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(54) Title: VIRUS-LIKE PARTICLES COMPRISING ZIKA ANTIGEN

(57) Abstract: The invention is related to chimeric Virus-Like Particles (VLPs) containing and displaying epitopes and antigen from Zika Virus (ZIKV); and to methods for creation and production of such chimeric VLPs to their applications, including but not limited to vaccines, diagnostics, clinical studies, assay development and antibody discovery.

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VIRUS-LIKE PARTICLES COMPRISING ZIKA ANTIGEN

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the priority benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 62/524,440, filed June 23, 2017, the disclosure of which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] This application contains, as a separate part of the disclosure, a Sequence Listing in computer-readable form which is incorporated by reference in its entirety and identified as follows: Filename: 52049A_Seqlisting.txt; Size-123,456 bytes, created : June 22, 2018.

FIELD OF THE INVENTION

[0003] This invention is related to improved tools for detection of Zika Virus (ZIKV), ZIKV vaccines, and ZIKV diagnostics.

BACKGROUND

[0004] Zika virus (ZIKV) is an arbovirus belonging to the Flavivirus genus. ZIKV was first isolated from an infected sentinel monkey in the Zika forest in Uganda (1947), and later in mosquitos [1, 2] and humans in 1954 [3]. No outbreaks were described until 2007 when a ZIKV epidemic on the Island of Yap in the Federated States of Micronesia showed that the virus had the propensity to cause serious disease [4, 5]. Subsequently, ZIKV spread to French Polynesia and Pacific Islands (2013-2014), and recently to the Americas causing very large outbreaks in more than twenty countries including Brazil, Mexico and the Caribbean Islands (2015-present). There is evidence that ZIKV transmission can also occur sexually [6, 7], by blood transfusion and possibly via placenta to infect the fetus [8].

[0005] ZIKV is transmitted by mosquitoes of the widely distributed species *Aedes aegypti* and *Aedes albopictus* [4, 5]. According to the Centers for Disease Control (CDC), *Aedes* mosquito species are distributed in many territories of the United States harboring either subtropical or temperate climates. Indeed, ZIKV has caused multiple local infections in the US and US territories, including Puerto Rico, Florida and Texas [9, 10]. ZIKV may continue to spread globally and be introduced in Europe and Australia, and is likely to reemerge in Africa and Asia. ZIKV infection is asymptomatic in a majority (approximately 80%) of people exposed to the virus. Symptoms of infection are similar to other arbovirus diseases, such as Dengue virus (DENV) and Chikungunya Virus (CHIKV), and include fever, maculopapular rash, conjunctivitis, and arthralgia, confounding accurate diagnosis. Importantly, there is a strong

association of ZIKV with the autoimmune disease, Guillain-Barré Syndrome (GBS), and congenital malformations resulting in Microcephaly [4, 11].

[0006] To date, no prophylactic or therapeutic treatment is commercially available and licensed for ZIKV, despite intensive efforts in this direction.

SUMMARY

[0007] The present invention includes a novel ZIKV virus like particle (VLP) and materials and methods for making and using such particles, including formulations and uses as a vaccine, a prophylactic, therapeutic, and diagnostic.

[0008] The development of a safe and effective vaccine to protect against ZIKV infection is a high priority objective to reduce the incidence and spread of the severe forms of the disease. An urgent need for the vaccine is also underlined by the impact of the infection on pregnant women and the still unknown implications to men who may become infected.

[0009] An ideal vaccine candidate, in addition to having a high safety profile, should also be cost effective and economically viable with ease of large scale manufacture. Live virus vaccines and inactivated virus vaccines are expensive to manufacture due the requirement of highly stringent processes and containment facilities (BSL2 or BSL3), using sophisticated biological production systems (e.g., mammalian cells, eggs). An important caveat with an attenuated vaccine is the safety profile, particularly due to the potential for reversions that may reduce/eliminate attenuation. In the context of a virus like ZIKV, especially if required to be administered to pregnant women, this will be a critical concern.

[0010] In the case of inactivated whole virus vaccines, often the inactivation methodologies render epitopes ineffective. Epitope stability is important to the production of completely neutralizing antibodies or protective antibodies.

[0011] With the unresolved issue of the role of non-neutralizing and cross-reacting Dengue antibodies enhancing ZIKV infection, similar phenomena may be anticipated in the case of non-neutralizing or partially neutralizing cross-reacting ZIKV antibodies. With the prevalence of diverse strains of ZIKV, and pending information on effective cross-protection between diverse strains, a VLP strategy engineered using highly conserved regions of ZIKV surface glycoproteins, as provided herein, provides significant advantages. This approach will maintain the ZIKV epitope architecture while increasing the possibility of cross-protection across multiple ZIKV strains and cost-effective scale-up. During the last decade, advances in VLP production, purification, and adjuvant optimization led to several licensed vaccines for viral diseases [12]

such as human papilloma virus (HPV), hepatitis B virus (HBV), hepatitis E virus (HEV), and influenza. VLPs are more efficient for stimulating the immune system with respect to the subunit proteins because they have the ability to mimic the native morphology of the target virion and they display a repetitive array of epitopes in high concentration. In addition, VLPs are safe due to the absence of replicating viral genetic material [12].

[0012] In some aspects, the VLPs disclosed herein are able to cross-protect across different strains of ZIKV. Contrary to other vaccination strategies such as live attenuated vaccines, a VLP strategy as disclosed herein possesses a higher safety profile, particularly for high risk populations such as immunocompromised individuals and pregnant women. In contrast to purified protein vaccines, VLPs of the disclosure express the immunological entity in higher concentration, in an appropriate confirmation (folding) that expresses the epitopes effectively, and with a higher stability profile. From a product development perspective, the technology disclosed herein will also lend itself to facilitated scale-up with defined quality control strategies for large scale production.

[0013] Accordingly, in some aspects the invention includes isolated peptides suitable for making Zika vaccines or Zika antibodies or for detecting Zika antibodies. For example, the invention includes an isolated peptide or protein comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51. Genera of peptides with higher minimum percent identity, including 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% minimum identity to a reference sequence also are contemplated. In some embodiments, the isolated peptide or protein comprises or consists of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51, or an immunogenic fragment thereof. In some aspects, the disclosure provides an isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11. In some embodiments, the isolated peptide comprises or consists of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11. In some aspects, the disclosure provides an isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 22-33. In some embodiments, the isolated peptide comprises or consists of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 22-33. In some aspects, the disclosure provides an isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or

more of SEQ ID NOs: 46-47. In some embodiments, the isolated peptide comprises or consists of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 46-47. In some aspects, an isolated peptide is provided comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 50-51. In some embodiments, the isolated peptide comprises or consists of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 50-51.

[0014] In related aspects, the invention includes a chimeric peptide comprising a peptide derived from Zika as described herein, including those of the preceding paragraph, linked to a heterologous peptide or protein having an amino acid sequence that is at least 90% identical to a Woodchuck Hepatitis core Antigen protein (WHcAg) comprising or consisting of an amino acid sequence as set out in SEQ ID NO: 1, or comprising or consisting of at least one fragment of SEQ ID NO: 1. In some variations, the Zika-derived peptide portion of the chimeric peptide is inserted within the WHcAg-derived portion. Higher minimum percent identities also are contemplated, as described in the preceding paragraph. In some embodiments, the chimeric peptide further includes at least one peptide linker of 1-10 amino acids linking the sequence that is at least 80% identical to any one or more of SEQ ID NOs: 2-11 or 22-33 or 46-47 or 50-51 to the sequence that is at least 90% identical to a WHcAg protein. In some embodiments, the chimeric peptide comprises or consists of an amino acid sequence at least 90% or at least 95% identical to any one of SEQ ID NOs: 12-21, 34-45, 48-49, and 52-53.

[0015] For example, the invention includes a chimeric peptide comprising a Zika-derived peptide as described herein linked to a heterologous peptide having an amino acid sequence that is at least 90% identical to a Woodchuck Hepatitis core Antigen protein (WHcAg) comprising or consisting of an amino acid sequence as set out in SEQ ID NO: 1, wherein the Zika-derived peptide is inserted into the WHcAg-derived peptide at a position between amino acids 77 and 82 of SEQ ID NO: 1.

