. The use according to Q16, wherein the composition for immunization against a virus infection is an infection caused by Zika virus.

Q18. A composition comprising the virus particles obtainable or obtained by the process of any one of Q1 to 17 for treating and/or preventing an infection, such as e.g. a Zika virus infection.

Q19. A Zika virus vaccine comprising an inactivated Zika virus particle grown on vero cells, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability and comprises minor amounts of protamine sulphate, preferably below the detection limit.

Q20. The Zika virus vaccine of Q19, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability, preferably a 80% probability.

Q21. The vaccine of Q19 or 20, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 2-13, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 2-13 and able to pack a virulent Zika virus.

Q22. The vaccine of any one of Q19, 20 and 21, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 14-69 or 72, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 14-69 or 72 and able to pack a virulent Zika virus.

Q23. The vaccine of any one of Q19, 20 to 22, wherein the Zika virus obtained by culturing on Vero cells is purified by protamine sulfate precipitation and sucrose gradient centrifugation.

Q24. The vaccine of Q23, wherein the sucrose gradient centrifugation is an optimized sucrose gradient centrifugation.

Q25. The vaccine of Q24, wherein the optimized sucrose gradient centrifugation comprises a virus comprising fraction in a 10% (w/w) sucrose solution and three layers of sucrose with different densities, i.e. a first sucrose solution with 15% (w/w) sucrose solution, a second sucrose solution with 35% (w/w) sucrose solution, and a third sucrose solution with a 50% (w/w) sucrose solution.

Q26. The vaccine of any one of Q19, 20 to 25, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

Q27. The vaccine of Q26, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.

Q28. The vaccine of Q27, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

Q29. The vaccine of Q28, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

Q30. The vaccine of any one of Q27-29, wherein the chemical activation is performed at about +4°C or about +22°C.

Q31. The vaccine of any one of Q19 to 30, further comprising an adjuvant.

Q32. The vaccine of Q31, wherein the adjuvant is an aluminum salt adjuvant.

Q33. The vaccine of Q32, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

Q34. The vaccine of Q32, wherein the aluminum salt adjuvant is aluminium hydroxide with less than 1.25ppb Cu based on the final pharmaceutical composition comprising the Zika virus, preferably the inactivated Zika virus.

Q35. The vaccine of any one of Q19 to 34, further comprising one or more pharmaceutically acceptable excipient.

R1. Use of protamine, preferably a protamine salt, to separate infectious and non-infectious Zika virus particles, host cell proteins and/or undefined low molecular weight materials.

R2. A process of purification of infectious Zika virus particles, comprising the steps of:

(a) providing a crude harvest (a) comprising Zika virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

(b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b), wherein the enrichment of infectious virus particles in the virus preparation (b) relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

R3. The use of R1 or the process of R2, wherein the virus particles are from Zika virus.

R4. A process of purification of infectious Zika virus particles, comprising the steps of:

(a) providing a crude harvest (a) comprising Zika virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate;

(b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising protamine, preferably a protamine salt, more preferably a protamine sulphate, even more preferably a recombinant protamine sulphate, to obtain a virus preparation (b);

(c) further purifying the virus preparation (b) by one or more size exclusion methods such as (i) a sucrose density gradient centrifugation, (ii) a solid-phase matrix packed in a column comprising a ligand-activated core and an inactive shell comprising pores, wherein the molecular weight cut off of the pores excludes the virus particles from entering the ligand-activated core, and wherein a molecule smaller than the molecular weight cutoff of the pores can enter the ligand-activated core and collecting the virus particles, and/or (iii) size exclusion chromatography to obtain a virus preparation (c) comprising the infectious virus particles, wherein the residual host cell DNA of the virus preparation (c) is less than 100 ng/mL and the residual host cell protein and the residual aggregates of infectious virus particles of the final virus preparation (c) is less than 1 μg/mL.

R5. The process of R4, wherein the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL and the residual host cell protein of the final virus preparation (c) is less than 100 ng/mL.

R6. The process of any of R2 to 5, wherein the crude harvest (a) comprising virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b).

R7. The process of R6, wherein the one or more pre-purification step(s) comprises

(a) filtration using a filter having a pore size equal to or less than 0.2 μm; and/or

(b) digestion of host cell genomic DNA by enzymatic treatment; and/or

(c) ultra/diafiltration using a hollow fiber membrane having a pore size equal to or greater than 300 kDa, preferably equal to or greater than 100 kDa.

R8. The process of any one of R2 to 7, wherein the concentration of protamine sulphate is 0.5 to 3 mg/ml, more preferably 1 to 2 mg/ml, more preferably 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml, most preferably 1.6 mg/ml.

R9. The process of any one of R2 to 8, wherein the enrichment of infectious virus particles in the virus preparation (c) or any final virus preparation relative to total virus products in the crude harvest (a) is in the range from at least 50% to 95%, preferably at least 80%.

R10. The process of any one of R6 to 9, wherein the one or more pre-purification step(s) prior to step (b) of any of R6 to 9 is performed using a filter having a pore size equal to or less than 1 μm, preferably 0.2 μm.

R11. The process of any one of R2 to 10, wherein the residual impurity of the virus preparation (c) is less than 10%.

R12. The process of any one of R2 to 11, wherein the virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line.

R13. The process of R12, wherein said cell line is a Vero cell line.

R14. The process of any one of R2 to 13, wherein the Zika virus is a live virus, an attenuated live virus, a chimeric virus, a modified live virus, or a recombinant live virus.

R15. The process of any one of R2 to 14, wherein the Zika virus is a Zika virus strain of the Asian lineage or an immunogenic variant thereof.

R16. The process of any one of R2 to 15, wherein said process resulting in final virus preparation (c) is followed by an inactivation step, wherein the virus is inactivated preferably by formaldehyde.

R17. Use of the process according to any one of R1 to 16 for manufacturing a composition for immunization against a virus infection.

R18. The use according to R17, wherein the composition for immunization against a virus infection is an infection caused by a Zika virus.

R19. A composition comprising the virus particles obtainable or obtained by the process of any one of R2 to 16 for treating and/or preventing an infection.

ZIKA VIRUS VACCINE

RELATED APPLICATIONS

This Application is a continuation of U.S. Application Serial No. 16/063,007, filed June 15, 2018, which is a national stage filing under 35 U.S.C. § 371 of International Patent Application Serial No. PCT/EP2016/082664, filed December 23, 2016, the contents of each of which is herein incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 15, 2020, is named I042270125US01-SUBSEQ-CEW and is 455 kilobytes in size.

FIELD OF INVENTION

The disclosure relates to methods for the purification of Zika viruses for use in vaccines and in particular relates to an improved sucrose gradient process step allowing the separation of impurities such as protamine sulphate. The disclosure also relates to Zika virus vaccines and compositions and methods for producing said vaccines and administering the vaccines to subjects for the generation of an anti-Zika virus immune response.

BACKGROUND OF THE INVENTION

Adverse responses to protamine sulfate have been known for many years. Previous exposure to protamine can induce a humoral immune response and predispose susceptible individuals to the development of untoward reactions from the subsequent use of this drug. Patients exposed to protamine through the use of protamine-containing insulin or during heparin neutralization may experience life-threatening reactions and fatal anaphylaxis upon receiving large doses of protamine intravenously. Severe reactions to intravenous protamine can occur in the absence of local or systemic allergic reactions to subcutaneous injection of protamine-containing insulin. Although there is no clear evidence for hypersensitivity reactions of protamine sulphate linked to vaccination, vaccines containing protamine impurities have a precaution and contraindication warning in their

labels stating that a serious allergic reaction after a previous dose of such a protamine containing vaccine (e.g. IXIARO®, see CDC site www.cdc.gov/japaneseencephalitis/vaccine/) is a contraindication to further doses. Thus elimination of said impurity is a medical request for an improved safety profile. On the other hand protamine sulphate is an excellent tool (and often better than other tools such as benzonase) to purify crude harvests of viruses grown on cell substrates.

In 2007, Zika virus was detected for the first time outside of the endemic regions of Asia and Africa since its discovery in a Rhesus monkey in Uganda in 1947. Since then, the virus has caused a large epidemic in French Polynesia, spreading through islands in the Pacific and into South and Central America by 2015 (WHO "Zika Situation Report" February 5, 2016). Evidence suggests that in addition to being transmitted by *Aedes* species mosquitos, other vectors may exist, and the virus may be transmitted by blood transfusion, transplacentally, and through sexual transmission (WHO Zika Virus Fact Sheet, Feb. 2016). Though the symptoms of Zika virus infection include mild fever, rash, and conjunctivitis, there is a likely correlation between infection and neurological disorders, including Guillain-Barré syndrome and microcephaly in fetuses/neonates subsequent to infection during pregnancy. There is currently no specific treatment or vaccine for Zika virus and the only preventative measures involve control of the mosquito vector. Zika virus presents a substantial public health threat due to the wide circulation of the *Aedes* mosquito, multiple routes of transmission, and potentially severe neurological effects of infection.

A preventative vaccine against Zika virus is a pressing medical need in endemic areas and in geographical areas where the vector is spreading. Furthermore, as Zika infection has dire consequences on embryonic and fetal development, a safe and effective vaccine for women of child-bearing potential or pregnant women is needed. Vaccines administered during pregnancy must be very safe for both the mother and the developing fetus. While live attenuated viral vaccines are highly effective, they are often not considered safe enough for administration to pregnant women. In this regard, inactivated viral vaccines, which lack the ability to propagate in the vaccinated subject, are considered much safer. Development of an inactivated Zika virus vaccine for administration to at-risk patients would fill this need.

SUMMARY OF THE INVENTION

During the course of virus purification, it was observed that addition of protamine sulfate to a virus harvest produced on a cell substrate removed not only contaminating DNA derived from host cells, as expected, but surprisingly also virtually eliminated immature and otherwise non-infectious virus

particles from the preparation. This finding provided a streamlined, gentle, reproducible and broadly-applicable process for obtaining highly-purified infectious virus particles for applications such as vaccine preparation. In addition, it was surprisingly found that said protamine sulfate can be very efficiently separated from the virus fraction allowing for a safer vaccine produced at high yields.

Disclosed herein are virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering said Zika virus vaccines for the prevention of Zika virus infection and/or for the production of an anti-virus immune response in subjects, for example subjects at risk of being exposed to Zika virus. In particular, the invention is directed to a virus vaccine comprising an optimally inactivated Zika virus particle, wherein the Zika virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability. Another advantage of the invention is that related methods of producing said vaccines and compositions are very efficient and provide pure compositions largely devoid of impurities, in particular protamine sulphate, allowing for high volume production of vaccines. Detail experimental examples to the above are provided for Zika virus.

The herein disclosed in vivo data regarding immunogenicity of the inactivated Zika virus vaccine of the current invention indicates that the virus is surprisingly potently immunogenic and also highly cross-protective (very similar immunogenicity in African and Asian strains). Data indicate that immunogenicity was unexpectedly higher than the recently reported inactivated Zika virus vaccine candidate (Larocca, et. al, 2016, Nature doi:10.1038/nature18952.). Inactivated viruses are among the safest vaccines and especially preferred for delivery to populations where safety is especially concerning, such as pregnant women, children and immunocompromised individuals, which makes the herein disclosed inactivated Zika virus particularly suitable. Obtaining a high titer of inactivated virus is a challenge in the field. The herein disclosed process for purifying inactivated Zika virus results in not only a high yield, but also a very pure drug substance.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing, alignments were performed with the multi alignment package Jalview (Waterhouse et al., 2009, Bioinformatics 25 (9) 1189-1191). In the drawings:

FIG. 1: Average distance tree (by % identity, nt), complete genomes.

FIG. 2: Neighbor joining tree (by % identity, nt), complete genomes.

FIG. 3: Pairwise alignment-Jalview (% identity, nt), complete genomes.

FIG. 4: Average distance tree (by % identity, aa), E-protein.

FIG. 5: Neighbor joining tree (by % identity. aa), E-protein.

FIG. 6: Pairwise alignment-Jalview (% identity, aa), E-protein.

FIGs. 7A-7C: Alignment (shading: % identity, aa), E-protein.

FIG. 8: An example of virus particle maturation in the host cell. As observed in flaviviruses, full maturation of the particles requires proteolytic cleavage of the precursor membrane glycoprotein (prM) by the host protease furin. Not all prM molecules are cleaved, resulting in the release of mature, mosaic or immature-like conformations from the cells. Mosaic and immature forms are generally not infectious—only mature virions are infective and have hemagglutinin (HA) / TCID50 activity. (Figure adapted from Plevka, et al., Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres, EMBO reports (2011) 12, 602–606).

FIGs. 9A-9B: An exemplary downstream virus purification process from the crude harvest to formulation of the drug substance (vaccine), a preferred embodiment of the process of the invention (**FIG. 9A**). A flow-chart of an exemplary virus inactivation process is shown in (**FIG. 9B**).

FIG. 10: PS treatment results in selective removal of Zika virus aggregates and Vero HCP and LMW impurities (SEC-HPLC of 30x concentrated Zika Virus harvest day 5).

FIG. 11: SEC-HPLC of individual 30x concentrated Zika harvest prior to PS treatment at different time points.

FIG. 12: SEC-HPLC of individual 30x concentrated Zika harvest post PS treatment at different time points. The smaller graph indicates the observed cytopathic effect (CPE) over time.

FIG. 13: Preparation of the sucrose gradient.

FIG. 14: Representative SDS-PAGE from the sucrose gradient harvest of a Zika purification is shown.

FIG. 15: Comparison of JEV and ZikaV harvest schedules/yields.

FIG. 16: SEC-HPLC elution profile of ZikaV NIV. Data were processed on Dionex Ultimate 3000 / Superose 6 Increase column. Both panels are from the same chromatogram. The upper graph is the complete elution profile; the lower graph is an enlargement of the ZIKAV elution peak.

FIG. 17: SEC-MALLS analysis of inactivated ZikaV.

FIG. 18: Cumulative particle size distribution of Zika NIV.

FIG. 19: Graphical representation of the neutralization of the Zika virus H/PF/2013 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated using the 3-parameter method.

FIG. 20: Graphical representation of the neutralization of the Zika virus MR766 with pooled mouse sera. The number of plaques without serum was set to 100%. The EC50 was calculated using the 3-parameter method.

FIG. 21: Correlation between JEV antigen content in neutralized inactivated virus (NIV) analysed by ELISA and SEC-HPLC (Dionex Ultimate 3000, Superose 6 column).

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering said virus vaccines for the prevention of virus infection and/or for the production of an anti-virus immune response in subjects, for example subjects at risk of being exposed to virus. In particular, the invention is directed to a virus vaccine comprising an optimally inactivated virus particle, wherein the virus particle in an appropriate dose is able to seroconvert a subject that is administered the virus vaccine with at least a 70% probability, preferably an 80% probability. Another advantage of the invention is that related methods of producing said vaccines and compositions are very efficient and provide pure compositions largely devoid of impurities, in particular protamine sulphate, allowing for high volume production of vaccines. Examples to the above are provided for Zika virus.

Disclosed herein are downstream processes for purifying Zika virus particles from a crude preparation. The downstream process can be applied to either a virus which has not adapted for propagation on a particular cell substrate or for a partially / fully cell substrate adapted Zika virus particle.