[0016] In related aspects, the invention includes a polynucleotide comprising a nucleotide sequence encoding any of the polypeptides described herein. For example, the invention includes a polynucleotide that comprises a nucleotide sequence that encodes a chimeric peptide described herein. Polynucleotides that are DNA, RNA, and that comprises synthetic or modified nucleotides are contemplated. In some embodiments, the polynucleotide comprises a nucleotide sequence at least 90% identical to or at least 95% identical to any one of SEQ ID NOs: 65-74, 87-98, 101-102, and 105-106.

[0017] The invention further includes, in some aspects, a vector comprising a polynucleotide as disclosed herein. In some variations, the vector comprises an expression vector comprising a polynucleotide of the disclosure operably linked to an expression control sequence. Vectors suitable for expression in all varieties of host cells are contemplated, including prokaryotic expression vectors and eukaryotic expression vectors. Exemplary eukaryotic expression vectors include vectors for expression in mammalian cells, insect cells, plant cells, avian cells, amphibian cells, and fungal cells, including yeast cells.

[0018] In further aspects, the invention includes a recombinant host cell comprising a vector or expression vector as disclosed herein. In some embodiments, the host cell is (a) a eukaryotic cell selected from the group consisting of mammalian, fungal (*e.g.*, yeast), insect, plant, amphibian and avian cells; or (b) a prokaryotic cell.

[0019] The invention also includes a composition comprising a vector as described herein in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant.

[0020] In some aspects, the invention includes a virus-like particle (VLP) comprising or consisting essentially of one or more chimeric peptides or proteins described herein. In further aspects, the invention includes an article comprising a chimeric peptide or protein as described herein or a VLP as described herein attached to a solid support. In some embodiments, the solid support is a microbead, an assay plate, a test strip, or a filter. In some variations, the article further includes a distinct peptide attached to the article, optimally at a spatially distinct location, that can serve as a positive or negative control in assays described herein. In still other variations, the article is packaged as part of a kit with at least one assay reagent, such as an immunoassay reagent.

[0021] The invention also includes a composition comprising a peptide or protein described herein, or a chimeric peptide or protein described herein, and a pharmaceutically acceptable diluent, adjuvant, excipient, stabilizer, preservative, or carrier. In some variations, the disclosure provides an antigenic composition comprising a VLP as disclosed herein, wherein the VLP is present in the composition at a concentration of about 0.1-2000 µg/ml of core antigen, in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant.

[0022] In still additional variations, the invention includes an antigenic composition comprising two or more different polypeptides, chimeric polypeptides, or VLP's described herein. For instance, the invention includes first and second VLP described herein, wherein the first and second VLP comprise different sequences independently selected from amino acid sequences at least 80% identical to SEQ ID NOs: 2-11, 22-33, 46-47, and 50-51. For

embodiments of this nature, ordinals such as "first" or "second" are intended simply to differentiate one from another, and are not intended to imply an order. Such compositions optionally further includes a pharmaceutically acceptable diluent, adjuvant, excipient, stabilizer, preservative, or carrier.

[0023] In a related embodiment, the invention is an antigenic composition comprising first, second, and third VLP as described herein. For instance, the first, second, and third VLP comprise different sequences independently selected from amino acid sequences at least 80% identical to SEQ ID NOs: 2-11, 22-33, 46-47, and 50-51.

[0024] In another related embodiment, the invention is an antigenic composition comprising first, second, third, fourth, fifth, sixth, and seventh VLP as described herein. For example, the first, second, third, fourth, fifth, sixth, and seventh VLP comprise different sequences independently selected from SEQ ID NOs: 2-11, 22-33, 46-47, and 50-51.

[0025] The invention also includes a kit comprising a VLP as described herein, or comprises an article of manufacture as described herein, packaged with at least one reagent useful for performing an immunoassay. Exemplary suitable reagents include an enzyme substrate, a detection antibody, positive and negative control reagents, substrate/s detection solution, and washing, blocking, and diluent buffer. In some embodiments, the kit includes a specific apparatus used for the execution of the protocol and for the detection.

[0026] The invention further includes an antigenic composition comprising a peptide, a chimeric peptide or chimeric protein, or a VLP as described herein in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant, wherein the composition is capable of generating an immune response to a Zika virus. An exemplary immune response includes antibody generation or a protective immune response in a mammalian subject. Desirably, the antibody response generated by the composition is improved relative to or compared to an immune response achieved with live Zika virus or Zika Envelope (E) recombinant protein. In some variations, the antibody response is a protective and functional against Zika virus by neutralizing activity, and/or antibody dependent cell-mediated cytotoxicity (ADCC), and/or antibody dependent cell-mediated phagocytosis (ADCP), and/or complement-dependent cytotoxicity (CDC), and/or T cell response (e.g. CD4+ and CD8+) and/or other protective immune mechanisms.

[0027] In still additional embodiments, the invention includes a vaccine comprising a peptide, chimeric peptide, chimeric protein, VLP, or antigenic composition as described herein and an adjuvant. In some embodiments, the adjuvant is a polymeric particle, cholera toxin, or

imidazoquinoline. In further embodiments, the adjuvant formulations include the classical aluminum-based adjuvants, and novel classes of adjuvants such as liposomes (e.g., CAF01), agonists of pathogen recognition receptors (e.g. Immune stimulating complexes (ISCOMs), Lipid A analogs (MPL, RC-529, and GLA), double stranded RNA analogs (e.g.Poly I:C and Poly ICLC), cytidine monophosphate guanosine oligodeoxynucleotide (e.g. CpG, CpG ODN), flagellin, imidazoquinoline (Imiquimod and Resiquimod), polymeric particles (e.g. Chitosan), emulsions (e.g. squalene oil-based), cytokines (e.g. Interleukin-12), bacterial toxins (e.g Cholera Toxin (CT) or Escherichia coli enterotoxin (LT)), Quil A and other saponins known in the art, and the plant polysaccharide inulin [12].

[0028] The invention also includes methods of making and methods of using any of the foregoing compounds, compositions, articles of manufacture, apparatuses, and/or materials. Furthermore, it should be understood that aspects of the inventions that are described herein as methods can alternatively be described as “uses” of the compounds, compositions, articles, apparatuses and/or materials. All equivalent “uses” are also contemplated as aspects of the invention.

[0029] In some variations, the invention includes a method of producing an immune response to a Zika virus in a subject, the method comprising administering to the subject an effective amount of an antigenic composition or a vaccine as described herein, thereby producing (causing the subject’s immune system to generate) an immune response to a Zika virus in the subject. In related variations, the disclosure provides an antigenic composition or vaccine for use in producing an immune response to a Zika virus in a subject characterized in that producing the immune response comprises administering to the subject an effective amount of an antigenic composition or a vaccine as described herein, thereby producing (causing the subject’s immune system to generate) an immune response to a Zika virus in the subject.

[0030] In some variations, the invention includes a method of treating a Zika virus infection in a subject in need thereof, the method comprising administering to the subject an effective amount of an antigenic composition described herein, thereby treating a Zika virus infection in the subject. In related variations, the disclosure provides an antigenic composition for use in treating a subject in need thereof, characterized in that the treating comprises administering to the subject an effective amount of an antigenic composition described herein, thereby treating a Zika virus infection in the subject.

[0031] In still additional variations, the invention includes a method of preventing a disease or disorder caused by a Zika virus infection in a subject, the method comprising

administering to the subject an effective amount of an antigenic composition or a vaccine as described herein, in an amount effective to prevent a disease or disorder caused by a Zika virus infection in the subject. In related variations, the disclosure provides an antigenic composition or a vaccine for use in preventing a disease or disorder caused by a Zika virus infection in a subject, characterized in that the use comprises administering to the subject an effective amount of an antigenic composition or a vaccine as described herein, in an amount effective to prevent a disease or disorder caused by a Zika virus infection in the subject.

[0032] The invention also includes a method of protecting a subject from developing one or more symptoms of Zika virus infection, the method comprising administering to the subject a vaccine composition as described herein, in an amount effective to protect the subject from developing one or more symptoms of Zika virus infection. In related variations, the method is effective to reduce the number, severity, or duration of symptoms of a Zika virus infection. In some embodiments, the reduction in symptoms is measured as a reduction in viral load or viral copy number in the subject. In further aspects, the disclosure provides a vaccine composition for use in protecting a subject from developing symptoms of Zika virus infection, characterized in that the protecting comprises administering to the subject a vaccine composition as described herein, in an amount effective to protect the subject from developing symptoms of Zika virus infection.

[0033] In still another related embodiment, the invention includes a method of immunizing a mammalian subject against a Zika virus infection comprising administering to the subject an effective amount of an antigenic composition described herein or a vaccine described herein. In some aspects, the disclosure provides an antigenic composition or a vaccine of the disclosure for use in immunizing a mammalian subject against a Zika virus infection, characterized in that the immunizing comprises administering to the subject an effective amount of an antigenic composition described herein or a vaccine described herein.

[0034] In some aspects, the disclosure provides a method of protecting a subject from sexual transmission of Zika virus, comprising administering to the subject an effective amount of an antigenic composition or vaccine of the disclosure, thereby protecting the subject from sexual transmission of Zika virus. In some aspects, the disclosure provides an antigenic composition or a vaccine for use in protecting a subject from sexual transmission of Zika virus, characterized in that the protecting comprises administering to the subject an effective amount of an antigenic composition or vaccine of the disclosure, thereby protecting the subject from sexual transmission of Zika virus. In some embodiments, the administering is mucosal

administration. In further embodiments, the mucosal administration is nasal, vaginal, rectal, or oral.

[0035] The materials and methods described herein also are useful for quantifying or detecting a Zika immune response after infection and/or vaccination. For instance, the invention includes a method of detecting or measuring antibodies to Zika virus in a biological sample comprising:

a) contacting a VLP as described herein with a biological sample under conditions suitable for the formation of an antigen-antibody complex; and

b) measuring or detecting antibodies to Zika virus by detecting or measuring an antigen-antibody complex formed between antibodies in the biological sample and the VLP.

[0036] The invention further includes a method of detecting a Zika virus infection comprising steps of:

a) contacting the VLP as described herein with a biological sample from a mammalian subject under conditions suitable for the formation of an antigen-antibody complex; and

b) detecting the antigen-antibody complex formed between the VLP and antibodies in the biological sample, thereby detecting the Zika virus infection.

[0037] In some variations, the foregoing method further comprises a step of detecting the Zika virus in the biological sample, wherein presence of the Zika virus indicates a current Zika virus infection.

[0038] The invention also includes a method for screening antibodies comprising steps of:

a) measuring binding of an antibody or fragment thereof to a VLP as described herein;

b) measuring binding of the antibody or fragment thereof to a Woodchuck Hepatitis core Antigen protein (WHcAg) VLP or protein; and

c) determining that the antibody or fragment thereof is an anti-Zika antibody when the antibody or fragment thereof binds to the VLP but not the WHcAg.

Such a method is particularly useful for evaluating antibodies produced following an immunization with VLP described herein and/or Zika virus infection.

[0039] In any of the foregoing methods, some variations involving using the VLP in solution or suspension. In other variations, the VLP is attached to a solid support, such as any of a microbead, an assay plate, a test strip, or a filter.

[0040] The invention also includes methods of making the VLP described herein. For instance, the invention includes a method of producing a VLP comprising introducing into a host cell the vector of claim 6 under conditions such that the cell produces the VLP. In some variations, the host cell is a eukaryotic cell, such as a mammalian cell, a fungal or yeast cell, an insect cell, a plant cell, an amphibian cell, or an avian cell. In still other variations, the cell is a prokaryotic cell, such as a bacterial cell. An exemplary yeast host cell is a *Pichia pastoris* cell (*e.g.*, *Komagataella phaffii* Kurtzman (ATCC® 76273™)). In some variations, the vector is introduced into the host cell via transformation, transfection, transduction, or electroporation. In some variations, the cells are cultured at temperatures ranging from 25°C to 37°C in an incubator or fermenter or shaker, in continuous agitation and oxygenation. Optionally, the VLP produced according to such a method is purified from the host cell or a culture media of the host cell. Exemplary suitable procedures for VLP purification include precipitation, ultracentrifugation, density gradient ultracentrifugation, ultrafiltration such as tangential flow filtration (TFF) and other methods, chromatography, or a combination thereof.

[0041] The invention also includes a VLP produced by any of the foregoing methods.

[0042] Reference throughout this specification to "one embodiment", "some embodiments" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. The particular features, structures, or characteristics described herein may be combined in any suitable manner, and all such combinations are contemplated as aspects of the invention.

[0043] Unless otherwise specified the use of the ordinal adjectives "first", "second", "third", etc., to describe a common object, merely indicate that different instances of like objects are being referred to, and are not intended to imply that the objects so described must be in a given sequence, either temporally, spatially, in ranking, or in any other manner.

[0044] The headings herein are for the convenience of the reader and not intended to be limiting. Additional aspects, embodiments, and variations of the invention will be apparent from the Detailed Description and/or Drawing and/or claims.

[0045] Although the Applicant invented the full scope of the invention described herein, the Applicant does not intend to claim subject matter described in the prior art work of others. Therefore, in the event that statutory prior art within the scope of a claim is brought to the attention of the Applicant by a Patent Office or other entity or individual, the Applicant reserves the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a claim to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] **Figure 1** depicts elements of the WHcAg VLP system as disclosed herein for epitope delivery. The depictions on the left show a WHcAg core antigen peptide and VLP comprised of such peptides. The images on the right show a chimeric peptide of the invention and a VLP comprised of such peptides. The dark black portions depict the displayed ZIKV epitope.

[0047] **Figure 2** depicts an example of a DNA construct for WHcAg chimeric VLP expression in a yeast system.

[0048] **Figures 3A and 3B** depict the structural vaccinology strategy that was applied for developing WHcAg-ZIKV chimeric VLPs using the Envelope protein Domain III (EDIII). In Figure 3B the EDIII sub-structural domain CD Loop is included for composition of the WHcAg-ZIKV chimeric VLP.

[0049] **Figure 4** shows a flow chart for production, purification, and quality testing of WHcAg-ZIKV chimerics.

[0050] **Figure 5** shows a WHcAg VLP analyzed by electron microscopy. Scale bar = 50 nanometers (nm).

[0051] **Figure 6** illustrates dot blot and Western blot analysis showing WHcAg-ZIKV chimeric VLP production and antigenicity. Figure 6A demonstrates WHcAg production and purification form Pichia culture, WHcAg VLPs are detected using the commercially available monoclonal antibody HepBcAg. Figures 6B and 6C show WHcAg-ZIKV chimeric VLPs

antigenicity using commercially available monoclonal antibodies such as ZV-2 and ZV-54 specific for ZIKV EDIII .

[0052] **Figure 7** illustrates dot blot analysis for WHcAg-ZIKV chimeric VLPs antigenicity using anti-Zika virus antibody for mouse serum, prME VLPs and ZIKV E recombinant protein are used as a positive controls for the assay.

[0053] **Figure 8** illustrates ELISA analysis of mouse serum immunized with different WHcAg-ZIKV chimeric VLPs for IgG titer (A), IgG1 titer (B) and IgG2a titer (C). The limit for level of detection is 100 (dotted line).

[0054] **Figure 9** illustrates dot blot analysis of serum pools from animals immunized with different WHcAg-ZIKV chimeric VLPs using Zika Virus (ZIKV) Envelope (E) recombinant protein and Dengue Virus 2 (DENV-2) E recombinant protein as antigen (Figure 9A). Commercially available monoclonal antibodies (mAb) are used for assay control (Figure 9B).

[0055] **Figure 10** shows immunofluorescence microscopy experiment demonstrating that serum form immunized mice with WHcAg CD loop VLP vaccine candidate induces antibodies able to recognize Zika virus in infected Vero cell in culture (left panel); the serum from the placebo control is used as a negative control in such experiment (right panel).