Aspects of the invention provide processes for the purification of infectious Zika virus particles comprising the steps of (a) providing a crude harvest (a) comprising virus particles and impurities, wherein the impurities are generated from growing said virus particles on a cell substrate; (b) reducing impurities from the crude harvest (a) by precipitation with an agent comprising a protamine salt, preferably a protamine sulphate, to obtain a virus preparation (b); and further purifying the virus preparation (b) by an optimized sucrose density gradient centrifugation to obtain a virus preparation (c) comprising the infectious virus particles.

In some embodiments, the concentration of protamine sulphate in step (b) is about 1 to 10 mg/ml, more preferably about 1 to 5 mg/ml, more preferably about 1 to 2 mg/ml. In one embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/mL. In one embodiment, the concentration of protamine sulphate is 1.2 to 1.8 mg/ml, more preferably 1.4 to 1.6 mg/ml. In a preferred embodiment, the concentration of protamine sulphate in step (b) is about 2 mg/ml.

In some embodiments, the residual host cell DNA of the virus preparation (c) is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL.

In a preferred embodiment, the residual host cell DNA of the virus preparation (c) is less than 10 ng/mL. In some embodiments, the residual host cell protein of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the residual host cell protein of the virus preparation (c) is less than 100 ng/mL. In some embodiments, the residual non-infectious virus particles of the final virus preparation (c) is less than 10 µg/mL, especially less than 9, 8, 7, 6, 5, 4, 3 or 2 µg/mL, preferably less than 1 µg/mL. In a preferred embodiment, the content of residual non-infectious virus particles of the virus preparation (c) is less than 100 ng/mL.

In some embodiments, the residual protamine is less than 1 µg/mL, especially less than 900, 800, 700, 600, 500, 400, 300 or 200 ng/mL, preferably less than 100 ng/mL, more preferably is below the detection limit of HPLC, in particular below the detection limit in the final drug substance. In some embodiments, the PS content is tested by HPLC or size exclusion chromatography (SEC). For example, HPLC is validated for PS determination in JEV sucrose gradient pool samples as a routine release assay and is very sensitive (i.e., LOQ 3 µg/mL; LOD 1 µg/mL). In the current invention, PS content in in Zika virus DS samples was <LOD. In one embodiment, the HPLC assessment of PS content can be performed on a Superdex Peptide 10/300GL column (GE: 17-5176-01) using 30% Acetonitrile, 0,1% Trifluoroacetic acid as solvent with a flow rate of 0,6 ml/min at 25°C and detection at 214 nm. A more sensitive method of measurement for residual protamine in a purified virus preparation is mass spectrometry (MS). In some embodiments, the residual PS levels in a Zika virus preparation are tested by MS or other such highly sensitive method, e.g. nuclear magnetic resonance (NMR). With this method, residual PS, as well as fragments and/or break-down products of PS, can be detected at trace amounts, such as levels as low as, for example, 10^6, 10^7 or 10^8 molecules per typical sample load. In some embodiments, the PS levels are tested in the sucrose gradient pool. In some embodiments, the PS levels are tested in the drug product. In some embodiments, the PS levels are tested in the drug substance.

In some embodiments, the crude harvest (a) comprising the virus particles and impurities is subjected to one or more pre-purification step(s) prior to step (b). In some embodiments, the one or more pre-purification step(s) comprises digesting host cell genomic DNA in the crude harvest (a) comprising the virus particles and impurities by enzymatic treatment. In some embodiments, the one or more pre-purification step(s) comprises filtration, ultrafiltration, concentration, buffer exchange and/or diafiltration. In some embodiments, the one or more pre-purification steps is filtration using a filter having a pore size equal to or less than 1 µm. In some embodiments, the filter has a pore size equal to or less than 0.2 µm. In a preferred embodiment, the filter has a pore size of 0.2 µm. In some

embodiments, the concentration and/or ultra/diafiltration and/or buffer exchange is performed by tangential flow filtration (TFF). In some embodiments, ultra/diafiltration of the crude harvest (a) comprising the virus particles and impurities is performed using a hollow fiber membrane having a cut-off of equal to or less than 300 kDa. In a preferred embodiment, the hollow fiber membrane has a cut-off of about 100 kDa.

The process according to the current invention may also comprise the use of a sucrose gradient, preferably an optimized sucrose gradient. The sucrose gradient is preferably optimized for the removal of protamine sulfate, also for the removal of immature viral particles or other viral particles which are non-infectious or host cell proteins or nucleic acids (DNA, RNA, mRNA, etc) or other host cell debris. In the current invention the optimized sucrose gradient comprises at least two, at least three, at least four layers of sucrose solutions with different densities. In one embodiment, the virus preparation to be purified is provided in a sucrose solution which has a density of about 8%, about 9%, about 10%, about 11%, about 12% sucrose (w/w), preferably about 10%. In one embodiment, one sucrose solution in the gradient has a density of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55% sucrose (w/w), preferably about 50%. In one embodiment, one sucrose solution in the gradient has a density of about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40% sucrose (w/w), preferably about 35%. In one embodiment, one sucrose solution in the gradient has a density of about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20% sucrose (w/w), preferably about 15% sucrose. In a preferred embodiment, the sucrose gradient comprises three layers of sucrose solutions of about 50%, about 35% and about 15% (w/w) sucrose and the virus composition to be purified is contained in about 10% (w/w) sucrose. Because the invention provided for means to not only test for host cell DNA but also immature viral particles, the skilled person in the art is able to more precisely optimize the sucrose gradient for most efficient purification and include additional tools such as PRNT assay to monitor purification success.

In some embodiments, the virus particle is a live virus, a chimeric virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In a further step, the virus particles of the invention may be optionally inactivated. In some embodiments, the virus particle is an attenuated form of the virus particle. For example, the virus may have reduced infectivity, virulence, and/or replication in a host, as compared to a wild-type virus. In some embodiments, the virus is a mutated or modified virus, for example the nucleic acid of the virus may contain at least one mutation relative to the wild-type virus. In some embodiments, the virus is a recombinant live virus, meaning a virus that is generated recombinantly and may contain nucleic acid from different sources.

In some embodiments, the Zika virus particle is a live virus, an attenuated live virus, a modified live virus, or a recombinant live virus. In preferred embodiments, the Zika virus is a Zika virus from the Asian lineage.

In some embodiments, the relative reduction of impurity of the final virus preparation relative to the liquid medium (a) comprising the virus particles and impurities is in a range from 60 to 95%. In some embodiments, the residual impurity of the final virus preparation is less than 1%.

In some embodiments, the Zika virus is propagated in a cell line selected from the group consisting of an EB66 cell line, a Vero cell line, a Vero-αHis cell line, a HeLa cell line, a HeLa-S3 cell line, a 293 cell line, a PC12 cell line, a CHO cell line, a 3T3 cell line, a PerC6 cell line, a MDSK cell line, a chicken embryonic fibroblast cell line, a duck cell line, and a diploid avian cell line. In some embodiments, said cell line is a duck cell line. In some embodiments, said cell line is a diploid avian cell line. In some embodiments, said cell line is an EB66 cell line. In a preferred embodiment, said cell line is a Vero cell line.

Aspects of the invention provide a use of any of the processes described herein for manufacturing a composition for immunization against a viral infection. In a preferred embodiment, the composition or vaccine is directed against Zika virus such as e.g. a Zika virus of the Asian lineage.

Other aspects provide compositions comprising the Zika virus particles obtainable by any of the processes described herein for treating and/or preventing (i.e. protecting from) a viral infection. In a preferred embodiment, the viral infection is caused by Zika virus such as e.g. a Zika virus of the Asian lineage.

Furthermore, disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering the Zika virus vaccines for the prevention of Zika virus infection and/or for the production of an anti-Zika virus immune response in subjects, for example subjects at risk of being exposed to Zika virus.

Zika virus is a flavivirus closely related to Dengue virus and is similarly transmitted by the *Aedes* species mosquito, although other arthropod vectors for Zika virus are possible. Since it was first isolated from a Rhesus monkey in the Zika forest of Uganda in 1947, there were very few reported

incidents of human infection, especially outside of the endemic regions of Africa and Asia until a large outbreak in French Polynesia in 2007 (Haddow *et al. PLoS Neglected Tropical Diseases* (2012) 6(2), Malone *et al. PLoS Neglected Tropical Diseases* (2016) 10(3),). The virus has since spread through islands of the Pacific, including Oceania, and into South and Central America (WHO "Zika Situation Report" February 5, 2016).

In addition to being spread by the bite of an infected mosquito, evidence also suggests transmission may occur between individuals, such as from the blood of an infected individual, *in utero*/transplacental transmission from an infected mother to the fetus, sexual transmission between sexual partners, and possibly by other local transmission routes. There is a possible association between Zika virus infection during pregnancy and microcephaly in the fetus/neonate. Microcephaly is a rare condition in which a baby's head circumference is significantly less than expected based on the average for their age, sex, and ethnicity. This is a result of the brain failing to undergo proper embryonic development, and in 90% of cases is associated with mental retardation (Rocha *et al.* (2016) *Bull World Health Organ* 8 Feb. 2016).

There is a probable association between individuals having had a prior Zika virus infection and the incidence of Guillain-Barré syndrome, a neurological disorder in which the individual's immune system destroys the myelin sheath surrounding axons of the peripheral nervous system (WHO "Zika Situation Report" February 5, 2016).

No specific treatments or vaccines for Zika virus currently exist, and the only measures at this time to prevent infection are through vector control and avoiding travel to regions experiencing outbreaks.

Described herein are Zika virus vaccines and compositions comprising inactivated Zika virus that provide a safe method for generating an immune response to Zika virus, including virus-neutralizing antibodies, that may help prevent against Zika virus infection.

Any strain of Zika virus may be used in the methods and compositions described herein. In some embodiments, the Zika virus is an isolate from an infected subject during a Zika virus outbreak. In some embodiments, the Zika virus is a strain isolated from Africa or from the African virus lineage. In some embodiments, the Zika virus is a strain isolated from Asia or from the Asian lineage (includes also strains from French Polynesia). In some embodiments, the Zika virus is a strain isolated from the Americas (South America, Central America, or North America), such as a Suriname Zika virus strain. In some embodiments, the Zika virus has an RNA genome corresponding (but not limited) to the

DNA sequence provided by GenBank Accession No. AY632535.2, KU321639.1, KU497555.1, KU501215.1, KU509998.1, KU527068.1, KU681081.3, KU681082.3, KU707826.1, KU744693.1, or LC002520.1 or RNA genome disclosed partially or fully herein (SEQ ID NO: 2 to 69).

In some embodiments, the process of the invention results in an enrichment of infectious Zika virus particles from the crude harvest comprising infectious Zika virus particles and non-infectious virus particles and other virus products such that the enrichment of the infectious virus particles is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, preferably at least 80%, especially at least 85% relative to the total virus particle content of the crude harvest (a) comprising the virus particles and impurities.

In some embodiments, the residual impurity of the final virus preparation with respect to all impurities in the crude harvest is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, preferably less than 5% as determined by SEC-HPLC (Size Exclusion Chromatography – HPLC).

A unique aspect of the current invention is the realization that know-how related to the vaccine design and purification approach used for the Japanese Encephalitis Vaccine (JEV) IXIARO® (see Srivastava A.K. et al., 2001, Vaccine 19, 4557-4565, WO99/11762) may be employed and improved upon in order to expedite the development of a Zika virus vaccine and provide it to the subjects in need as soon as possible. The industrial process as disclosed for IXIARO®, providing a very effective vaccine against JEV, was complemented by further significant improvements disclosed herein in order to provide a more efficient (higher yield) and safer (less or no protamine sulphate with its allergic potential) Zika vaccine compared to the available JEV vaccine. A particular innovation of the herein disclosed vaccines is their greatly reduced protamine salt (SEQ ID NO: 1) content in the final drug substance facilitated by the development of an improved sucrose gradient. Said sucrose gradient not only allowed the separation of protamine sulphate but also allowed for a very effective inactivation by formaldehyde and resulted in the case of Zika with over 90% yield with the improved process disclosed herein vs about 35% yield with the published JEV process, see experimental part for comparison).

Aspects of the disclosure relate to methods of producing a virus in Vero tissue culture cells. Vero cells are a commonly used tissue culture cell line derived from the kidney of an African green monkey. The Vero cells used in the methods described herein are the Vero (WHO) cell line, obtained from the Health Protection Agency general cell collection under catalogue number 88020401.

Vero cells can be grown to confluent monolayers, for example in tissue culture flasks; in suspension (on microcarriers), for example in roller bottles; or in any other cell culture system for viral production. In some embodiments, the Vero cells are grown in a bioreactor for viral production. For plaque assays or the plaque reduction neutralization test (PRNT), Vero cells are grown in monolayers in tissue culture flasks, dishes, or wells of a plate. To infect the Vero cells with the virus, the culture medium is inoculated with virus and the cells are incubated with the virus for a period of time. The cells may be washed after inoculation to remove any virus that did not adsorb to the cells in a given amount of time.

The methods provided herein involve passaging the virus in Vero cells. As used herein, the terms "passage" or "passaging" refer to infecting a population of Vero cells with virus and subsequently inoculating a second population of Vero cells with virus produced by infection of the first Vero cell population. In some embodiments, a portion of the culture medium from the infected Vero cells (containing virus that was released from the infected cells) is used to inoculate a second population of Vero cells. This is referred to as one passage or one round of passaging. The passaging may be performed serially, for example, a portion of the culture medium from the infected second population of Vero cells is used to inoculate a third population of Vero cells, and so on. In some embodiments, virus obtained from a single plaque is used to inoculate another population of cells.

In some embodiments, the virus is passaged in Vero cells several times, such as at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times. In some embodiments, the virus is passaged in Vero cells at least 4 times or 5 times. In some embodiments, the virus is passaged in Vero cells at least 30 times. It is important that the virus population, i.e. the virus sequences, stays as much as possible constant over said passaging. If adaption of the virus occurs (i.e. appearance of mutated viruses in the original virus population), it is preferred that said passages are not used in the context of manufacturing of said virus, e.g. for Zika it was found that up to passage 3 and culturing to day 7 can be used without major shifts in virus population, i.e. introduction of virus population with mutations. However this observation needs to be done for each virus strain and may be different.

In some embodiments, the Vero cells are incubated for at least 2 days after inoculation with the virus at e.g. a typical 0.01 MOI (multiplicity of infection) to allow for viral production prior to passaging. In some embodiments, the Vero cells are incubated for at least 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 days e.g. at least 7 days after inoculation with the virus prior to passaging. The number of days the Vero cells are incubated after viral inoculation may depend on factors such as the multiplicity of

infection used to inoculate the cells and the viral titer desired in the culture medium. Serial passaging of the virus in Vero cells may result in generation of a Vero cell adapted virus strain.

The culture medium from the infected Vero cells may be harvested (collected) to obtain the virus. In some embodiments, the culture medium is harvested from infected Vero cells and is replaced with fresh culture medium, which is then harvested after another period of time. In some embodiments, the culture medium harvested from infected Vero cells is pooled from independent Vero cell cultures and/or from independent days. Harvesting can be repeated up to 4 times by 7 or 9 days post infection, for example, and result in a high yield of virus per unit cell culture. In order to minimize the adaption of Zika virus strain to Vero cells, it was found that Vero cells could be incubated for at least 7 days, more preferably 5 days, prior to passaging and subsequently supernatants could be harvested at days 2, 3, 5 and 7 or 2, 3, and 5 (see also experimental part). The harvested culture medium can be stored at +4°C prior to purification of the virus from the culture medium up to 2 weeks.