[0056] **Figure 11** demonstrates that WHcAg CD loop VLP vaccine candidate induced protective antibodies against Zika Virus in a mouse model. Figure 11A shows antibody dependent cell-mediated cytotoxicity (ADCC) assay: mouse serum immunized with WHcAg CD loop VLPs exert protective activity of antibodies against Zika Virus; the serum from animals immunized with placebo control WHcAg CTRL is included as a negative control and serum from an animal immunized with live Zika virus (#426) is used as an additional control. Figure 11B illustrates complement dependent cytotoxicity (CDC) assay: WHcAg CD loop VLPs induces CDC activity in mice immunized with such vaccine candidate in respect placebo controls (WHcAg CTRL) and an animal immunized with live Zika virus (#426).

[0057] **Figure 12** depicts an exemplary plate, test strip, and microbead of the invention.

[0058] **Figure 13** is a depiction of a test strip of the invention and of detection of Zika virus infection using viral epitopes expressed in VLPs using a Lateral Flow Immunoassay (LFIA) system (see Example 5).

[0059] **Figure 14** shows WHcAg-ZIKV chimeric VLP Lateral Flow Immunoassay Application (LFIA).

[0060] **Figure 15** shows mouse models utilized for testing efficacy, safety and protection for WHcAg-ZIKV chimera VLPs vaccine candidates.

[0061] **Figure 16** shows a mouse model utilized for testing ZIKV intrauterine transmission protection by WHcAg-ZIKV chimera VLPs vaccine candidates.

[0062] **Figure 17** shows results of experiments analyzing serum viremia in mice 3 days viral post-injection using quantitative Real-Time PCR (qRT-PCR).

DETAILED DESCRIPTION

[0063] The morphology of VLPs is pivotal for their strong immune-stimulatory activity: i) VLPs are more efficiently recognized by antigen presenting cells (APCs); ii) VLPs are trafficked from the site of injection to the lymph nodes; iii) the VLP structure presents a repetitive arrangement of antigens that stimulates B-cells for the humoral immune response, and T-cells for cell mediated immune response [13, 14].

[0064] The majority of FDA approved VLP-based vaccines are currently manufactured in yeast due to ease of scalability. Aspects of the present invention are directed to a ZIKV VLP (ZIK-VLP)-based vaccine and uses of it. In some embodiments, the VLP is produced using a yeast expression system, applying structural vaccinology for the optimization of VLP immunogenicity: antigen determinants are selectively engineered for achieving high level of immunogenicity, ZIKV specificity, and enhanced inter-strain protection [15, 16].

[0065] Terms used herein generally have the meaning that scientists in the field would ascribe to them. The following definitions will assist understanding of the invention.

[0066] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0067] "Conservative amino acid substitution" refers to the interchange of a residue having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine- valine, and asparagine-glutamine.

[0068] The term "nucleic acid" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end.

[0069] The term "encoding" refers to a polynucleotide sequence encoding one or more amino acids. The term does not require a start or stop codon. An amino acid sequence can be encoded in any one of six different reading frames provided by a double-stranded polynucleotide sequence. In some variations, encoding sequences further include a start and/or a stop codon.

[0070] A "vector" refers to a polynucleotide, which when independent of the host chromosome, is capable of replication in a host organism. Examples of vectors include plasmids. Vectors typically have an origin of replication. Vectors can comprise, e.g., transcription and translation terminators, transcription and translation initiation sequences, and promoters useful for regulation of the expression of the particular nucleic acid.

[0071] The term "recombinant' when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified and that retains the modification, such as a daughter cell. Thus, for example, recombinant cells express genes that are not found within the native (nonrecombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all.

[0072] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. "Substantially identical" refers to two or more nucleic acids or polypeptide sequences having a specified percentage (or specified minimum percentage) of amino acid

residues or nucleotides that are the same (*i.e.*, (at least) 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the sequence comparison algorithms below or by manual alignment and visual inspection. This definition also refers to the complement of a test sequence. Optionally, the identity or substantial identity exists over a region that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides or amino acids in length.

[0073] A "non-native amino acid" in a protein sequence refers to any amino acid other than the amino acid that occurs in the corresponding position in an alignment with a naturally-occurring polypeptide with the lowest smallest sum probability where the comparison window is the length of the monomer domain queried and when compared to a naturally-occurring sequence in the non-redundant ("nr") database of Genbank using BLAST 2.0. BLAST 2.0 is described in the art [17], respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

[0074] As used herein, the terms "virus-like particle" and "VLP" refer to a structure that resembles a virus. VLPs of the present disclosure lack a viral genome and are therefore noninfectious. Preferred VLPs of the present disclosure are derived from Woodchuck Hepatitis core Antigen (WHcAg) and thus have a VLP structure or arrangement similar to WHcAg VLPs. Virus-like particles show improved efficiency in stimulating the immune system because they resemble the morphology of a virion displaying a densely repetitive array of epitopes in a limited space. Furthermore, VLPs are very safe candidates for vaccine development due to their lack of replicating viral genetic material rendering them unable to cause viral disease. During the last decade, advancement in VLP production, purification, and adjuvant optimization has led to the licensing of several VLP-based vaccines for the prevention of infectious diseases [12] such as human papilloma virus (HPV), hepatitis B virus (HBV), hepatitis E virus (HEV), and influenza. Furthermore, several clinical trials are currently ongoing for VLP vaccines against influenza, norovirus, and chikungunya virus (CHIK) (https://clinicaltrials.gov/).

[0075] The term "Woodchuck Hepatitis Virus" is used interchangeably herein with the term "Woodchuck Hepadnavirus" and refers to the virus species that expresses the core Antigen protein used as a platform for recombinant VLPs.

[0076] The term "chimeric" refers to a fusion of polypeptide and/or peptides sequences. "Chimeric" as used in reference to a Woodchuck Hepatitis core Antigen (WHcAg) refers to a fusion protein of the WHcAg and an unrelated antigen (e.g., a viral peptide and variants thereof). For instance, in some embodiments, the term "chimeric peptide" or "chimeric protein" refers to a fusion protein comprising both a WHcAg component (full length, or partial) and a Zika peptide or a fragment thereof. As described herein, some fusions take the form of insertions, where a Zika sequence is inserted within a WHcAg sequence.

[0077] The term "heterologous" with respect to a nucleic acid, or a polypeptide component, indicates that the component occurs where it is not normally found in nature (e.g., relative to an adjacent component) and/or that it originates from a different source or species.

[0078] An "effective amount" or a "sufficient amount" of a substance is that amount necessary to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. In the context of administering an antigenic composition, an effective amount contains sufficient antigen (e.g., a VLP comprising a chimeric peptide of the disclosure) to elicit an immune response. An effective amount can be administered in one or more doses. Efficacy can be shown in an experimental or clinical trial, for example, by comparing results achieved with a substance of interest compared to an experimental control.

[0079] The term "dose" as used herein in reference to an antigenic composition refers to a measured portion of the antigenic composition taken by (administered to or received by) a subject at any one time.

[0080] The term "about" as used herein in reference to a value, encompasses from 90% to 110% of that value (e.g., about 200 µg VLP refers to 180 µg to 220 µg VLP).

[0081] The term "vaccination" as used herein refers to the introduction of vaccine into a body of an organism.

[0082] A "subject" is a living multi-cellular vertebrate organism. In the context of this disclosure, the subject can be an experimental subject, such as a non-human mammal (e.g., a mouse, a rat, or a non-human primate). Alternatively, the subject can be a human subject.

[0083] An "antigenic composition" is a composition of matter suitable for administration to a human or animal subject (e.g., in an experimental or clinical setting) that is capable of eliciting a specific immune response, e.g., against a pathogen, such as Zika virus. As such, an antigenic composition includes one or more antigens (for example, peptide

antigens) or antigenic epitopes. An antigenic composition can also include one or more additional components capable of eliciting or enhancing an immune response, such as an excipient, carrier, and/or adjuvant. In certain instances, antigenic compositions are administered to elicit an immune response that protects the subject against symptoms or conditions induced by a pathogen. In some cases, symptoms or disease caused by a pathogen is prevented (or reduced or ameliorated) by inhibiting replication of the pathogen (e.g., virus) following exposure of the subject to the pathogen. In the context of this disclosure, the term antigenic composition will be understood to encompass compositions that are intended for administration to a subject or population of subjects for the purpose of eliciting a protective or palliative immune response against a virus.