In some embodiments, debris from infected and lysed Vero cells may be removed from the harvested culture medium, referred to as a "clarification" of the culture medium. The harvested culture medium may be clarified by common methods known in the art, such as low-speed centrifugation, for example, at 1500 *g* for 10 min, and/or by filtration through a filter of pore size of 0.45 µm. The harvested culture medium can be stored at +4°C prior to concentration.

To concentrate the titer of the Zika virus in the harvested culture medium, it may be subjected to concentration by any method known in the art. For example, the harvested culture medium may be concentrated by methods including, without limitation, ultrafiltration, ultracentrifugation, centrifugal concentrator, vacuum centrifugation, and lyophilization. In some embodiments, the harvested culture medium is concentrated by ultrafiltration and the retentate containing the Zika virus is collected. In some embodiments, the harvested culture medium is concentrated by precipitation in which polyethylene glycol (PEG) 8000 is dissolved in the culture medium (up to 10%) and the precipitate is dissolved in a buffer, for example phosphate-buffered saline (PBS, pH 7.0).

The harvested culture medium may be precipitated to produce a virus supernatant. In some embodiments, the harvested culture medium is precipitated to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. In some embodiments, the harvested culture medium is concentrated prior to precipitation. In some embodiments, the harvested culture medium is precipitated by adding protamine sulfate (e.g. SEQ ID NO: 1) to the harvested culture medium and incubating the mixture, for example at +4°C or on ice. In

some embodiments, the harvested culture medium is treated with benzonase to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. However, it was found that the treatment with protamine sulfate is preferred (see experimental part). In some embodiments, the precipitated culture medium is centrifuged to collect precipitated material and the supernatant containing the virus, referred to as a "virus supernatant", is collected.

The virus supernatant may be further purified after precipitation, for example density gradient ultracentrifugation. In some embodiments, the virus supernatant is further purified by sucrose gradient. Fractions may be collected from the sucrose gradients and assayed for presence of the virus. Methods for assaying for virus positive fractions include plaque assay, hemagglutination assay, polyacrylamide gel electrophoresis, and antigen assays such as Western blotting and ELISA. The fractions containing virus may be pooled based on titer of the virus and level of other impurities. The level or amount of impurities present in the virus supernatant can be estimated by testing for Vero cell DNA, virus aggregates and/or Vero cell protein (see experimental part). A particular embodiment of the invention is the improved sucrose gradient that allows for an efficient protamine separation as shown in the experimental part. It was surprisingly found that the addition of a virus-containing fraction with 10% (w/w) sucrose to a simple three layer sucrose density gradient (e.g. a gradient comprising a 15% (w/w) sucrose solution, a 35% (w/w) sucrose solution, and a 50% (w/w) sucrose solution) resulted in efficient separation of protamine sulphate without much loss of virus. Thus a particularly preferred embodiment of the invention is the use of a sucrose density gradient that is able to efficiently separate protamine sulphate, wherein said sucrose density gradient is used in the purification of virus such as the viruses described herein, i.e. a Zika virus.

To achieve a safe vaccine or composition for the administration to subjects, the virus supernatant may be inactivated (see experimental part for Zika virus). As used herein, the terms "inactivated" and "optimally inactivated" may be used interchangeably and refer to a process (or its result) by which the virus is rendered unable to infect a host cell (non-infectious), but that does not affect or substantially affect the antigenicity of the virus, for example, the immunogenic antigens exposed on the surface of the virus are able to stimulate an immune response in a subject (*e.g.*, antigen-specific antibodies). By "does not affect or substantially affect the antigenicity of the virus" is meant that the inactivated virus retains at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even essentially 100% of the antigenicity of a virus that is not subjected to inactivation.

A variety of methods are known in the art for inactivating viruses. In some embodiments, the virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

In some embodiments, the inactivating is by chemical inactivation and involves contacting the virus with one or more chemical inactivation agents for a period of time under conditions such that the virus is inactivated but the antigenic epitopes are substantially intact. In some embodiments, the virus is inactivated for a period of time that is longer than is required to completely inactivate the virus. In some embodiments, the virus supernatant is inactivated for the number of days required to inactivate the virus plus at least one additional day. Samples of the virus supernatant may be taken at one or more times throughout the inactivation process and assessed for viral viability (infectivity) by any method known in the art, such as by infecting a monolayer of host cells (*i.e.*, plaque assay). Using such a procedure, the period of time that is required to completely inactivate the virus can be determined, and a longer period of time is selected to ensure complete inactivation.

In some embodiments, the virus is contacted with a chemical inactivation agent for between 1 day and 50 days, between 2 days and 40 days, between 2 days and 30 days, between 2 days and 20 days, between 2 days and 10 days, between 3 days and 9 days, between 4 days and 8 days, between 5 days and 7 days, between 2 days and 5 days, or between 5 and 10 days. In some embodiments, the virus is contacted with one or more chemical inactivation agents for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days, 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, 42 days, 43 days, 44 days, 45 days, 46 days, 47 days, 48 days, 49 days, or at least 50 days.

In some embodiments, the chemical inactivation is performed at about +5°C, +10°C, +15°C, +20°C, +25°C, +30°C, +35°C, +40°C, or about +45°C. In some embodiments, the chemical inactivation is performed at about +4°C. In some embodiments, the chemical inactivation is performed at about +22°C.

Any chemical inactivation agent known in the art may be suitable for inactivating the virus in the methods described herein. It will be appreciated by one of skill in the art that factors such as the chemical inactivation agent and the temperature at which inactivation is performed may affect the length of time (number of days) required to completely inactivate the virus. Examples of chemical inactivation agents include, without limitation, formaldehyde, enzymes, β-propiolactone, ethanol,

trifluroacetic acid, acetonitrile, bleach, urea, guanidine hydrochloride, tri-n-butyl phosphate, ethylene-imine or a derivatives thereof, and organic solvents such as Tween, Triton, sodium deoxycholate, and sulphobetaine. A preferred inactivation is inactivation with formaldehyde at 22°C +/-2°C for about 10 days.

In some embodiments, the inactivating agent is neutralized after chemical inactivation of the virus. In some embodiments, the inactivating agent is formaldehyde and is neutralized after chemical inactivation using sodium thiosulphate or sodium metabisulfite.

In some embodiments, the virus is inactivated by thermal inactivation. In some embodiments, the thermal inactivation involves exposing the virus to heat, such as dry heat or vapor heat, for a period of time. In some embodiments, the thermal inactivation involves exposing the virus to temperatures of about +40°C, +45°C, +50°C, +55°C, +60°C, +65°C, +70°C, +75°C, +80°C, +85°C, +90°C,+ 95°C, or about +100°C. In some embodiments, the virus is exposed to heat for at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, about 96 hours, or longer. A preferred thermal inactivation involves exposing the virus to temperatures of about +56°C for 60 minutes.

In some embodiments, the virus is inactivated by exposing the virus to acidic or alkaline conditions for a period of time such that the virus is completely inactivated. The pH of a virus preparation may be adjusted to a desired pH, for example by the addition of an acid, a base, or a buffer with a particular pH to the virus preparation. In some embodiments, the virus is inactivated at an acidic pH of about 2, 2.5, 3, 3.5, 4, 4.5, 5 or about 5.5. In other embodiments, the virus is inactivated at an alkaline pH of about 8, 8.5, 9, 9.5, 10, or about 10.5.

In some embodiments, the virus is inactivated using UV inactivation. UV inactivation involves exposing the virus to energy-rich radiation, such as UV-A, UV-B, or UV-C light for a period of time. It will be appreciated that any two or more methods of inactivation may be combined and performed concurrently or serially.

The inactivated virus may be subsequently dialyzed to remove any undesired material, including the inactivating agent and any neutralizing agent, and/or to replace the buffer with a buffer that is pharmaceutically acceptable for administration to subjects. In some embodiments, the inactivated

virus is dialyzed with PBS. In addition or alternatively, the inactivated virus may be filtered, such as sterile filtered, through a 0.22 μm filter.

Any of the methods or uses described herein may be for the prevention of a Zika virus infection in a subject. As used herein, the terms "prevent," "preventing" and "protection from" include the administration of a virus vaccine or composition to a subject to reduce, or delay the onset of the manifestation of clinical or subclinical symptoms, complications, pathologies or biochemical indicia of a disease or infection, or to reduce or inhibit the spread/transmission of the Zika virus. As used herein, antigen(s), such as an inactivated Zika virus, that is administered to a subject prophylactically (e.g., prior to infection) may be referred to as a vaccine.

Zika Vaccine

As described herein Zika virus may cause any of a variety of symptoms upon infection of a subject, and is generally characterized by mild fever; rash (exanthema) on face, neck, trunk, upper arms; headache; sensitivity to light; non-inflammatory joint pain; conjunctivitis; lack of appetite; diarrhea; abdominal pain; and/or dizziness. Zika virus infection during pregnancy is associated with microcephaly in the fetus/neonate. There is also a probable association between the onset of Guillain-Barré syndrome or symptoms thereof. Diagnosis of Zika virus infection in subjects exposed to Zika virus or suspected of being exposed to Zika virus involves detecting the presence of virus-specific antibodies and/or molecular testing, such as PCR or real-time PCR detection of Zika virus.

Provided herein are methods for administering a dose of a therapeutically effective amount of a Zika virus vaccine to a subject in need thereof. In some embodiments, the subject is a mammalian subject, such as a human, non-human primate, rodent, rabbit, sheep, dog, cat, horse, or cow. In some embodiments, the subject is a mouse. In some embodiments, the subject is a human subject, such as a child, an adult, or an elderly adult. In some embodiments, the subject is a female subject. In some embodiments, the subject is pregnant or planning on becoming pregnant. In some embodiments, the subject is at risk of being exposed to Zika virus. In some embodiments, the subject is living in or traveling to an area where Zika virus is present or is thought to be present. In some embodiments, the subject has been previously infected with or vaccinated against Dengue virus; i.e., at risk for antibody-dependent enhancement of disease. In some embodiments, the subject is living in or traveling to an area that is experiencing a Zika virus infection outbreak. In some embodiments, the subject is living in or traveling to an area where an arthropod vector capable of transmitting the Zika virus vector is present or is thought to be present.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject in a therapeutically effective amount or a dose of a therapeutically effective amount. As used herein, a "therapeutically effective amount" of vaccine is any amount that results in a desired response or outcome in a subject, such as those described herein, including but not limited to prevention of infection, an immune response or an enhanced immune response to Zika virus, or prevention or reduction of symptoms associated with Zika disease.

In some embodiments, the therapeutically effective amount of a Zika virus vaccine or composition described herein is an amount sufficient to generate antigen-specific antibodies (*e.g.*, anti-Zika virus antibodies). In some embodiments, the therapeutically effective amount is sufficient to provide seroprotection in a subject; i.e., to generate sufficient antigen-specific antibodies to prevent/protect from infection. In some embodiments, seroprotection is conferred on at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or at least 99% of vaccinated subjects. In some embodiments, seroprotection is defined by a reduction in the number of Zika virus plaques by 50% or more in a plaque reduction neutralization test (PRNT) by a 1:10 or higher dilution of sera from a vaccinated subject. In some embodiments, an effective amount of the Zika vaccine is sufficient to seroconvert a subject with at least 70% probability. In some embodiments, the therapeutically effective amount is sufficient to seroconvert a subject with at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98%, or at least 99% probability. Whether a subject has been seroconverted can be assessed by any method known in the art, such as obtaining a serum sample from the subject and performing an assay to detect anti-Zika virus antibodies. In some embodiments, a subject is seroconverted if a serum sample from the subject contains an amount of anti-Zika virus antibodies that surpasses a threshold or predetermined baseline. A subject is generally considered seroconverted if there is at least a 4-fold increase in anti-Zika virus antibodies (*i.e.,* anti-Zika E protein IgG antibodies) in a serum sample from the subject as compared to a serum sample previously taken from the same subject.

In some embodiments, seroconversion of a subject is assessed by performing a plaque reduction neutralization test (PRNT). Briefly, PRNT is used to determine the serum titer required to reduce the number of Zika virus plaques by 50% (PRNT50) as compared to a control serum/antibody. The PRNT50 may be carried out using monolayers of Vero cells or any other cell type/line that can be infected with Zika virus. Sera from subjects are diluted and incubated with live, non-inactivated Zika virus. The serum/virus mixture may be applied to the Vero cells and incubated for a period of time. Plaques formed on the Vero cell monolayers are counted and compared to the number of plaques formed by the Zika virus in the absence of serum or a control antibody. A threshold of neutralizing

antibodies of 1:10 dilution of serum in a PRNT50 is generally accepted as evidence of protection (Hombach *et. al.* Vaccine (2005) 23:5205-5211).

In some embodiments, the Zika virus may be formulated for administration in a composition, such as a pharmaceutical composition. The term "pharmaceutical composition" as used herein means a product that results from the mixing or combining of at least one active ingredient, such as an inactivated Zika virus, and one or more inactive ingredients, which may include one or more pharmaceutically acceptable excipient.

Pharmaceutical compositions of the invention, including vaccines, can be prepared in accordance with methods well known and routinely practiced in the art (see *e.g.*, Remington: The Science and Practice of Pharmacy, Mack Publishing Co. 20th ed. 2000; and Ingredients of Vaccines – Fact Sheet from the Centers for Disease Control and Prevention, e.g., adjuvants and enhancers such as alum to help the vaccine improve its work, preservatives and stabilizers to help the vaccine remain unchanged (e.g., albumin, phenols, glycine)). Pharmaceutical compositions are preferably manufactured under GMP conditions. Typically a therapeutically effective dose of the inactivated Zika virus preparation is employed in the pharmaceutical composition of the invention. The inactivated Zika virus is formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, the prophylactic response).

Dosages of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired pharmaceutical response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors.

A physician, veterinarian or other trained practitioner, can start doses of the inactivated Zika virus vaccine employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect (*e.g.*, production of anti-Zika virus antibodies) is achieved. In general, effective doses of the compositions of the present

invention, for the prophylactic treatment of groups of people as described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and the titer of anti-Zika virus antibodies desired. Dosages need to be titrated to optimize safety and efficacy. In some embodiments, the dosing regimen entails subcutaneous or intramuscular administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 7. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 14. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 28. In some embodiments, the inactivated Zika virus is administered to the subject once.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject with, prior to, or after administration of one or more adjuvants. An adjuvant is a molecule that enhances a response in a subject, such as an immune response, to an antigen or other molecule. In some embodiments, an adjuvant may stabilize an antigen or other molecule. Determining whether a Zika virus vaccine or compositions thereof are administered with an adjuvant depends on various factors (e.g., type and extent of response desired) and will be evident to one of skill in the art. In some embodiments, administering any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may enhance the production of virus neutralizing (anti-Zika virus) antibodies. In some embodiments, a subject that is administered any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may only require a single administration of the Zika virus vaccine or composition to be seroconverted (produce a level of anti-Zika virus antibodies). Examples of adjuvants may include, without limitation, aluminium salt (aluminium hydroxide or aluminium phosphate), calcium phosphate hydroxide, paraffin oil, killed bacteria, bacterial toxins, toxoids, subunits of bacteria, squalene, thimerosal, detergents, IL-1, IL-2, IL-12, 2-component adjuvants, such as 2-component adjuvants containing an antibacterial peptide and a TLR9 agonist (*e.g.*, IC31®), and combinations such as Freund's complete adjuvant and Freund's incomplete adjuvant. In some embodiments, the Zika virus vaccines or compositions is administered with aluminium hydroxide. In some embodiments, the inactivated Zika virus vaccine or composition is administered with aluminium phosphate salt. A preferred aluminium salt is the aluminium hydroxide with reduced Cu content, e.g. lower than 1,25ppb based on the weight of the Zika composition, an adjuvant described in detail in WO 2013/083726 or Schlegl et al., Vaccine 33 (2015) 5989-5996.