[0084] "Adjuvant" refers to a substance which, when added to a composition comprising an antigen, nonspecifically enhances or potentiates an immune response to the antigen in the recipient upon exposure. Common adjuvants include suspensions of minerals (alum, aluminum hydroxide, aluminum phosphate) onto which an antigen is adsorbed; emulsions, including water-in-oil, and oil-in-water (and variants thereof, including double emulsions and reversible emulsions), liposaccharides, lipopolysaccharides, immunostimulatory nucleic acids (such as CpG oligonucleotides), liposomes, Pattern Recognition Receptor (PRR) agonists (e.g. NALP3. RIG-I-like receptors (RIG-I and MDA5), and Toll-like Receptor agonists (particularly, TLR2, TLR3, TLR4, TLR7/8 and TLR9 agonists)), and various combinations of such components [12].

[0085] An "immune response" is a response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus, such as a pathogen or antigen (e.g., formulated as an antigenic composition or a vaccine). An immune response can be a B cell response, which results in the production of specific antibodies, such as antigen specific neutralizing antibodies. An immune response can also be a T cell response, such as a $CD4^+$ response or a $CD8^+$ response. B cell and T cell responses are aspects of a "cellular" immune response. An immune response can also be a "humoral" immune response, which is mediated by antibodies. In some cases, the response is specific for a particular antigen (that is, an "antigen-specific response"). If the antigen is derived from a pathogen, the antigen-specific response is a "pathogen-specific response." A "protective immune response" is an immune response that inhibits a detrimental function or activity of a pathogen, reduces infection by a pathogen, or decreases symptoms (including death) that result from infection by the pathogen. A protective immune response can be measured, for example, by viral and immune assays using a serum sample from an immunized subject for testing the ability of serum antibodies for inhibition of viral

replication, such as: plaque reduction neutralization test (PRNT), ELISA-neutralization assay, antibody dependent cell-mediated cytotoxicity assay (ADCC), complement-dependent cytotoxicity (CDC), antibody dependent cell-mediated phagocytosis (ADCP). In addition, vaccine efficacy can be tested by measuring the T cell response CD4+ and CD8+ after immunization, using flow cytometry (FACS) analysis or ELISpot assay. The protective immune response can be tested by measuring resistance to pathogen challenge *in vivo* in an animal model. In humans, a protective immune response can be demonstrated in a population study, comparing measurements of infection, symptoms, morbidity, mortality, etc. in treated subjects compared to untreated controls. Exposure of a subject to an immunogenic stimulus, such as a pathogen or antigen (*e.g.*, formulated as an antigenic composition or vaccine), elicits a primary immune response specific for the stimulus, that is, the exposure "primes" the immune response. A subsequent exposure, *e.g.*, by immunization, to the stimulus can increase or "boost" the magnitude (or duration, or both) of the specific immune response. Thus, "boosting" a preexisting immune response by administering an antigenic composition increases the magnitude of an antigen (or pathogen) specific response, (*e.g.*, by increasing antibody titer and/or affinity, by increasing the frequency of antigen specific B or T cells, by inducing maturation effector function, or a combination thereof).

[0086] An "improved" antibody response is measured by a difference such as: protection from Zika Virus replication and viremia; neutralizing antibody titer; antibody dependent cell-mediated cytotoxicity (ADCC); complement dependent cytotoxicity (CDC), antibody dependent cell-mediated phagocytosis (ADCP); stimulation of B cell immune memory; activation of immune cells such as B cells, T cell and Antigen Presenting Cells (APC); protection from disease symptoms such as fever, pain, weight loss; weakness, maculopapular rash, Zika Congenital Syndrome (microcephaly), Guillain-Barré Syndrome. Such differences are measured in a population study in which treated subjects are compared with untreated control subjects.

[0087] The phrase "specifically (or selectively) binds," when referring to the interaction between an antibody or fragment thereof and a VLP, a peptide, a chimeric protein, or a chimeric peptide as disclosed herein, refers to a binding reaction that can be determinative of the presence of the polypeptide in a heterogeneous population of proteins (*e.g.*, a cell or tissue lysate) and other biologics. Thus, under standard conditions used in antibody binding assays, the specified VLP, peptide, or chimeric peptide binds to a particular target antibody or fragment thereof above background (*e.g.*, 2X, 5X, 10X or more above background) and does not bind in a

significant amount to other molecules present in the sample. Of particular interest herein are antibodies that recognize Zika virus but not Dengue virus or other flaviviruses.

[0088] As used herein, an "expression vector" is a DNA construct that contains a structural gene operably linked to an expression control sequence so that the structural gene can be expressed when the expression vector is transformed into an appropriate host cell. Two DNA sequences are said to be "operably linked" if the biological activity of one region will affect the other region and also if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of the desired sequence, or (3) interfere with the ability of the desired sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a desired DNA sequence if the promoter were capable of effecting transcription of that desired DNA sequence. As described herein, vectors suitable for expression in all varieties of host cells are contemplated, including prokaryotic expression vectors and eukaryotic expression vectors. Exemplary eukaryotic expression vectors include vectors for expression in mammalian cells, avian cells, insect cells, amphibian cells, plant cells, and fungal cells, including yeast cells.

[0089] Conventional or known techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology can be used to implement many elements of the invention. Such techniques are not always described herein in detail because they are known and/or are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989); Current Protocols in Molecular Biology (Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Culture of Animal Cells: A Manual of Basic Technique (Freshney, 1987); Harlow et al., Antibodies: A Laboratory Manual (Harlow et al., 1988); and Current Protocols in Immunology (Coligan et al., eds., 1991).

[0090] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

Zika virus

[0091] Zika virus (ZIKV), a Flaviviridae family member, is a single-stranded, positive-sense RNA virus with an approximate 10.7 Kb genome encoding a single polyprotein that is cleaved into three structural proteins (C, prM/M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) by viral and host proteases [4, which is incorporated by reference herein in its entirety]. The overall structure of ZIKV soluble envelope

(E) protein resembles previously reported flavivirus E protein structures and has three distinct domains: a central b-barrel (domain I or domain 1), an elongated finger-like structure (domain II or domain 2), and a C-terminal immunoglobulin-like module (domain III or domain 3) [18]).

Chimeric Peptide Constructs

[0092] Some aspects of the invention comprise chimeric peptide or protein constructs having at least one portion comprised of, or derived from, a rodent hepadnavirus core antigen attached to at least one portion comprised of, or derived from, a Zika virus protein antigen. In some embodiments, the portions are joined by peptide bonds to form a chimeric polypeptide, as described below in greater detail.

A. Rodent Hepadnavirus Core Antigens

[0093] In some aspects, the chimeric hepadnavirus portion of the chimeric construct is engineered from a rodent hepadnavirus core antigen amino acid sequence. For instance, one or more endogenous B cell epitopes from the native core antigen amino acid sequence are effectively removed. Hepadnavirus core antigens are generally described in U.S. Patent Application Publication No. 2016/0022801, which is incorporated by reference herein in its entirety.

[0094] Exemplary rodent hepadnavirus core antigens suitable for this component/portion of the chimeric construct include woodchuck (WHcAg), ground squirrel (GScAg), arctic ground squirrel (AGScAg) and human (HBcAg) hepadnavirus core antigens. An exemplary amino acid sequence of woodchuck hepadnavirus core antigen is set out in SEQ ID NO: 1, and is also available as GenBank accession number NP_671816. Rodent hepadnavirus core antigens have a number of properties that make them particularly useful for making the chimeric constructs described herein. For instance, they will self-aggregate/assemble into a multimeric complex or VLP. The basic subunit of the core particle is a 21 kDa protein monomer (schematically depicted in Figure 1, top left) that spontaneously assembles into a 240 subunit particulate structure of about 34 nm in diameter (Figure 1, bottom left). The tertiary and quaternary structures of hepadnavirus core particles have been elucidated [19, incorporated herein by reference]. The immunodominant B cell epitope on WHcAg is localized around amino acids 76-82 of SEQ ID NO: 1 [20] forming a loop connecting adjacent alpha-helices. This observation is consistent with the finding that a heterologous antigen inserted within the 76-82 loop region of HBcAg was significantly more antigenic and immunogenic than the antigen inserted at the N- or C-termini and, importantly, more immunogenic than the antigen in the context of its native protein [20].