In some embodiments, the adjuvant is comprised of two components. In some embodiments, the 2-component adjuvant comprises an antibacterial peptide and a TLR9 agonist. In some embodiments, the antibacterial peptide is provided by the amino acid sequence $KLKL_5KLK$ (SEQ ID NO: 71). In some embodiments, the TLR9 agonist is a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN). In some embodiments, the I-ODN comprises the nucleic acid sequence $(dIdC)_{13}$ (SEQ ID NO: 70). In some embodiments, the adjuvant is IC31®. In some embodiments, the adjuvant is in nanoparticle form (See, *e.g.*, US Patent No. 8,765,148 B2, incorporated by reference in its entirety). In some embodiments, the adjuvant is IC31®, i.e. $KLKL_5KLK$ (SEQ ID NO: 71) and the nucleic acid sequence $(dIdC)_{13}$ (SEQ ID NO: 70), in combination with an aluminium salt such as aluminium hydroxide.

The Zika virus vaccines or compositions described herein may be administered to a subject concomitantly with one or more vaccines to another infectious agent, such as another infectious agent is that present or thought to be present in the same geographic area as Zika virus. In some embodiments, the other infectious agent is one that the subject is also at risk of being in contact with. In some embodiments, the other infectious agent is transmitted by the same arthropod vector as Zika virus. In some embodiments, the other infectious agent is Japanese Encephalitis virus, Yellow Fever virus, Dengue virus and/or Chikungunya virus.

Also within the scope of the present disclosure are kits for use in prophylactically administering to a subject, for example to prevent or reduce the severity of Zika virus infection. Such kits can include one or more containers comprising a composition containing inactivated Zika virus, such as an inactivated Zika virus vaccine. In some embodiments, the kit may further include one or more additional containing comprising a second composition, such as a second vaccine. In some embodiments, the second vaccine is a vaccine for another arbovirus. In some embodiments, the second vaccine is a Dengue virus vaccine and/or a Chikungunya virus vaccine.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the composition containing inactivated Zika virus to prevent, delay the onset, or reduce the severity of Zika virus infection. The kit may further comprise a description of selecting a subject suitable for administration based on identifying whether that subject is at risk for exposure to Zika virus or contracting a Zika virus infection. In still other embodiments, the instructions comprise a description of administering a composition containing inactivated Zika virus to a subject at risk of exposure to Zika virus or contracting Zika virus infection.

The instructions relating to the use of the composition containing inactivated Zika virus generally include information as to the dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine readable instructions are also acceptable.

The kits of the present disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as a syringe or an infusion device. The container may have a sterile access port, for example the container may be a vial having a stopper pierceable by a hypodermic injection needle. At least one active agent in the composition is an inactivated Zika virus, as described herein.

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including", "comprising", or "having,", "containing", "involving", and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, virology, cell or tissue culture, genetics and protein and nucleic chemistry described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated.

The present invention is further illustrated by the following examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove. However, the citation of any reference is not intended to be an admission that the reference is prior art.

Table 1. Overview of process buffers and stock solutions.

Buffer	Composition	Final pH	Final conductivity [mS/cm]
A	0.5 M NaOH		n.a.
B	0.1 M NaOH		n.a.
C	25 mM Tris, 150 mM NaCl	7.4±0.2	16.5
D	1 M Tris	7.4±0.2	n.a.
E	4.5 M NaCl	n.a.	n.a.
F	1 M NaCl	n.a.	n.a.
G	1 % SDS	n.a.	n.a.
H	50 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
I	35 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
J	15 % (w/w) Sucrose in 25 mM Tris, 150 mM NaCl	7.4±0.2	n.a.
K	10 x PBS	7.4±0.2	n.a.
L	50 mg/mL Protamine sulphate	7.4±0.2	n.a.
M	Drug substance formulation buffer (10mM Tris(hydroxymethyl)-aminomethan, 5 % Sucrose, 1% (10 mg/mL) rHSA)	7.4±0.2	1.3

Table 2. Abbreviations.

°Bx	Degrees Brix = sugar content (w/w) of an aqueous solution*
BSA	Bovine serum albumin
CC700	Capto™ Core 700
CPE	Cytopathic effect
EtOH	Ethanol
EU	Endotoxin units
DS	Drug Substance
DP	Drug Product
DSP	Downstream Process
HCP	Host cell protein
hcDNA	Host cell DNA
hpi	Hours post infection
HPLC	High Performance Liquid Chromatography
ID	Inner diameter
JEV	Japanese Encephalitis virus
LAL	Limulus amebocyte lysate
LDS buffer	Lithium dodecyl sulfate sample loading buffer
LOD	Limit of detection
LOQ	Limit of quantitation
MALLS	Multiangle light scattering
mAU	Milli absorbance units
MS	Mass spectroscopy

NIV	Neutralized inactivated virus
PBS	Phosphate buffered saline
PD	Process development
PFU	Plaque forming units
p.i.	Post-infection
PS	Protamine sulphate or protamine sulfate
rcf	Relative centrifugal force
rHSA	Recombinant human serum albumin
Rms radius	Root mean square radius
rMSB	Research master seed bank
RSD	Relative standard deviation
SEC	Size exclusion chromatography
SGC	Sucrose gradient centrifugation
SGP	Sucrose gradient purified
SDS	Sodium dodecyl sulphate
TBS	Tris buffered saline
TFF	Tangential flow filtration
TCID50	Tissue culture infectious dose 50%
UF/DF	Ultrafiltration/diafiltration
WFI	Water for injection
ZikaV	Zika virus

*Degrees Brix (°Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. °Bx corresponds to the sucrose content in percent (w/w), e.g., 45 °Bx equals 45 % (w/w) sucrose.

Table A. Primers for Zika virus sequencing: lower case letters indicate bases not included in ZIKA but containing restriction sites for later cloning when needed (therefore, two Tms provided).

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
1	9320_Zika_PF_1F	SEQ ID NO: 74 ttaggatccGTTGTTGATCTGTGTGAAT	69.9	74.6	707
	9321_Zika_PF_1R	SEQ ID NO: 75 taactcgagCGTACACAACCCAAGTT	69.3	75.6	
2	9322_Zika_PF_2F	SEQ ID NO: 76 ttaggatccTCACTAGACGTGGGAGTG	70	73.9	704
	9323_Zika_PF_2R	SEQ ID NO: 77 taactcgagAAGCCATGTCYGATATTGAT	69.8	73.7	
3	9324_Zika_PF_3F	SEQ ID NO: 78 ttaggatccGCATACAGCATCAGGTG	72.3	74.5	712
	9325_Zika_PF_3R	SEQ ID NO: 79 taactcgagTGTGGAGTTCCGGTGTCT	72	76.4	
4	9326_Zika_PF_4F	SEQ ID NO: 80 ttaggatccGAATAGAGCGAARGTTGAGATA	70.9	74	712
	9327_Zika_PF_4R	SEQ ID NO: 81 taactcgAGTGGTGGGTGATCTTCTTCT	70.5	73.7	
5	9328_Zika_PF_5F	SEQ ID NO: 82 ttaggatcCAGTCACAGTGGAGGTACAGTAC	70.3	75	704
	9329_Zika_PF_5R	SEQ ID NO: 83 taactcgagCRCAGATACCATCTTCCC	71.5	77.3	
6	9330_Zika_PF_6F	SEQ ID NO: 84 ttaggatCCCTTATGTGCTTGGCCTTAG	70.7	72.7	698
	9331_Zika_PF_6R	SEQ ID NO: 85 taactcgagTCTTCAGCCTCCATGTG	70.4	76.9	

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
7	9332_Zika_PF_7F	SEQ ID NO: 86 ttaggatccAATGCCCACTCAAACATAGA	71.9	75	716
	9333_Zika_PF_7R	SEQ ID NO: 87 taactcgagTCATTCTCTTCTTCAGCCCTT	71	74	
8	9334_Zika_PF_8F	SEQ ID NO: 88 ttaggatccAAGGGTGATCGAGGAAT	70.9	75.2	703
	9335_Zika_PF_8R	SEQ ID NO: 89 taactcgagTTCCCTTCAGAGAGAGGAGC	71.9	73.4	
9	9336_Zika_PF_9F	SEQ ID NO: 90 ttaggatccTCTTTTGCAAACTGCGATC	71.9	75	699
	9337_Zika_PF_9R	SEQ ID NO: 91 taactcgagTCCAGCTGCAAAGGGTAT	71	74.9	
10	9338_Zika_PF_10F	SEQ ID NO: 92 ttaggatccGTGTGGACATGTACATTGA	71.4	75.8	706
	9339_Zika_PF_10R	SEQ ID NO: 93 taactcgagCCCATTGCCATAAAGTC	70.4	75.8	
11	9340_Zika_PF_11F	SEQ ID NO: 94 ttaggatccTCATACTGTGGTCCATGGA	71.6	78.1	692
	9341_Zika_PF_11R	SEQ ID NO: 95 taactcgagGCCCATCTCAACCCTTG	74	78	
12	9342_Zika_PF_12F	SEQ ID NO: 96 ttaggatccTAGAGGGCTTCCAGTGC	70.9	74	707
	9343_Zika_PF_12R	SEQ ID NO: 97 taactcgAGATACTCATCTCCAGGTTTGTTG	70.2	72.2	
13	9344_Zika_PF_13F	SEQ ID NO: 98 ttaggatccGAAAACAAAACATCAAGAGTG	70.6	75.4	726
	9345_Zika_PF_13R	SEQ ID NO: 99 taactcgagGAATCTCTCTGTCATGTGTCCT	71.9	75.6	
14	9346_Zika_PF_14F	SEQ ID NO: 100 ttaggatccTTGATGGCACGACCAAC	73.1	75.6	715
	9347_Zika_PF_14R	SEQ ID NO: 101 ttaggatccGTTGTTGATCTGTGTGAAT	70.8	77.9	
15	9348_Zika_PF_15F	SEQ ID NO: 102 taactcgagCAGGTCAATGTCCATTG	71.9	75.4	719
	9349_Zika_PF_15R	SEQ ID NO: 103 ttaggatccTGTTGTGTTCCTATTGCTGGT	73.9	77.2	
16	9350_Zika_PF_16F	SEQ ID NO: 104 taactcgaGTGATCAGRGCCCCAGC	72.3	75.4	703
	9351_Zika_PF_16R	SEQ ID NO: 105 ttaggatccTGCTGCCCAGAAGAGAA	72	76.3	
17	9352_Zika_PF_17F	SEQ ID NO: 106 taactcgaGCACCAACAYGGGTTCTT	73.6	76	705
	9353_Zika_PF_17R	SEQ ID NO: 107 ttaggatcCTCAAGGACGGTGTGGC	72	75.5	
18	9354_Zika_PF_18F	SEQ ID NO: 108 taactcgagCAATGATCTTCATGTTGGG	71.7	75.8	699
	9355_Zika_PF_18R	SEQ ID NO: 109 ttaggatccTATGGGGGAGGACTGGT	71	74.1	
19	9356_Zika_PF_19F	SEQ ID NO: 110 taactcGAGCCCAGAACCTTGGATC	73.3	75.5	711
	9357_Zika_PF_19R	SEQ ID NO: 111 ttaggatcCAGACCCCCAAGAAGGC	71.3	76.9	
20	9358_Zika_PF_20F	SEQ ID NO: 112 taactcgagCCCCTTTGGTCTTGTCT	71.7	75	706
	9359_Zika_PF_20R	SEQ ID NO: 113 ttaggatccAGGAAGGATGTATGCAGATG	71.9	73.9	
21	9360_Zika_PF_21F	SEQ ID NO: 114 taactcgagACATTTGCGCATATGATTTTG	70.4	75.7	709
	9361_Zika_PF_21R	SEQ ID NO: 115 ttaggatccAGGAAGGACACACAAGAGT	71.8	75	
22	9362_Zika_PF_22F	SEQ ID NO: 116 taactcgagACAGGCTGCACAGCTTT	70	79.1	581

Primer Pair	Oligoname	Primer sequence (5'-3') restriction sites (lower case)	Tm (gene-specific)	Tm (entire primer)	Amplicon size [bp]
	9363_Zika_PF_22R	SEQ ID NO: 117 ttaggatccTCTCTCATAGGGCACAGAC	74.8	81.1	

SEQUENCES

SEQ ID NO: 1
A typical form of protamine
PRRRRSSSRP VRRRRRPRVS RRRRRRGGRR RR

Provided below are examples of nucleic acid sequences of the genomes of Zika viruses that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 2
KU321639.1 Zika virus strain ZikaSPH2015, Brazil, complete genome

GTTGTTACTGTTGCTGACTCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTGG
ATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACG
CGGAGTAGCCCGTGTGAGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGTCATGGGCCCATCAGG
ATGGTCTTGGCAATTCTAGCCTTTTTGAGATTCACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTG
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SEQ ID NO: 3

KU497555.1 Zika virus isolate Brazil-ZKV2015, Brazil, complete genome

CCAATCTGTGAATCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTTGGATTTG
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SEQ ID NO: 5
KU509998.1 Zika virus strain Haiti/1225/2014, Haiti, complete genome

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SEQ ID NO: 6
KU527068.1 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, complete genome
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SEQ ID NO: 7

KU681081.3 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, complete genome

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SEQ ID NO: 9
KU707826.1 Zika virus isolate SSABR1, Brazil, complete genome
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SEQ ID NO: 11
LC002520.1 Zika virus genomic RNA, strain: MR766-NIID, Uganda, complete genome
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SEQ ID NO: 12
AY632535.2 NC_012532.1 Zika virus strain MR 766, Uganda, complete genome
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CCACCACGCTGGCCGCCAGGCACAGATCGCCGAACTTCGGCGGCCGGTGTGGGGAAATCCATGGTTTCT

SEQ ID NO: 13
KJ776791.1, Zika virus strain H/PF/2013 polyprotein gene, complete cds
AGTATCAACAGGTTTTATTTTGGATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTC
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CAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAG

In some embodiments, the Zika virus has a RNA genome corresponding to the DNA sequence provided by the nucleic acid sequence of any one of SEQ ID NOs: 2-13 or 72. In some embodiments, the Zika virus has a variant genome that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%. 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-13 or 72.