[0095] In some embodiments, the chimeric constructs of the invention are comprised of a hepadnavirus portion that is based on a woodchuck hepadnavirus core antigen. For example, the portion used, when aligned with SEQ ID NO:1, has an amino acid sequence that is at least 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to SEQ ID NO:1. The amino acid variation, relative to wildtype, can be any variation that does not destroy the self-assembling properties of the wildtype protein. In some variations, the variation does not increase antigenicity of the protein, compared to wildtype. In some variations, the changed amino acids are conservative substitution variants. Sequence variation can also be expressed as a limited number (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid sequence differences between the wildtype sequence and the aligned sequence used in the present invention.

[0096] As described below, the chimeric construct preferably comprises a Zika peptide or polypeptide sequence insert that disrupts and/or replaces the B cell epitope region of the core antigen sequence. For purposes of sequence identity analysis in the preceding paragraphs, the changes to the B cell epitope and the Zika insert are ignored.

B. Zika-derived Peptides

[0097] A peptide or protein identical to or derived from a Zika virus amino acid sequence is used in the chimeric constructs of the invention. The Zika portion has been chosen for its immunogenicity properties. In preferred variations, the Zika portion comprises, or is derived from, a Zika Virus Envelope (E), NS1, prM, or C protein. In some variations, the Zika portion comprises, or is derived from, domain 3 of a Zika Virus E protein. An exemplary domain 3 sequence is set forth in SEQ ID NO: 2. The use of peptides with sequence variation is contemplated, so long as the peptide still comprises sequence that acts as an epitope that will generate an immune response that recognizes wildtype Zika protein or wildtype Zika virus. For instance, the peptide or protein used comprises an amino acid sequence that is at least 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to SEQ ID NO: 2. Sequence variation can also be expressed as a limited number (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid sequence differences between the wildtype sequence and the aligned sequence used in the present invention. In some variations, the Zika portion comprises, or is derived from, NS1. An exemplary NS1 sequence is set forth in SEQ ID NO: 22. The use of peptides with sequence variation is contemplated, so long as the peptide still comprises sequence that acts as an epitope that will generate an immune response that recognizes wildtype Zika NS1 protein or wildtype Zika virus. For instance, the peptide used comprises an

amino acid sequence that is at least 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to SEQ ID NO: 22. Sequence variation can also be expressed as a limited number (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid sequence differences between the wildtype sequence and the aligned sequence used in the present invention. In some variations, the Zika portion comprises, or is derived from, prM/M protein. An exemplary prM/M protein sequence is set forth in SEQ ID NO: 46. The use of peptides with sequence variation is contemplated, so long as the peptide still comprises sequence that acts as an epitope that will generate an immune response that recognizes wildtype Zika prM/M protein or wildtype Zika virus. For instance, the peptide used comprises an amino acid sequence that is at least 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to SEQ ID NO: 46. Sequence variation can also be expressed as a limited number (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid sequence differences between the wildtype sequence and the aligned sequence used in the present invention.

[0098] In some embodiments, the peptide derived from Zika is a polypeptide of from 4 to 200 amino acids in length. In some embodiments, the peptide is from 5 to 150 amino acids in length, or from 5 to 100 amino acids in length, or from 5 to 55 amino acids in length, preferably 10 to 50 amino acids in length, preferably 15 to 45 amino acids in length, or preferably 20 to 40 amino acids in length. In some embodiments, the length of the peptide is within any range having a lower limit of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids and an independently selected upper limit of 200, 195, 190, 185, 180, 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 125, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25 or 20 amino acids in length, provided that the lower limit is less than the upper limit. All integer lengths from 4 – 200 amino acids are specifically contemplated.

[0099] In some embodiments, the peptide derived from Zika is itself a fusion protein comprising fragments of two, three, four or five different Zika peptides. In various embodiments, the peptide comprises or consists of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51. In further embodiments, the peptide is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51.

[0100] As described more fully below, the core antigen used herein is modified to include one or more Zika virus epitopes.

C. Combinatorial Technology

[0101] In some embodiments, the peptide derived from Zika is inserted into the peptide derived from the Hepadnavirus core protein (schematically depicted in Figure 1, right top) at a location that preserves the self-assembly properties of the core protein and that presents the peptide or protein derived from Zika in an antigenic manner (Figure 1, right bottom).

[0102] Several groups working with the HBcAg or with other VLP technologies (*e.g.*, the L1 protein of the human papillomavirus and Qβ phage) have opted to chemically link the foreign epitopes to the VLPs rather than inserting the epitopes into the particles by recombinant methods. Such embodiments are contemplated as one aspect of the invention. The chemically conjugation approach for linking heterologous antigens has been circumvented by identification of suitable insertions sites for chimeric proteins, identifiable, e.g., by combinatorial technology. (See [21]). Such techniques were used to determine 17 different insertion sites and 28 modifications of the WHcAg C-terminus that together favor assembly of chimeric particles, as well as the identification of a number of additional improvements (see, *e.g.*, U.S. Pat. Nos. 7,144,712; 7,320,795; and 7,883,843, all incorporated herein by reference). ELISA-based screening systems have been developed that measure expression levels, VLP assembly, and insert antigenicity using crude bacterial lysates, avoiding the need to employ labor-intensive purification steps for VLPs that do not express and/or assemble well.

[0103] A number of insertion sites inside the loop region (positions 76-82), as well as outside the loop region are tolerated by WHcAg. In some embodiments, the peptides or proteins are inserted directly or optionally with linker(s) at one or both ends of the Zika peptide. For example, the chimeric peptides or proteins set out in SEQ ID NOs: 12-21, 34-45, 48-49, and 52-53 contain portions that originate from the WHcAg (the non-underlined sequences in each of SEQ ID NOs: 12-21, 34-45, 48-49, and 52-53) and portions that are the peptide derived from Zika (the underlined sequences in SEQ ID NOs: 12-21, 34-45, 48-49, and 52-53).

[0104] SEQ ID NOs: 2-11 were obtained via structure analysis of Envelope (E) protein (see Examples and Figure 3). The sequences were selected for their adaptability with the scaffolding system, i.e., the Woodchuck Hepatitis core Antigen (WHcAg) protein (Table 1). Specifically, SEQ ID NOs: 2-7 were generated from the Envelope Domain 3 with amino acid sequence very specific for Zika Virus. SEQ ID NO: 8 was generated from Fusion Loop Domain that shares very similar amino acid sequence between flavivirus (*e.g.*, Dengue Virus, Yellow Fever Virus, West Nile Virus). SEQ ID NOs: 9 and 10 were generated from Envelope Domain 2 with amino acid sequence very specific for Zika Virus. Finally, SEQ ID NO: 11 was generated from Envelope Domain 1 with amino acid sequence very specific for Zika Virus.