Provided below are amino acid sequences of the E-proteins of Zika strains that may be used in the methods, compositions, and/or vaccines described herein.

SEQ ID NO: 14
isol-ARB15076.AHF49784.1.Central_African_Republic/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG

TDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 15
isol-IbH_30656.AEN75265.1.Nigeria/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHSGADTETPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
RDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSIIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 16
ArB1362.AHL43500.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXXXXXXXNR
AEVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 17
ArD128000.AHL43502.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMXXXXXGHETDEN
RAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHRLVRKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWLKKGSSIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 18
ArD158095.AHL43505.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 19
ArD158084.AHL43504.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 20

isol-ARB13565.AHF49783.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 21
isol-ARB7701.AHF49785.1.Central_African_Republic/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGVHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 22
isol-ArD_41519.AEN75266.1.Senegal/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVE
FKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVT
VEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKA
FEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 23
MR766-NIID.BAP47441.1.Uganda/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 24
LC002520.1/326-829 Zika virus genomic RNA, strain: MR766-NIID, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMTVNDIGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 25
isol-MR_766.AEN75263.1.Uganda/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGYETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV

EVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 26
ArD7117.AHL43501.1.-/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAVCTAAKVPAETLHGTVT
VEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKA
FEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGG
VMIFLSTAVSA

SEQ ID NO: 27
AY632535.2/326-825 NC_012532.1 Zika virus strain MR 766, Uganda, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 28
MR_766.AAV34151.1.Uganda/291-790 Flavivirus envelope glycoprotein E. |Q32ZE1|Q32ZE1_9FL
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 29
MR_766.YP_009227198.1.Uganda/1-500 envelope protein E [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIGYETDEDRAKVE
VTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAH
AKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQY
AGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATV
RGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFL
STAVSA

SEQ ID NO: 30
KU681081.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHTGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVLNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 31
isol-Zika_virus%H.sapiens-tc%THA%2014%SV0127-_14.AMD61710.1.Thailand/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHTGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITEGTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGVLNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 32
CK-ISL_2014.AIC06934.1.Cook_Islands/1-504 Flavivirus envelope glycoprotein E. (Fragment) OS=Zika virus GN=E PE=4 SV=1
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 33
Natal_RGN.AMB18850.1.Brazil:_Rio_Grande_do_Norte,_Natal/291-794 Flavivirus envelope glycoprotein E.]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 34
isol-Si323.AMC37200.1.Colombia/1-504 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 35
KU707826.1/317-820 Zika virus isolate SSABR1, Brazil, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 36
KU509998.1/326-829 Zika virus strain Haiti/1225/2014, Haiti, Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 37
isol-GDZ16001.AML82110.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 38
BeH819015.AMA12085.1.Brazil/291-794 Flavivirus envelope glycoprotein E.]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 39
MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 40
KU501215.1/308-811 Zika virus strain PRVABC59, Puerto Rico, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 41
Haiti%1225%2014.AMB37295.1.Haiti/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF

EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 42
KU527068.1/308-811 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 43
isol-Z1106027.ALX35662.1.Suriname/5-508 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 44
isol-FLR.AMM39804.1.Colombia:_Barranquilla/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 45
PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.Canada/254-757 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 46
BeH818995.AMA12084.1.Brazil/291-794 Flavivirus envelope glycoprotein E. [Zika virus].
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 47

H/PF/2013.AHZ13508.1.French_Polynesia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 48
PRVABC59.AMC13911.1.Puerto_Rico/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 49
KU321639.1/326-829 Zika virus strain ZikaSPH2015, Brazil, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 50
ZikaSPH2015.ALU33341.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 51
103344.AMC13912.1.Guatemala/291-794 polyprotein [Zika virus]. 103344.AMC13912.1.Guatemala Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEIRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 52
isol-Brazil-ZKV2015.AMD16557.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF

KDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 53
KU497555.1/308-811 Zika virus isolate Brazil-ZKV2015, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 54
isol-ZJ03.AMM39806.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGARRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 55
isol-FSS13025.AFD30972.1.Cambodia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 56
isol-Z1106032.ALX35660.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 57
isol-Z1106033.ALX35659.1.Suriname/291-794 Flavivirus envelope glycoprotein E. [Zika virus]
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 58
isol-BeH828305.AMK49165.1.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDTQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFE
ATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGV
LIFLSTAVSA

SEQ ID NO: 59
isol-GD01.AMK79468.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNGTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 60
isol-Z1106031.ALX35661.1.Suriname/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVLAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSA

SEQ ID NO: 61
ACD75819.1.Micronesia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 62
KU681082.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 63
isol-Zika_virus%H.sapiens-tc%PHL%2012%CPC-0740.AMD61711.1.Philippines/291-794 Flavivirus envelope glycoprotein E.

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 64
isol-BeH823339.AMK49164.2.Brazil/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVSTTVSNMAEVRSYCYEATISDIASDSRCPTQGEAYLD
KQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENR
AKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFK
DAHAKRQTAVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVE
VQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFE
ATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGV
LIFLSTAVSA

SEQ ID NO: 65
isol-P6-740.AEN75264.1.Malaysia/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDXGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTV
EVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWXRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
LIFLSTAVSA

SEQ ID NO: 66
KU744693.1/326-829 Zika virus isolate VE_Ganxian, China, Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMLVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETVDGTVTV
EGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSG

SEQ ID NO: 67
isol-VE_Ganxian.AMK79469.1.China/291-794 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTAMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMLVNDTGHETDEN
RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLAHKEWFHDIPLPWHAGAATGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETVDGTVTV
EGQYGGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAF
EATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIIGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGG
VLIFLSTAVSG

SEQ ID NO: 68
ArD157995.AHL43503.1.-/291-794 Flavivirus envelope glycoprotein E.
ISCIGVSNRDLVEGMSGGTWVDVVLEHGGCVTEMAQDKPTVDIELVTMTVSNMAEVRSYCYEASLSDMASASRCPTQGEPSL
DKQSDTQSVCKRTLGDRGWGNGCGIFGKGSLVTCSKFTCCKKMPGKSIQPENLEYRIMLPVHGSQHSGMIVNDIGHETDENR
AKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEF
KDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTV
EVQSAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAF

EATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGV
MIFLSTAVSA

SEQ ID NO: 69
MR_766.ABI54475.1.Uganda/291-788 Flavivirus envelope glycoprotein E.
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYL
DKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDENRAKVEVT
PNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAK
RQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAG
TDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG
AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTA
VSA

SEQ ID NO: 70
5'-(dIdC)$_{13}$-3'
dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC dIdC

SEQ ID NO: 71
KLK peptide
KLKLLLLLKLK

SEQ ID NO: 72
ZIKV Sequence H/PF/2013 as sequenced
CAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTTATTTTGGATTTGGAAACGAGAGTT
TCTGGTCATGAAAAACCCAAAAAAGAAATCCGGAGGATTCCGGATTGTCAATATGCTAAAACGCGGAGTAGCCCGTGTG
AGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGTCATGGGCCCATCAGGATGGTCTTGGCGATTCT
AGCCTTTTTGAGATTCACGGCAATCAAGCCATCACTGGGTCTCATCAATAGATGGGGTTCAGTGGGGAAAAAAGAGGCTA
TGGAAATAATAAAGAAGTTCAAGAAAGATCTGGCTGCCATGCTGAGAATAATCAATGCTAGGAAGGAGAAGAAGAGAC
GAGGCGCAGATACTAGTGTCGGAATTGTTGGCCTCCTGCTGACCACAGCTATGGCAGCGGAGGTCACTAGACGTGGGAG
TGCATACTATATGTACTTGGACAGAAACGACGCTGGGGAGGCCATATCTTTTCCAACCACATTGGGGATGAATAAGTGTT
ATATACAGATCATGGATCTTGGACACATGTGTGATGCCACCATGAGCTATGAATGCCCTATGCTGGATGAGGGGGTGGAA
CCAGATGACGTCGATTGTTGGTGCAACACGACGTCAACTTGGGTTGTGTACGGAACCTGCCATCACAAAAAAGGTGAAGC
ACGGAGATCTAGAAGAGCTGTGACGCTCCCCTCCCATTCCACTAGGAAGCTGCAAACGCGGTCGCAAACCTGGTTGGAAT
CAAGAGAATACACAAAGCACTTGATTAGAGTCGAAAATTGGATATTCAGGAACCCTGGCTTCGCGTTAGCAGCAGCTGCC
ATCGCTTGGCTTTTGGGAAGCTCAACGAGCCAAAAAGTCATATACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGC
ATCAGGTGCATAGGAGTCAGCAATAGGGACTTTGTGGAAGGTATGTCAGGTGGGACTTGGGTTGATGTTGTCTTGGAAC
ATGGAGGTTGTGTCACCGTAATGGCACAGGACAAACCGACTGTCGACATAGAGCTGGTTACAACAACAGTCAGCAACAT
GGCGGAGGTAAGATCCTACTGCTATGAGGCATCAATATCGGACATGGCTTCGGACAGCCGCTGCCCAACACAAGGTGAA
GCCTACCTTGACAAGCAATCAGACACTCAATATGTCTGCAAAAGAACGTTAGTGGACAGAGGCTGGGGAAATGGATGTG
GACTTTTTGGCAAAGGGAGCCTGGTGACATGCGCTAAGTTTGCATGCTCCAAGAAAATGACCGGGAAGAGCATCCAGCC
AGAGAATCTGGAGTACCGGATAATGCTGTCAGTTCATGGCTCCCAGCACAGTGGGATGATCGTTAATGACACAGGACATG
AAACTGATGAGAATAGAGCGAAGGTTGAGATAACGCCCAATTCACCAAGAGCCGAAGCCACCCTGGGGGGTTTTGGAAG
CCTAGGACTTGATTGTGAACCGAGGACAGGCCTTGACTTTTCAGATTTGTATTACTTGACTATGAATAACAAGCACTGGTT
GGTTCACAAGGAGTGGTTCCACGACATTCCATTACCTTGGCACGCTGGGGCAGACACCGGAACTCCACACTGGAACAACA
AAGAAGCACTGGTAGAGTTCAAGGACGCACATGCCAAAAGGCAAACTGTCGTGGTTCTAGGGAGTCAAGAAGGAGCAG
TTCACACGGCCCTTGCTGGAGCTCTGGAGGCTGAGATGGATGGTGCAAAGGGAAGGCTGTCCTCTGGCCACTTGAAATG
TCGCCTGAAAATGGATAAACTTAGATTGAAGGGCGTGTCATACTCCTTGTGTACCGCAGCGTTCACATTCACCAAGATCCC
GGCTGAAACACTGCACGGGACAGTCACAGTGGAGGTACAGTACGCAGGGACAGATGGACCTTGCAAGGTTCCAGCTCAG
ATGGCGGTGGACATGCAAACTCTGACCCCAGTTGGGAGGTTGATAACCGCTAACCCCGTAATCACTGAAAGCACTGAGAA
CTCTAAGATGATGCTGGAACTTGATCCACCATTTGGGGACTCTTACATTGTCATAGGAGTCGGGGAGAAGAAGATCACCC
ACCACTGGCACAGGAGTGGCAGCACCATTGGAAAAGCATTTGAAGCCACTGTGAGAGGTGCCAAGAGAATGGCAGTCTT
GGGAGACACAGCCTGGGACTTTGGATCAGTTGGAGGCGCTCTCAACTCATTGGGCAAGGGCATCCATCAAATTTTTGGAG
CAGCTTTCAAATCATTGTTTGGAGGAATGTCCTGGTTCTCACAAATTCTCATTGGAACGTTGCTGATGTGGTTGGGTCTGA
ACACAAAGAATGGATCTATTTCCCTTATGTGCTTGGCCTTAGGGGGAGTGTTGATCTTCTTATCCACAGCTGTCTCTGCTGA
TGTGGGGTGCTCGGTGGACTTCTCAAAGAAGGAGACGAGATGCGGTACAGGGGTGTTCGTCTATAACGACGTTGAAGCC