Table 1

SEQUENCE ID NO	VIRUS-LIKE PARTICLE PROTEIN	AMINO ACID SEQUENCE
1	Woodchuck Hepatitis Core Antigen (WHcAg)	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYE EELTGREHCSPHHTAIRQALVCWDELTKLIAWMSSNITS EQVRTIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQE FLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGAR ASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
SEQUENCE ID NO	**ZIKV E ENVELOPE ANTIGEN**	**AMINO ACID SEQUENCE**
2	Envelope domain 3 full length	HLKCRLKMDKLRLKGVSYSLCTAAFTFTKIPAETLHGTV TVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSG STIGKAFEATVRGAKRMAV
3	Envelope domain 3 G (EDIII) G loop-truncated	AFTFTKIPAETLHGTVTVELQYAGTDGPCKVPAQMAVDM QTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIV IG
4	Envelope domain 3, A-B loop	AFTFTKIPAETLHGTVTVELQYA
5	Envelope domain 3, CXCDDX loop (CD loop)	PCKVPAQMAVDMQTLTPVGRLITANPVIT
6	Envelope domain 3, DX-E loop	RLITANPVITESTENSKMMLELDP
7	Envelope domain 3, F-G loop	GDSYIVIGVGEKKITHHWHR
8	Envelope fusion loop	DRGWGNGCGLFGK
9	Envelope domain 2 (ED2) sequence A-E	TTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQ SDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKK MTGKSIQPENLEYR
10	Envelope domain 2 sequence B-D	EASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRG WGNGCGLFGKGSLVTCAKFACS
11	Envelope domain 1 glycan loop	MTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDEN RAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLY YLTM

[0105] Table 2 depicts chimeric peptide sequences that comprise the Woodchuck Hepatitis core Antigen (WHcAg) sequence (SEQ ID NO: 1) together with each of SEQ ID NOs: 2-11 inserted (double underline) in the region of amino acids 77 and 82 of SEQ ID NO: 1. Amino acids in bold and italics indicate linker sequence.

Table 2

SEQ ID NO	WHcAg (SEQ ID NO: 1) PLUS SEQ ID NO:	AMINO ACID SEQUENCE OF CHIMERIC PEPTIDE WITH ZIKV ENVELOPE (E) ANTIGEN
12	2	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***HLKCRLKMDKL RLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQ MAVDMQTLTPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIG VGEKKITHHWHRSGSTIGKAFEATVRGAKRMAV***GGGG***TIIVNHVND TWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNA PILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQS PSANC
13	3	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNIAFTFTKIPAETLHGTV TVELQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENS KMMLELDPPFGDSYIVIGTIIVNHVNDTWGLKVRQSLWFHLSCLTF GQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGAR ASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
14	4	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNIAFTFTKIPAETLHGTV TVELQYATIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVS FGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPS PRRRRSQSPRRRRSQSPSANC
15	5	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNIPCKVPAQMAVDMQTLT PVGRLITANPVITTIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTV QEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSP RRRTPSPRRRRSQSPRRRRSQSPSANC
16	6	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNIRLITANPVITESTENS KMMLELDPTIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLV SFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTP SPRRRRSQSPRRRRSQSPSANC
17	7	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNIGDSYIVIGVGEKKITH HWHRTIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGV WIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRR RRSQSPRRRRSQSPSANC
18	8	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGT***DRGWGNGCGLFGK ***GG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWI RTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRR SQSPRRRRSQSPSANC

19	9	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNI***GG***TTTVSNMAEVRSYC YEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCG LFGKGSLVTCAKFACSKKMTGKSIQPENLEYR***GG***TIIVNHVNDTWG LKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPIL STLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSA NC
20	10	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNI***GG***EASISDMASDSRCP TQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFAC S***GG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVW IRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRR RSQSPRRRRSQSPSANC
21	11	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGRE HCSPHHTAIRQALVCWDELTKLIAWMSSNI***GG***MTGKSIQPENLEYR IMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFG SLGLDCEPRTGLDFSDLYYLTM***GG***TIIVNHVNDTWGLKVRQSLWFH LSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIR RRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC

[0106] Sequence ID NOs: 22-33 were obtained via structure analysis of NS1. The sequences were selected for their adaptability with the scaffolding system, *i.e.*, the Woodchuck Hepatitis core Antigen (WHcAg) protein (Table 3). Structural information of the Zika Virus NS1 Protein was obtained from published scientific literature [22].

Table 3.

SEQ ID NO	ZIKV NS1 antigen	AMINO ACID SEQUENCE
22	NS1 Beta 1-2	DVGCSVDFSKKETRCGT
23	NS1 Beta 3-4	DRYKYHPDSPRRLAAAVKQAWEDGICGISSVSR
24	NS1 Alpha 2-Beta 5	MENIMWRSVEGELNAILEENGVQLTVVVGSV
25	NS1 Beta 4-5-6	CGISSVSRMENIMWRSVEGELNAILEENGVQLTVVVGSV KNPMWRGPQRLPVPVNELPHGWKAWGKSYFVRAAKTNNS FVVDGDTLKEC
26	NS1 Intertwined Loop-Beta 6	KNPMWRGPQRLPVPVNELPHGWKAWGKSYFVRAAKTNNS FVVDG
27	NS1 Beta 7-8-9	DTLKECPLKHRAWNSFLVEDHGFGVFHTSVWLKVREDYS LE
28	NS1 Beta 10-11-12-13	CDPAVIGTAVKGKEAVHSDLGYWIESEKNDTWRLKRAHL IEMKTC
29	NS1 Beta 12-13	GYWIESEKNDTWRLKRAHLI
30	NS1 Spaghetti Loop-Beta 14	RAHLIEMKTCEWPKSHTLWTDGIEESDLIIPKSLAGPLS HHNTREGYRTQMKGPWHSEELEIR
31	NS1 Beta 14-15-16-17	LEIRFEECPGTKVHVEETCGTRGPSLRSTTASGRVIEEW CCRECTMPPLSFRAK

32	NS1 Beta 15-16-17-18	CPGTKVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGC
33	NS1 Beta 14-15-16-17-18-19-C terminus	MKGPWHSEELEIRFEECPGTKVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGCWYGMEIRPRKEPESNLVRSMVTA

[0107] Table 4 depicts chimeric peptide sequences that comprise the Woodchuck Hepatitis core Antigen (WHcAg) sequence (Sequence ID NO: 1) together with each of Sequence ID NOs: 22-33 inserted (double underline) in the region of amino acids 77 and 82 of Sequence ID NO: 1. Amino acids in bold and italics indicate linker sequence.

Table 4.

SEQ ID NO	WHcAg (SEQ. ID NO: 1) PLUS SEQ. ID NO:	AMINO ACID SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV NS1 ANTIGEN
34	22	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***DVGCSVDFSKKETRCGT***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
35	23	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***DRYKYHPDSPRRLAAAVKQAWEDGICGISSVSR***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
36	24	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***MENIMWRSVEGELNAILEENGVQLTVVVGSV***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
37	25	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***CGISSVSRMENIMWRSVEGELNAILEENGVQLTVVVGSVKNPMWRGPQRLPVPVNELPHGWKAWGKSYFVRAAKTNNSFVVDGDTLKEC***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
38	26	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSPHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***KNPMWRGPQRLPVPVNELPHGWKAWGKSYFVRAAKTNNSFVVDG***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC

39	27	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***DTLKECPLKHRAWNSFL VEDHGFGVFHTSVWLKVREDYSLE***GGGG***TIIVNHVNDTWGLKVRQSLWF HLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRR GGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
40	28	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***CDPAVIGTAVKGKEAVH SDLGYWIESEKNDTWRLKRAHLIEMKTC***GGGG***TIIVNHVNDTWGLKVRQ SLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTV IRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
41	29	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***GYWIESEKNDTWRLKRA HLI***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGV WIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRS QSPRRRRSQSPSANC
42	30	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***RAHLIEMKTCEWPKSHT LWTDGIEESDLIIPKSLAGPLSHHNTREGYRTQMKGPWHSEELEIR***GGG G***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPA PYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRR RSQSPSANC
43	31	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***LEIRFEECPGTKVHVEE TCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAK***GGGG***TIIVNHVN DTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPI LSTLPEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
44	32	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***CPGTKVHVEETCGTRGP SLRSTTASGRVIEEWCCRECTMPPLSFRAKDGC***GGGG***TIIVNHVNDTWG LKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTL PEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
45	33	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***MKGPWHSEELEIRFEEC PGTKVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGC WYGMEIRPRKEPESNLVRSMVTA***GGGG***TIIVNHVNDTWGLKVRQSLWFH LSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRG GARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC

[0108] Sequence ID NOs: 46-47 were obtained via structure analysis of prM/M protein. The sequences were selected for their adaptability with the scaffolding system, *i.e.*, the Woodchuck Hepatitis core Antigen (WHcAg) protein (Table 5). Structural information of the Zika Virus prM/M protein was obtained from the literature [23]. prM sequence (Sequence ID NO: 46) has been mutagenized to prevent furin protease cleavage (R89G/R90G/R92G/R93G see underlined amino acids).