TGGAGGGACAGGTACAAGTACCATCCTGACTCCCCCCGTAGATTGGCAGCAGCAGTCAAGCAAGCCTGGGAAGATGGTA
TCTGTGGGATCTCCTCTGTTTCAAGAATGGAAAACATCATGTGGAGATCAGTAGAAGGGGAGCTCAACGCAATCCTGGAA
GAGAATGGAGTTCAACTGACGGTCGTTGTGGGATCTGTAAAAAACCCCATGTGGAGAGGTCCACAGAGATTGCCCGTGC
CTGTGAACGAGCTGCCCCACGGCTGGAAGGCTTGGGGGAAATCGTACTTCGTCAGAGCAGCAAAGACAAATAACAGCTT
TGTCGTGGATGGTGACACACTGAAGGAATGCCCACTCAAACATAGAGCATGGAACAGCTTTCTTGTGGAGGATCATGGGT
TCGGGGTATTTCACACTAGTGTCTGGCTCAAGGTTAGAGAAGATTATTCATTAGAGTGTGATCCAGCCGTTATTGGAACAG
CTGTTAAGGGAAAGGAGGCTGTACACAGTGATCTAGGCTACTGGATTGAGAGTGAGAAGAATGACACATGGAGGCTGA
AGAGGGCCCATCTGATCGAGATGAAAACATGTGAATGGCCAAAGTCCCACACATTGTGGACAGATGGAATAGAAGAGAG
TGATCTGATCATACCCAAGTCTTTAGCTGGGCCACTCAGCCATCACAATACCAGAGAGGGCTACAGGACCCAAATGAAAG
GGCCATGGCACAGTGAAGAGCTTGAAATTCGGTTTGAGGAATGCCCAGGCACTAAGGTCCACGTGGAGGAAACATGTGG
AACAAGAGGACCATCTCTGAGATCAACCACTGCAAGCGGAAGGGTGATCGAGGAATGGTGCTGCAGGGAGTGCACAAT
GCCCCCACTGTCGTTCCGGGCTAAAGATGGCTGTTGGTATGGAATGGAGATAAGGCCCAGGAAAGAACCAGAAAGTAAC
TTAGTAAGGTCAATGGTGACTGCAGGATCAACTGATCACATGGATCACTTCTCCCTTGGAGTGCTTGTGATTCTGCTCATG
GTGCAGGAAGGGCTGAAGAAGAGAATGACCACAAAGATCATCATAAGCACATCGATGGCAGTGCTGGTAGCTATGATCC
TGGGAGGATTTTCAATGAGTGACCTGGCTAAGCTTGCAATTTTGATGGGTGCCACCTTCGCGGAAATGAACACTGGAGGA
GATGTAGCTCATCTGGCGCTGATAGCGGCATTCAAAGTCAGACCAGCGTTGCTGGTATCTTTCATCTTCAGAGCTAATTGG
ACACCCCGTGAAAGCATGCTGCTGGCCTTGGCCTCGTGTCTTTTGCAAACTGCGATCTCCGCCTTGGAAGGCGACCTGATG
GTTCTCATCAATGGTTTTGCTTTGGCCTGGTTGGCAATACGAGCGATGGTTGTTCCACGCACTGATAACATCACCTTGGCA
ATCCTGGCTGCTCTGACACCACTGGCCCGGGGCACACTGCTTGTGGCGTGGAGAGCAGGCCTTGCTACTTGCGGGGGGTT
TATGCTCCTCTCTCTGAAGGGAAAAGGCAGTGTGAAGAAGAACTTACCATTTGTCATGGCCCTGGGACTAACCGCTGTGA
GGCTGGTCGACCCCATCAACGTGGTGGGACTGCTGTTGCTCACAAGGAGTGGGAAGCGGAGCTGGCCCCCTAGCGAAGT
ACTCACAGCTGTTGGCCTGATATGCGCATTGGCTGGAGGGTTCGCCAAGGCAGATATAGAGATGGCTGGGCCCATGGCC
GCGGTCGGTCTGCTAATTGTCAGTTACGTGGTCTCAGGAAAGAGTGTGGACATGTACATTGAAAGAGCAGGTGACATCAC
ATGGGAAAAAGATGCGGAAGTCACTGGAAACAGTCCCCGGCTCGATGTGGCGCTAGATGAGAGTGGTGATTTCTCCCTG
GTGGAGGATGACGGTCCCCCCATGAGAGAGATCATACTCAAGGTGGTCCTGATGACCATCTGTGGCATGAACCCAATAGC
CATACCCTTTGCAGCTGGAGCGTGGTACGTATACGTGAAGACTGGAAAAAGGAGTGGTGCTCTATGGGATGTGCCTGCTC
CCAAGGAAGTAAAAAAGGGGGAGACCACAGATGGAGTGTACAGAGTAATGACTCGTAGACTGCTAGGTTCAACACAAGT
TGGAGTGGGAGTTATGCAAGAGGGGGTCTTTCACACTATGTGGCACGTCACAAAAGGATCCGCGCTGAGAAGCGGTGAA
GGGAGACTTGATCCATACTGGGGAGATGTCAAGCAGGATCTGGTGTCATACTGTGGTCCATGGAAGCTAGATGCCGCCT
GGGACGGGCACAGCGAGGTGCAGCTCTTGGCCGTGCCCCCCGGAGAGAGAGCGAGGAACATCCAGACTCTGCCCGGAA
TATTTAAGACAAAGGATGGGGACATTGGAGCGGTTGCGCTGGATTACCCAGCAGGAACTTCAGGATCTCCAATCCTAGAC
AAGTGTGGGAGAGTGATAGGACTTTATGGCAATGGGGTCGTGATCAAAAATGGGAGTTATGTTAGTGCCATCACCCAAG
GGAGGAGGGAGGAAGAGACTCCTGTTGAGTGCTTCGAGCCTTCGATGCTGAAGAAGAAGCAGCTAACTGTCTTAGACTT
GCATCCTGGAGCTGGGAAAACCAGGAGAGTTCTTCCTGAAATAGTCCGTGAAGCCATAAAAACAAGACTCCGTACTGTGA
TCTTAGCTCCAACCAGGGTTGTCGCTGCTGAAATGGAGGAAGCCCTTAGAGGGCTTCCAGTGCGTTATATGACAACAGCA
GTCAATGTCACCCACTCTGGAACAGAAATCGTCGACTTAATGTGCCATGCCACCTTCACTTCACGTCTACTACAGCCAATCA
GAGTCCCCAACTATAATCTGTATATTATGGATGAGGCCCACTTCACAGATCCCTCAAGTATAGCAGCAAGAGGATACATTT
CAACAAGGGTTGAGATGGGCGAGGCGGCTGCCATCTTCATGACCGCCACGCCACCAGGAACCCGTGACGCATTTCCGGA
CTCCAACTCACCAATTATGGACACCGAAGTGGAAGTCCCAGAGAGAGCCTGGAGCTCAGGCTTTGATTGGGTGACGGATC
ATTCTGGAAAAACAGTTTGGTTTGTTCCAAGCGTGAGGAACGGCAATGAGATCGCAGCTTGTCTGACAAAGGCTGGAAA
ACGGGTCATACAGCTCAGCAGAAAGACTTTTGAGACAGAGTTCCAGAAAACAAAACATCAAGAGTGGGACTTTGTCGTG
ACAACTGACATTTCAGAGATGGGCGCCAACTTTAAAGCTGACCGTGTCATAGATTCCAGGAGATGCCTAAAGCCGGTCAT
ACTTGATGGCGAGAGAGTCATTCTGGCTGGACCCATGCCTGTCACACATGCCAGCGCTGCCCAGAGGAGGGGGCGCATA
GGCAGGAATCCCAACAAACCTGGAGATGAGTATCTGTATGGAGGTGGGTGCGCAGAGACTGACGAAGACCATGCACACT
GGCTTGAAGCAAGAATGCTCCTTGACAATATTTACCTCCAAGATGGCCTCATAGCCTCGCTCTATCGACCTGAGGCCGACA
AAGTAGCAGCCATTGAGGGAGAGTTCAAGCTTAGGACGGAGCAAAGGAAGACCTTTGTGGAACTCATGAAAAGAGGAG
ATCTTCCTGTTTGGCTGGCCTATCAGGTTGCATCTGCCGGAATAACCTACACAGATAGAAGATGGTGCTTTGATGGCACGA
CCAACAACACCATAATGGAAGACAGTGTGCCGGCAGAGGTGTGGACCAGACACGGAGAGAAAAGAGTGCTCAAACCGA
GGTGGATGGACGCCAGAGTTTGTTCAGATCATGCGGCCCTGAAGTCATTCAAGGAGTTTGCCGCTGGGAAAAGAGGAGC
GGCTTTTGGAGTGATGGAAGCCCTGGGAACACTGCCAGGACACATGACAGAGAGATTCCAGGAAGCCATTGACAACCTC
GCTGTGCTCATGCGGGCAGAGACTGGAAGCAGGCCTTACAAAGCCGCGGCGGCCCAATTGCCGGAGACCCTAGAGACCA
TTATGCTTTTGGGGTTGCTGGGAACAGTCTCGCTGGGAATCTTTTTCGTCTTGATGAGGAACAAGGGCATAGGGAAGATG
GGCTTTGGAATGGTGACTCTTGGGGCCAGCGCATGGCTCATGTGGCTCTCGGAAATTGAGCCAGCCAGAATTGCATGTGT
CCTCATTGTTGTGTTCCTATTGCTGGTGGTGCTCATACCTGAGCCAGAAAAGCAAAGATCTCCCCAGGACAACCAAATGGC
AATCATCATCATGGTAGCAGTAGGTCTTCTGGGCTTGATTACCGCCAATGAACTCGGATGGTTGGAGAGAACAAAGAGTG

ACCTAAGCCATCTAATGGGAAGGAGAGAGGAGGGGGCAACCATAGGATTCTCAATGGACATTGACCTGCGGCCAGCCTC
AGCTTGGGCCATCTATGCTGCCTTGACAACTTTCATTACCCCAGCCGTCCAACATGCAGTGACCACTTCATACAACAACTAC
TCCTTAATGGCGATGGCCACGCAAGCTGGAGTGTTGTTTGGTATGGGCAAAGGGATGCCATTCTACGCATGGGACTTTGG
AGTCCCGCTGCTAATGATAGGTTGCTACTCACAATTAACACCCCTGACCCTAATAGTGGCCATCATTTTGCTCGTGGCGCAC
TACATGTACTTGATCCCAGGGCTGCAGGCAGCAGCTGCGCGTGCTGCCCAGAAGAGAACGGCAGCTGGCATCATGAAGA
ACCCTGTTGTGGATGGAATAGTGGTGACTGACATTGACACAATGACAATTGACCCCCAAGTGGAGAAAAAGATGGGACA
GGTGCTACTCATAGCAGTAGCCGTCTCCAGCGCCATACTGTCGCGGACCGCCTGGGGGTGGGGGGAGGCTGGGGCCCTG
ATCACAGCGGCAACTTCCACTTTGTGGGAAGGCTCTCCGAACAAGTACTGGAACTCCTCTACAGCCACTTCACTGTGTAAC
ATTTTTAGGGGAAGTTACTTGGCTGGAGCTTCTCTAATCTACACAGTAACAAGAAACGCTGGCTTGGTCAAGAGACGTGG
GGGTGGAACAGGAGAGACCCTGGGAGAGAAATGGAAGGCCCGCTTGAACCAGATGTCGGCCCTGGAGTTCTACTCCTAC
AAAAAGTCAGGCATCACCGAGGTGTGCAGAGAAGAGGCCCGCCGCGCCCTCAAGGACGGTGTGGCAACGGGAGGCCAT
GCTGTGTCCCGAGGAAGTGCAAAGCTGAGATGGTTGGTGGAGCGGGGATACCTGCAGCCCTATGGAAAGGTCATTGATC
TTGGATGTGGCAGAGGGGGCTGGAGTTACTACGCCGCCACCATCCGCAAAGTTCAAGAAGTGAAAGGATACACAAAAGG
AGGCCCTGGTCATGAAGAACCCATGTTGGTGCAAAGCTATGGGTGGAACATAGTCCGTCTTAAGAGTGGGGTGGACGTC
TTTCATATGGCGGCTGAGCCGTGTGACACGTTGCTGTGTGACATAGGTGAGTCATCATCTAGTCCTGAAGTGGAAGAAGC
ACGGACGCTCAGAGTCCTCTCCATGGTGGGGGATTGGCTTGAAAAAAGACCAGGAGCCTTTTGTATAAAAGTGTTGTGCC
CATACACCAGCACTATGATGGAAACCCTGGAGCGACTGCAGCGTAGGTATGGGGGAGGACTGGTCAGAGTGCCACTCTC
CCGCAACTCTACACATGAGATGTACTGGGTCTCTGGAGCGAAAAGCAACACCATAAAAAGTGTGTCCACCACGAGCCAGC
TCCTCTTGGGGCGCATGGACGGGCCCAGGAGGCCAGTGAAATATGAGGAGGATGTGAATCTCGGCTCTGGCACGCGGG
CTGTGGTAAGCTGCGCTGAAGCTCCCAACATGAAGATCATTGGTAACCGCATTGAAAGGATCCGCAGTGAGCACGCGGA
AACGTGGTTCTTTGACGAGAACCACCCATATAGGACATGGGCTTACCATGGAAGCTATGAGGCCCCCACACAAGGGTCAG
CGTCCTCTCTAATAAACGGGGTTGTCAGGCTCCTGTCAAAACCCTGGGATGTGGTGACTGGAGTCACAGGAATAGCCATG
ACCGACACCACACCGTATGGTCAGCAAAGAGTTTTCAAGGAAAAAGTGGACACTAGGGTGCCAGACCCCCAAGAAGGCA
CTCGTCAGGTTATGAGCATGGTCTCTTCCTGGTTGTGGAAAGAGCTAGGCAAACACAAACGGCCACGAGTCTGTACCAAA
GAAGAGTTCATCAACAAGGTTCGTAGCAATGCAGCATTAGGGGCAATATTTGAAGAGGAAAAAGAGTGGAAGACTGCAG
TGGAAGCTGTGAACGATCCAAGGTTCTGGGCTCTAGTGGACAAGGAAAGAGAGCACCACCTGAGAGGAGAGTGCCAGA
GTTGTGTGTACAACATGATGGGAAAAAGAGAAAAGAAACAAGGGGAATTTGGAAAGGCCAAGGGCAGCCGCGCCATCT
GGTATATGTGGCTAGGGGCTAGATTTCTAGAGTTCGAAGCCCTTGGATTCTTGAACGAGGATCACTGGATGGGGAGAGA
GAACTCAGGAGGTGGTGTTGAAGGGCTGGGATTACAAAGACTCGGATATGTCCTAGAAGAGATGAGTCGCATACCAGGA
GGAAGGATGTATGCAGATGACACTGCTGGCTGGGACACCCGCATCAGCAGGTTTGATCTGGAGAATGAAGCTCTAATCA
CCAACCAAATGGAGAAAGGGCACAGGGCCTTGGCATTGGCCATAATCAAGTACACATACCAAAACAAAGTGGTAAAGGT
CCTTAGACCAGCTGAAAAAGGGAAGACAGTTATGGACATTATTTCGAGACAAGACCAAAGGGGGAGCGGACAAGTTGTC
ACTTACGCTCTTAACACATTTACCAACCTAGTGGTGCAACTCATTCGGAATATGGAGGCTGAGGAAGTTCTAGAGATGCAA
GACTTGTGGCTGCTGCGGAGGTCAGAGAAAGTGACCAACTGGTTGCAGAGCAACGGATGGGATAGGCTCAAACGAATG
GCAGTCAGTGGAGATGATTGCGTTGTGAAGCCAATTGATGATAGGTTTGCACATGCCCTCAGGTTCTTGAATGATATGGG
AAAAGTTAGGAAGGACACACAAGAGTGGAAACCCTCAACTGGATGGGACAACTGGGAAGAAGTTCCGTTTTGCTCCCAC
CACTTCAACAAGCTCCATCTCAAGGACGGGAGGTCCATTGTGGTTCCCTGCCGCCACCAAGATGAACTGATTGGCCGGGC
CCGCGTCTCTCCAGGGGCGGGATGGAGCATCCGGGAGACTGCTTGCCTAGCAAAATCATATGCGCAAATGTGGCAGCTC
CTTTATTTCCACAGAAGGGACCTCCGACTGATGGCCAATGCCATTTGTTCATCTGTGCCAGTTGACTGGGTTCCAACTGGG
AGAACTACCTGGTCAATCCATGGAAAGGGAGAATGGATGACCACTGAAGACATGCTTGTGGTGTGGAACAGAGTGTGGA
TTGAGGAGAACGACCACATGGAAGACAAGACCCCAGTTACGAAATGGACAGACATTCCCTATTTGGGAAAAAGGGAAGA
CTTGTGGTGTGGATCTCTCATAGGGCACAGACCGCGCACCACCTGGGCTGAGAACATTAAAAACACAGTCAACATGGTGC
GCAGGATCATAGGTGATGAAGAAAAGTACATGGACTACCTATCCACCCAAGTTCGCTACTTGGGTGAAGAAGGGTCTACA
CCTGGAGTGCTGTAAGCACCAATCTTAGTGTTGTCAGGCCTGCTAGTCAGCCACAGCTTGGGGAAAGCTGTGCAGCCTGT
GACCCCCCCAGGAGAAGCTGGGAAACCAAGCCTATAGTCAGGCCGAGAACGCCATGGCACGGAAGAAGCCATGCTGCCT
GTGAGCCCCTCAGAGGACACTGAGTCAAAAAACCCCACGCGCTTGGAGGCGCAGGATGGGAAAAGAAGGTGGCGACCT
TCCCCACCCTTCAATCTGGGGCCTGAACTGGAGATCAGCTGTGGATCTCCAGAAGAGGGACTAGTGGTTAGAGGAGACCC
CCCGGAAAACGCAAAACAGCATATTGACGCTGGGAAAGACCAGAGACTCCATGAGTTTCCACCACGCTGGCCGCCAGGC
ACAGATCGCCGAATAGCGGCGGCCGGTGTGGGG

SEQ ID NO: 73

AHZ13508.1, Zika virus polyprotein from Polynesian outbreak (H/PF/2013)

MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIKPSLGLINRWGSVGKKEAMEIIKKFKK
DLAAMLRIINARKEKKRRGADTSVGIVGLLLTTAMAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDA
TMSYECPMLDEGVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREYTKHLIRVENWI

FRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIEL
VTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTG
KSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKH
WLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLK
CRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKM
MLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLF
GGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSADVGCSVDFSKKETRCGTGVFVYNDVEAWRDRYKYHP
DSPRRLAAAVKQAWEDGICGISSVSRMENIMWRSVEGELNAILEENGVQLTVVVGSVKNPMWRGPQRLPVPVNELPHGWK
AWGKSYFVRAAKTNNSFVVDGDTLKECPLKHRAWNSFLVEDHGFGVFHTSVWLKVREDYSLECDPAVIGTAVKGKEAVHSDL
GYWIESEKNDTWRLKRAHLIEMKTCEWPKSHTLWTDGIEESDLIIPKSLAGPLSHHNTREGYRTQMKGPWHSEELEIRFEECPG
TKVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGCWYGMEIRPRKEPESNLVRSMVTAGSTDHMDHFSLG
VLVILLMVQEGLKKRMTTKIIISTSMAVLVAMILGGFSMSDLAKLAILMGATFAEMNTGGDVAHLALIAAFKVRPALLVSFIFRA
NWTPRESMLLALASCLLQTAISALEGDLMVLINGFALAWLAIRAMVVPRTDNITLAILAALTPLARGTLLVAWRAGLATCGGFM
LLSLKGKGSVKKNLPFVMALGLTAVRLVDPINVVGLLLLTRSGKRSWPPSEVLTAVGLICALAGGFAKADIEMAGPMAAVGLLIV
SYVVSGKSVDMYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEDDGPPMREIILKVVLMTICGMNPIAIPFAAGAWYVY
VKTGKRSGALWDVPAPKEVKKGETTDGVYRVMTRRLLGSTQVGVGVMQEGVFHTMWHVTKGSALRSGEGRLDPYWGDVK
QDLVSYCGPWKLDAAWDGHSEVQLLAVPPGERARNIQTLPGIFKTKDGDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGVVIK
NGSYVSAITQGRREEETPVECFEPSMLKKKQLTVLDLHPGAGKTRRVLPEIVREAIKTRLRTVILAPTRVVAAEMEEALRGLPVRY
MTTAVNVTHSGTEIVDLMCHATFTSRLLQPIRVPNYNLYIMDEAHFTDPSSIAARGYISTRVEMGEAAAIFMTATPPGTRDAFP
DSNSPIMDTEVEVPERAWSSGFDWVTDHSGKTVWFVPSVRNGNEIAACLTKAGKRVIQLSRKTFETEFQKTKHQEWDFVVTT
DISEMGANFKADRVIDSRRCLKPVILDGERVILAGPMPVTHASAAQRRGRIGRNPNKPGDEYLYGGGCAETDEDHAHWLEAR
MLLDNIYLQDGLIASLYRPEADKVAAIEGEFKLRTEQRKTFVELMKRGDLPVWLAYQVASAGITYTDRRWCFDGTTNNTIMEDS
VPAEVWTRHGEKRVLKPRWMDARVCSDHAALKSFKEFAAGKRGAAFGVMEALGTLPGHMTERFQEAIDNLAVLMRAETGS
RPYKAAAAQLPETLETIMLLGLLGTVSLGIFFVLMRNKGIGKMGFGMVTLGASAWLMWLSEIEPARIACVLIVVFLLLVVLIPEPE
KQRSPQDNQMAIIIMVAVGLLGLITANELGWLERTKSDLSHLMGRREEGATIGFSMDIDLRPASAWAIYAALTTFITPAVQHAV
TTSYNNYSLMAMATQAGVLFGMGKGMPFYAWDFGVPLLMIGCYSQLTPLTLIVAIILLVAHYMYLIPGLQAAAARAAQKRTAA
GIMKNPVVDGIVVTDIDTMTIDPQVEKKMGQVLLIAVAVSSAILSRTAWGWGEAGALITAATSTLWEGSPNKYWNSSTATSLC
NIFRGSYLAGASLIYTVTRNAGLVKRRGGGTGETLGEKWKARLNQMSALEFYSYKKSGITEVCREEARRALKDGVATGGHAVSR
GSAKLRWLVERGYLQPYGKVIDLGCGRGGWSYYAATIRKVQEVKGYTKGGPGHEEPMLVQSYGWNIVRLKSGVDVFHMAAE
PCDTLLCDIGESSSSPEVEEARTLRVLSMVGDWLEKRPGAFCIKVLCPYTSTMMETLERLQRRYGGGLVRVPLSRNSTHEMYWV
SGAKSNTIKSVSTTSQLLLGRMDGPRRPVKYEEDVNLGSGTRAVVSCAEAPNMKIIGNRIERIRSEHAETWFFDENHPYRTWAY
HGSYEAPTQGSASSLINGVVRLLSKPWDVVTGVTGIAMTDTTPYGQQRVFKEKVDTRVPDPQEGTRQVMSMVSSWLWKELG
KHKRPRVCTKEEFINKVRSNAALGAIFEEEKEWKTAVEAVNDPRFWALVDKEREHHLRGECQSCVYNMMGKREKKQGEFGKA
KGSRAIWYMWLGARFLEFEALGFLNEDHWMGRENSGGGVEGLGLQRLGYVLEEMSRIPGGRMYADDTAGWDTRISRFDLE
NEALITNQMEKGHRALALAIIKYTYQNKVVKVLRPAEKGKTVMDIISRQDQRGSGQVVTYALNTFTNLVVQLIRNMEAEEVLEM
QDLWLLRRSEKVTNWLQSNGWDRLKRMAVSGDDCVVKPIDDRFAHALRFLNDMGKVRKDTQEWKPSTGWDNWEEVPFC
SHHFNKLHLKDGRSIVVPCRHQDELIGRARVSPGAGWSIRETACLAKSYAQMWQLLYFHRRDLRLMANAICSSVPVDWVPTG
RTTWSIHGKGEWMTTEDMLVVWNRVWIEENDHMEDKTPVTKWTDIPYLGKREDLWCGSLIGHRPRTTWAENIKNTVNMV
RRIIGDEEKYMDYLSTQVRYLGEEGSTPGVL

SEQ ID NO: 74
9320_Zika_PF_1F
ttaggatccGTTGTTGATCTGTGTGAAT

SEQ ID NO: 75
9321_Zika_PF_1R
taactcgagCGTACACAACCCAAGTT

SEQ ID NO: 76
9322_Zika_PF_2F
ttaggatccTCACTAGACGTGGGAGTG

SEQ ID NO: 77
9323_Zika_PF_2R
taactcgagAAGCCATGTCYGATATTGAT

SEQ ID NO: 78
9324_Zika_PF_3F
ttaggatccGCATACAGCATCAGGTG

SEQ ID NO: 79
9325_Zika_PF_3R
taactcgagTGTGGAGTTCCGGTGTCT

SEQ ID NO: 80
9326_Zika_PF_4F
ttaggatccGAATAGAGCGAARGTTGAGATA

SEQ ID NO: 81
9327_Zika_PF_4R
taactcgAGTGGTGGGTGATCTTCTTCT

SEQ ID NO: 82
9328_Zika_PF_5F
ttaggatcCAGTCACAGTGGAGGTACAGTAC

SEQ ID NO: 83
9329_Zika_PF_5R
taactcgagCRCAGATACCATCTTCCC

SEQ ID NO: 84
9330_Zika_PF_6F
ttaggatCCCTTATGTGCTTGGCCTTAG

SEQ ID NO: 85
9331_Zika_PF_6R
taactcgagTCTTCAGCCTCCATGTG

SEQ ID NO: 86
9332_Zika_PF_7F
ttaggatccAATGCCCACTCAAACATAGA

SEQ ID NO: 87
9333_Zika_PF_7R
taactcgagTCATTCTCTTCTTCAGCCCTT

SEQ ID NO: 88
9334_Zika_PF_8F
ttaggatccAAGGGTGATCGAGGAAT

SEQ ID NO: 89
9335_Zika_PF_8R
taactcgagTTCCCTTCAGAGAGAGGAGC

SEQ ID NO: 90
9336_Zika_PF_9F
ttaggatccTCTTTTGCAAACTGCGATC

SEQ ID NO: 91
9337_Zika_PF_9R

taactcgagTCCAGCTGCAAAGGGTAT

SEQ ID NO: 92
9338_Zika_PF_10F
ttaggatccGTGTGGACATGTACATTGA

SEQ ID NO: 93
9339_Zika_PF_10R
taactcgagCCCATTGCCATAAAGTC

SEQ ID NO: 94
9340_Zika_PF_11F
ttaggatccTCATACTGTGGTCCATGGA

SEQ ID NO: 95
9341_Zika_PF_11R
taactcgagGCCCATCTCAACCCTTG

SEQ ID NO: 96
9342_Zika_PF_12F
ttaggatccTAGAGGGCTTCCAGTGC

SEQ ID NO: 97
9343_Zika_PF_12R
taactcgAGATACTCATCTCCAGGTTTGTTG

SEQ ID NO: 98
9344_Zika_PF_13F
ttaggatccGAAAACAAAACATCAAGAGTG

SEQ ID NO: 99
9345_Zika_PF_13R
taactcgagGAATCTCTCTGTCATGTGTCCT

SEQ ID NO: 100
9346_Zika_PF_14F
ttaggatccTTGATGGCACGACCAAC

SEQ ID NO: 101
9347_Zika_PF_14R
ttaggatccGTTGTTGATCTGTGTGAAT

SEQ ID NO: 102
9348_Zika_PF_15F
taactcgagCAGGTCAATGTCCATTG

SEQ ID NO: 103
9349_Zika_PF_15R
ttaggatccTGTTGTGTTCCTATTGCTGGT

SEQ ID NO: 104
9350_Zika_PF_16F
taactcgaGTGATCAGRGCCCCAGC

SEQ ID NO: 105

9351_Zika_PF_16R
ttaggatccTGCTGCCCAGAAGAGAA

SEQ ID NO: 106
9352_Zika_PF_17F
taactcgaGCACCAACAYGGGTTCTT

SEQ ID NO: 107
9353_Zika_PF_17R
ttaggatcCTCAAGGACGGTGTGGC

SEQ ID NO: 108
9354_Zika_PF_18F
taactcgagCAATGATCTTCATGTTGGG

SEQ ID NO: 109
9355_Zika_PF_18R
ttaggatccTATGGGGGAGGACTGGT

SEQ ID NO: 110
9356_Zika_PF_19F
taactcGAGCCCAGAACCTTGGATC

SEQ ID NO: 111
9357_Zika_PF_19R
ttaggatcCAGACCCCCAAGAAGGC

SEQ ID NO: 112
9358_Zika_PF_20F
taactcgagCCCCTTTGGTCTTGTCT

SEQ ID NO: 113
9359_Zika_PF_20R
ttaggatccAGGAAGGATGTATGCAGATG

SEQ ID NO: 114
9360_Zika_PF_21F
taactcgagACATTTGCGCATATGATTTTG

SEQ ID NO: 115
9361_Zika_PF_21R
ttaggatccAGGAAGGACACACAAGAGT

SEQ ID NO: 116
9362_Zika_PF_22F
taactcgagACAGGCTGCACAGCTTT

SEQ ID NO: 117
9363_Zika_PF_22R
ttaggatccTCTCTCATAGGGCACAGAC

In some embodiments, the Zika virus has polyprotein, including an envelope (E) protein, with an amino acid sequence provided by any one of SEQ ID NOs: 14-69 or 72. In some embodiments, the

polyprotein or E protein sequence is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%. 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to any one of SEQ ID NOs: 2-69 or 72.

The terms "identical" or percent "identity" in the context of two or more nucleic acids or amino acid sequences refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*e.g.*, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) over a specified region or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length. In some embodiments, the identity exists over the length of a protein, such as the E protein.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. Methods of alignment of sequences for comparison are well known in the art. See, *e.g.,* by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, J. MoI. Biol. 48:443, 1970, by the search for similarity method of Pearson and Lipman. Proc. Natl. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, Jalview and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group. 575 Science Dr., Madison. WI), by multi sequence alignment implementation using e.g. CLUSTALW (Larkin et al., (2007). Bioinformatics, 23, 2947-2948.) or MAFFT (Katoh & Toh 2008 Briefings in Bioinformatics 9:286-298), or by manual alignment and visual inspection (see. *e.g.,* Brent et al., Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (Ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977 and Altschul et al., J. MoI. Biol. 215:403-410, 1990, respectively.

EXAMPLES

Example 1: Production of a Zika drug substance suitable for application as a vaccine in humans and animals

Materials and Methods:

For the production of ZikaV the JEV process platform (Srivastava et al., Vaccine 19 (2001) 4557-4565; US 6,309,650B1) was used as a basis. Small changes of certain process steps were adapted to ZikaV properties and to improve purity. A short summary of the process steps is outlined below (see also Figure 9A and B). Briefly, the unexpected and novel purification properties of protamine sulphate (PS) were evaluated in purification processes for Zika Virus. As shown in Figure 10, non-infectious virus particle aggregates, HCP and other LMW impurities were removed by PS precipitation as shown by removal of aggregate shoulder in SEC-HPLC and no loss of infectious virus titer by PS treatment. Further optimization of the Zika purification protocol is provided below.

Upstream:

- Roller Bottle based Vero cell expansion (25x850cm2 CellBind):
- 5% CO_2, 35°C, MEM+2mM L-Glutamine + 10% FBS
- Infection with ZikaV research Master Seed Bank (rMSB) at MOI 0.01
- Virus Production without serum
- 5% CO_2, 35°C, MEM+2mM L-Glutamine
- Multiple harvests (days 2, 3, 5 and 7) with re-feed
- Sterile filtration of harvests and storage at 2-8°C until further processing

Downstream:

- Pooling of harvests and concentration by ultrafiltration (100kDa)
- Stabilization of concentrated harvest (Tris/10% sucrose) for storage if required (-80°C)
- Removal of hcDNA by Protamine Sulphate (2 mg/mL)
- Sucrose Gradient Purification (optimized three layered gradient)
- Formaldehyde Inactivation (0.02%, 22°C, 10 days), neutralization with Na-metabisulfite
- Dilution to DS antigen target content and formulation with Aluminium hydroxide (0.5mg Al/mL)

Zika Virus Strain H/PF/2013 was originally isolated from a 51-year-old woman (accession number KJ776791.1, also SEQ ID NO: 13 herein) from French Polynesia. A sample was obtained from the European Virus Archive (EVAg; Ref-SKU: 001v-EVA1545). Based on this material, a research

master seed bank (rMSB) was prepared on Vero cells as the cell substrate and the genomic sequence was checked by sequencing. Because the genomic sequence at the 5´and 3´flanking sequences of Zika virus strain H/PF/2013 was unknown, primers for sequencing were designed in those regions based on other Zika virus strains whereas the internal primers were designed from the published sequence (SEQ ID NOs: 74 to 117, see also Table A). The sequence obtained from the rMSB by use of these primers is provided by SEQ ID NO: 72. There was 100% overlap of the sequence with the published sequence of Zika Virus Strain H/PF/2013 (SEQ ID NO: 13). However, we sequenced additional regions 5´ (an additional 40 bp) and 3' (an additional 160 bp) represented in SEQ ID NO: 72. In a preferred embodiment, the Zika virus of the invention comprises SEQ ID NO: 72. The genomic RNA is somewhat longer than the sequence according to SEQ ID NO: 72 (perhaps an additional 200 bp). Additionally, a Zika virus adapted to a host cell such as e.g. Vero cells may be expected to contain one or more mutations. For these reasons, the Zika virus of the current invention comprises the sequence of SEQ ID NO: 72 or, preferably, a sequence with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 72. Furthermore, because the viral genome is likely to contain even further flanking regions to SEQ ID NO: 72; in one embodiment, the Zika virus of the invention contains the sequence of SEQ ID NO: 72 and optionally further comprises extensions at the 5' and/or 3' ends of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120 or at least 130 nucleotides. In a preferred embodiment, the Zika virus comprises at least the coding sequence for the entire polyprotein of Zika Virus Strain H/PF/2013 of the invention i.e. the amino acid sequence of SEQ ID NO: 73 or a polyprotein with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 73. Furthermore, the Zika virus comprises at least the coding sequence for the E-protein of Zika Virus Strain H/PF/2013 of the invention SEQ ID NO: 47 or an E-protein thereof with at least 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence provided by SEQ ID NO: 47.

Virus growth on Vero Cells

Vero cells were grown in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS). Roller bottle cultures of Vero cell monolayers were infected with Zika Virus Strain H/PF/2013 at a multiplicity of infection (moi) of 0.01 plaque forming units (pfu) per cell. After 2 hours of virus adsorption, the cultures were washed 3 times with PBS and fed with EMEM without FBS and incubated at +35°C with 5% CO_2. Infected Vero cell cultures were incubated until the virus titer reaches a desired level.

The culture medium was harvested at days 2, 3, 5 and 7 and were pooled from those harvest days and then centrifuged in a standard centrifuge. The supernatants were then filtered. Virus culture supernatants were concentrated by TFF ultrafiltration to remove cell culture media components and to reduce batch volume.

Evaluation of harvest Procedure

The current JEV harvest process has scheduled harvests on days 3, 5, 7 and 9 post infection. To mimic the JEV process roller bottles were infected with ZIKV bank P4-FBS at an MOI of 0.01 in infection medium (MEM with 2% FBS + 2mM L-glutamine) for 2 hours. After removing the inoculum the cells were washed twice with PBS and 200 mL production medium (MEM+ 2mM L-glutamine) was added.

After taking a sample on day 2 the first virus harvest was conducted on day 3 after infection. At this point significantly higher CPE could be observed compared to cells where virus was removed on day 2. Plaque assay analysis showed that the viral titers on day 2 were in the same range as for the standard harvesting schedule. However, starting with the day 3 harvest, the observed titers were significantly lower correlating with the increased CPE observed compared to the standard harvest schedule. On day 5 post infection no more living cells could be observed at all and the experiment was terminated with a final day 5 harvest.

Table 3: The calculated titers per plaque assay are summarized in the list below.

	Log 10 PFU/mL
sample day2	7.02
harvest day 3	6.66
harvest day 5	6.26

This finding led to an optimized harvest schedule to better control of CPE and allow additional harvest day 5 and 7, see Figure 15. For both harvest days the optimized ZikaV protocol yield significant higher virus titers compared to the modified protocol showing that the time of the first harvest is crucial for production yields. Additionally first harvesting at day 3 results in maximum 2 harvest points whereas first harvesting at day 2 allows for 4 harvest points further increasing the yield gain.

Downstream Purification of Zika virus

The purification process was carried out at room temperature (18-22°C) unless stated otherwise. Virus purification started with concentration of filtered combined harvest using 100 kDa cut-off TFF

ultrafiltration modules to remove cell culture media components and reduce batch volume. After concentration, the pooled filtered harvest material was adjusted to a final concentration of 25 mM Tris pH 7.5 and 10% sucrose (w/w) using stock solution of both components (see Figure 11 for SEC-HPLC of different harvests prior to PS treatment). This allowed for freezing the concentrated harvest at <-65°C if required.

Host cell DNA and protein reduction as well reduction of non-infectious virus aggregates in the concentrated material was achieved by precipitation with protamine sulphate (2 mg/mL) followed by sucrose density centrifugation (2-8°C) as final polishing step (see Figure 20 for SEC-HPLC of different harvests post PS treatment). The purification process was designed to be completed within 2 working days with SGC starting on end of day 1 followed by fractionation and SDS-PAGE analysis on day 2. The sucrose gradient fractions were stored at 2-8 °C during the SDS-PAGE analysis (Silver staining) to identify the pure fractions containing ZikaV (see Figure 21). After pooling the relevant fractions, the pool was diluted and inactivated by Formalin. After pooling the relevant fractions of sucrose gradient centrifugation, the pool was diluted 1:3 in PBS and inactivated by Formalin (0.02% v/v, 200ppm). Fractions were subjected to analysis by SDS-PAGE.

Effect of PS treatment on virus recovery

Samples of individual 30x concentrated harvests days 2, 3, 5 and 7 were analysed before (Figure 11) and after PS (Figure 12) treatment by SEC-HPLC and plaque assay. SEC-HPLC was used for determination of relative total ZikaV content (active + inactive) expressed as peak area, whereas the rel. ZikaV peak purity is given as relative content of virus monomer population to total virus peak. Plaque assay states the content of total active virus particles in each sample. Experimental results are summarized in Table 4. The virus peak recovery by SEC-HPLC was only between 12 to 36% with peak purity after PS treatment in the range of >90% (no virus aggregates detected). The recovery of active virus particles by plaque assay was all > 100% (130-700%, range within the variability of the assay) showing that no active virus particles were lost during PS treatment. These results show that during PS treatment only non-infective (immature and/or aggregated virus) particles were removed.

Table 4: ZikaV recovery by SEC-HPLC and plaque assay before and after PS treatment.

SEC-HPLC

	Peak area mAU*min			
Harvest day	30x conc	30x + PS	SEC Recovery (%)	rel. virus monomer content after PS (%)
Day 2	101.36	18.63	18	89%
Day 3	144.51	17.48	12	90%

Day 5	19.97	5.92	30	96%
Day 7	68.80	24.43	36	99%

Plaque Assay

	PFU/mL		
Harvest day	30x conc	30x + PS	Plaque Recovery (%)
Day 2	3E+08	5E+08	179
Day 3	2E+08	4E+08	193
Day 5	1E+08	9E+08	700
Day 7	3E+08	4E+08	132

Sucrose gradient centrifugation

The PS treated harvest was split in two parts and loaded on two centrifuge bottles. Sucrose density gradient centrifugation (SGC) was used for final concentration and polishing of the ZikaV material. The ZikaV PS treated concentrated harvest was loaded on top of a solution consisting of three layers of sucrose with different densities. The three sucrose layers were selected based on a preliminary study which showed the formation of a linear sucrose gradient and complete separation of the virus particles from residual contaminants as demonstrated for ChikV (Fig. 15D). The optimal volumes of the sucrose solutions were determined empirically. The volumes of individual layers for a centrifugation in 100 mL bottle scale are shown in Table 5.

Table 5: Individual layers/volumes for a centrifugation in bottle.

Solution	**Volume (mL)**
PS treated harvest in 10% sucrose (L)	40
15% sucrose (J)	15
35% sucrose (I)	15
50% sucrose (H)	20
Total volume	90

The sucrose gradient bottles were prepared by stratifying the individual sucrose layers. A plastic tube was attached to peristaltic pump tubing. The plastic tube was mounted on a laboratory stand using a clamp and placed into the centrifuge bottle. The nozzle of the plastic tube was touching the bottom of the bottle. Using a peristaltic pump the ZikaV material and the sucrose solutions were pumped into the cylinder. A measuring cylinder was used as feed vessel. The first solution pumped was the ZikaV material as it represented the solution of lowest density (10 % sucrose (w/w)). After the ZikaV material the sucrose solutions were pumped in ascending order starting with the 15 % (w/w) solution

J, followed by 35 % sucrose solution I and finishing with the highest density sucrose solution H (50 % (w/w)). The described setup is shown in Figure 14. After all sucrose solutions were transferred the plastic tubing was carefully removed in order not to disturb the layers.

Prior to centrifugation the centrifuge was pre-cooled to 4°C. The prepared SG bottles were carefully transferred into the pre-cooled rotor. (Note: Sudden movement of the bottles during transfer to the rotor must be avoided in order not to disturb the sucrose layers.) The bottles were centrifuged at ~11.000 RCF max at 4 °C for at least 20 hours, no brake/deceleration activated. In case a different centrifuge system with a different rotor is used the necessary speed and centrifugation times need to be calculated based on the k-factor in order to achieve comparable centrifugation efficiency.

Harvesting of the sucrose gradient was done manually using a peristaltic pump. A plastic tube attached to peristaltic pump tubing was used for harvesting the sucrose gradient. The bottle containing the gradient was mounted onto a laboratory stand in a tilted position (~12°) using a clamp. The plastic tubing was then placed into the bottle touching the bottom edge of the bottle and was fastened in position using a clamp. This resulted in a small gap of 1-2 mm between the tubing inlet and the bottom of the bottle (see Figure 14).

Using a peristaltic pump set to a flow rate of 30 mL per minute the gradient was harvested and manually split into 2 mL fractions. A total number of 32 fractions per bottle were harvested (~ 64 mL) and the remaining volume was discarded. The fractions were immediately tested by SDS-PAGE / silver stain to identify the virus containing fractions with sufficient high purity. Representative SDS-PAGE is shown in Figure 14. Fraction 10-14 were pooled and further processed.

The purified viral solution was inactivated by incubation with 0.02% formaldehyde over a period of ten days in a 22°C controlled-temperature incubator. The formaldehyde is neutralized by addition of sodium metabisulphite on the tenth day.

The sucrose gradient pool (~17mL after sampling) was further diluted 3-fold with PBS to a final volume of 51 mL in a PETG container. A volume of 1% formaldehyde (10,000 ppm) solution equivalent to 1/50 of the final volume of the pre-formaldehyde pool was added to this pool resulting in an effective concentration of 200 ppm. The formaldehyde-treated solution was mixed on a magnetic stirrer for 10 minutes. After sampling, the formaldehyde-treated viral solution was placed within a cooled incubator at 22°C ± 2°C. On Day 5 post addition of formaldehyde, the formaldehyde-treated viral solution was filtered through a 0.2 µm filter and then placed in the incubator at 22°C ±

2°C again. On Day 10, after removing the 10-Day inactivation final sample, a volume of 1 % (of the weight of the final formaldehyde-treated viral solution) of 200 mM-sodium metabisulphite solution (2mM final concentration) was aseptically transferred into the PETG container containing the formaldehyde-treated viral solution. After mixing for 5 minutes on a magnetic stirrer, the neutralized inactivated viral solution is held at room temperature (20 to 25°C) for a minimum of 30 minutes. After sampling, the neutralized inactivated viral solution is stored at 5°C ± 3°C until further processing.

Inactivation by formaldehyde

Critical parameters for this step are final formalin concentration, temperature, mixing and transfer into a new container. A preliminary acceptance criterion for maximum pfu/mL (determined by plaque assay) has been set on the diluted pool pre formaldehyde treatment.

The quality of the neutralized inactivated viral solution was monitored by the following parameters: Plaque assay on Day 10, SEC-HPLC, SDS-PAGE/Western Blot.

Interestingly, SEC-HPLC analysis of samples taken during the inactivation period followed by neutralization with bisulfite showed more or less constant peak area throughout the inactivation period. This is in contrast to JEV where losses of viral particles up to 60% are observed using the process disclosed by Srivastava et al. Vaccine 19 (2001) 4557-4565. In a scale-down model the viral losses were even much higher due to surface/area ratio at smaller scale and high losses due to unspecific adsorption. Differences of the ZikaV inactivation experiment and JEV inactivation were noticed as follows:

A) Much higher purity of ZikaV SGP pool with regard to residual PS (<2µg/mL) compared to JEV. The 3-fold ZikaV inactivated sample contained therefore <<1µg/mL of residual PS. Commercial JEV SGP pool contains on average ~120µg/mL (up to 152 µg/mL possible). The average dilution to inactivation solution of ~14-fold results in a residual PS content up to ~11 µg/mL. It may be that higher amount of residual PS could cause virus precipitation due to cross-linking / reaction with formalin.

B) ZikaV inactivation sample contained ~10% sucrose (3-fold dilution of SGP pool containing ~30-35% sucrose). Sucrose might have stabilizing effect of viral ZikaV particles during treatment with formalin.

Dilution to DS and Formulation with Aluminium hydroxide (DP)

For preparation of ZikaV drug substance used in mouse potency assay an antigen content (expressed as total viral particles or SEC peak area) of 5 times higher compared to Ixiaro was targeted. The basis for determination of antigen content was SEC-HPLC. Briefly, a Superose 6 10/300 Increase column

(GE Healthcare) equilibrated with PBS + 250 mM NaCl, pH 7.4 at 1 ml/min and 25°C, was used to detect ZikaV at 214 nm detection wavelength in harvest samples and throughout the downstream process. In the current JEV process the antigen content in NIV is determined by a specific ELISA. A good correlation was observed between antigen content determined by ELISA and SEC-HPLC. On average, the antigen content in commercial NIV samples is in the range of 33 AU/mL corresponding to ~5.2mAU JEV peak area, see Figure 21.

ZikaV NIV day10 (Zika peak ~36 mAU, analysed on Waters HPLC/Superose6 Increase column) was diluted with PBS to a target of 6.3 (~5.7x dilution). Aluminium hydroxide was added to a final concentration of 0.5 mg/mL Aluminium (1/20 v/v Alum 2% stock solution added) to prepare ZikaV Drug Product (DP). The DP was gently mixed for 5 min. An aliquot of the DP was removed, Alum sedimented by centrifugation and the clear supernatant analysed by SEC-HPLC. No ZikaV peak was detected in the supernatant indicating complete adsorption (estimated as >95%) of viral particles on the mineral adjuvant. Formulated ZikaV DP was stored at 2-8°C.

The impurity profile of the inactivated Zika virus DS is comparable to the profile of JEV DS with the exception of a lower PS content (Table 6).

Table 6: Determination of impurity profile in Zika and JEV DS samples:

	Specification (JEV DS)	**JEV**	**Zika**
HCP (ng/mL)	<100 LOQ 12 ng/mL	<LOQ	<LOQ
DNA (pg/mL)	<200 LOQ 40 pg/mL	<40	<40
Aggregates by SEC-MALLS (%)	Not specified, part of characterization LOQ 5%	<LOQ	<LOQ
PS (µg/mL)	Specification only at SGP pool to demonstrate consistent process performance (19-152µg/mL), *PS content in DS calculated based on PS content in SGP pool (~100 µg/mL) and average dilution factor (~28x) to DS; LOQ 2 µg/mL	~4*	<<LOQ

*Typical PS impurity in a JEV sample produced in accordance with protocol disclosed in Srivastava et al. Vaccine 19 (2001) 4557-4565.

SEC-MALLS Results

A representative SEC-HPLC elution profile of ZikaV NIV at 214 nm detection wave length is shown in Figure 16. Note that BSA (50µg/mL) was added to the sample to minimize losses in HPLC glass vial due to unspecific surface adsorption. ZikaV monomer content was estimated as ~98% with a multimer content of ~2%.