Table 5.

SEQ ID NO	ZIKV prM/M antigen	AMINO ACID SEQUENCE
46	prM Furin deficient	AEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMC DATMSYECPMLDEGVEPDDVDCWCNTTSTWVVYGTCHHKKGEAGG SGGAVTLPSHSTRKLQTRSQTWLESREYTKHLIRVENWIFRNPGF ALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYS
47	M full length	AVTLPSHSTRKLQTRSQTWLESREYTKHLIRVENWIFRNPGFALA AAAIAWLLGSSTSQKVIYLVMILLIAPAYS

[0109] Table 6 depicts chimeric peptide sequences that comprise the Woodchuck Hepatitis core Antigen (WHcAg) sequence (Sequence ID NO: 1) together with each of Sequence ID NOs: 46-47 inserted (double underline) in the region of amino acids 77 and 82 of Sequence ID NO: 1. Amino acids in bold and italics indicate linker sequence.

Table 6.

SEQ ID NO	WHcAg (SEQ ID NO: 1) PLUS SEQ. ID NO:	AMINO ACID SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV prM/M ANTIGEN
48	46	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***AEVTRRGSAYYMYLDRN DAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDEGVEPDDVD CWCNTTSTWVVYGTCHHKKGEAGGSGGAVTLPSHSTRKLQTRSQTWLES REYTKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIA PAYS***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSFG VWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRR SQSPRRRRSQSPSANC
49	47	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCS PHHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***AVTLPSHSTRKLQTRSQ TWLESREYTKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVM ILLIAPAYS***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEF LVSFGVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPS PRRRRSQSPRRRRSQSPSANC

[0110] Sequence ID NOs: 50-51 were obtained via structure analysis of Capsid C protein. The sequences were selected for their adaptability with the scaffolding system, *i.e.*, the Woodchuck Hepatitis core Antigen (WHcAg) protein (Table 7). Structural information of the Zika Virus Capsid protein were obtained from the literature [24].

Table 7.

SEQ ID NO	ZIKV C CAPSID ANTIGEN	AMINO ACID SEQUENCE
50	C full length	MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIR MVLAILAFLRFTAIKPSLGLINRWGSVGKKEAMETIKKFKKDLAA MLRIINARKEKKRR
51	C alpha 2	GHGPIRMVLAILAFLRFTAIKPSLG

[0111] Table 8 depicts chimeric peptide sequences that comprise the Woodchuck Hepatitis core Antigen (WHcAg) sequence (Sequence ID NO: 1) together with each of Sequence ID NOs: 50-51 inserted (double underline) in the region of amino acids 77 and 82 of Sequence ID NO: 1. Amino acids in bold and italics indicate linker sequence.

Table 8.

SEQ ID NO	WHcAg (SEQ ID NO: 1) PLUS SEQ ID NO:	AMINO ACID SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV C ANTIGEN
52	50	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSP HHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***MKNPKKKSGGFRIVNMLKR GVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIKPSLGLINRW GSVGKKEAMETIKKFKKDLAAMLRIINARKEKKRR***GGGG***TIIVNHVNDTW GLKVRQSLWFHLSCLTFGQHTVQEFLVSFGVWIRTPAPYRPPNAPILSTL PEHTVIRRRGGARASRSPRRRTPSPRRRRSQSPRRRRSQSPSANC
53	51	MDIDPYKEFGSSYQLLNFLPLDFFPDLNALVDTATALYEEELTGREHCSP HHTAIRQALVCWDELTKLIAWMSSNI***GGGGT***GHGPIRMVLAILAFLRFTA IKPSLG***GGGG***TIIVNHVNDTWGLKVRQSLWFHLSCLTFGQHTVQEFLVSF GVWIRTPAPYRPPNAPILSTLPEHTVIRRRGGARASRSPRRRTPSPRRRR SQSPRRRRSQSPSANC

Polynucleotides

[0112] The invention includes polynucleotides encoding the peptides as well as the chimeric peptides described herein. Exemplary sequences are set out in SEQ ID NOs: 22-53 (Tables 3-8, respectively). Because of the degeneracy of the genetic code, numerous polynucleotide sequences encode a given amino acid sequence, and all are contemplated as part of the invention. In some variations, codon selection is optimized for the type of host organism that will be used for expression.

Table 9. Polynucleotide sequences that encode the peptide and protein sequences of ZIKV E antigen shown in Table 1.

SEQ ID NO	VIRUS-LIKE PARTICLE PROTEIN	POLYNUCLEOTIDE SEQUENCE
54	Woodchuck Hepatitis Core Antigen (WHcAg)	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCACTTCTGAACAAGTTAGA ACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAA GGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTT TCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGT GTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACGC TCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAA GAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGA ACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAG AAGAAGATCTCAATCTCCATCTGCTAACTGT
SEQ ID NO	**ZIKV E ENVELOPE ANTIGEN**	**POLYNUCLEOTIDE SEQUENCE**
55	Envelope domain 3 full length	CACTTGAAGTGTAGATTGAAGATGGACAAGTTGAGATTGAA GGGTGTTTCTTACTCTTTGTGTACTGCTGCTTTCACTTTCA CTAAGATCCCAGCTGAAACTTTGCACGGTACTGTTACTGTT GAAGTTCAATACGCTGGTACTGACGGTCCATGTAAGGTTCC AGCTCAAATGGCTGTTGACATGCAAACTTTGACTCCAGTTG GTAGATTGATCACTGCTAACCCAGTTATCACTGAATCTACT GAAAACTCTAAGATGATGTTGGAATTGGACCCACCATTCGG TGACTCTTACATCGTTATCGGTGTTGGTGAAAAGAAGATCA CTCACCACTGGCACAGATCTGGTTCTACTATCGGTAAGGCT TTCGAAGCTACTGTTAGAGGTGCTAAGAGAATGGCTGTT
56	Envelope domain 3 (EDIII) G loop-truncated	GCTTTCACTTTCACTAAGATCCCAGCTGAAACTTTGCACGG TACTGTTACTGTTGAATTGCAATACGCTGGTACTGACGGTC CATGTAAGGTTCCAGCTCAAATGGCTGTTGACATGCAAACT TTGACTCCAGTTGGTAGATTGATCACTGCTAACCCAGTTAT CACTGAATCTACTGAAAACTCTAAGATGATGTTGGAATTGG ACCCACCATTCGGTGACTCTTACATCGTTATCGGT
57	Envelope domain 3, A-B loop	GCTTTCACTTTCACTAAGATCCCAGCTGAAACTTTGCACGG TACTGTTACTGTTGAATTGCAATACGCT
58	Envelope domain 3, CXCDDX loop (CD loop)	CCATGTAAGGTTCCAGCTCAAATGGCTGTTGACATGCAAA CTTTGACTCCAGTTGGTAGATTGATCACTGCTAACCCAGT TATCACT
59	Envelope domain 3, DX-E loop	AGATTGATCACTGCTAACCCAGTTATCACTGAATCTACT GAAAACTCTAAGATGATGTTGGAATTGGACCCA
60	Envelope domain 3, F-G loop	GGTGACTCTTACATCGTTATCGGTGTTGGTGAAAAGAAGAT CACTCACCACTGGCACAGA
61	Envelope fusion loop	GACAGAGGTTGGGGTAACGGTTGTGGTTTGTTCGGTAAG

SEQ ID NO	VIRUS-LIKE PARTICLE PROTEIN	POLYNUCLEOTIDE SEQUENCE
54	Woodchuck Hepatitis Core Antigen (WHcAg)	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCACTTCTGAACAAGTTAGA ACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAA GGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTT TCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGT GTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACGC TCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAA GAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGA ACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAG AAGAAGATCTCAATCTCCATCTGCTAACTGT
SEQ ID NO	**ZIKV E ENVELOPE ANTIGEN**	**POLYNUCLEOTIDE SEQUENCE**
62	Envelope domain 2 (ED2) sequence A-E	ACTACTACTGTTTCTAACATGGCTGAAGTTAGATCTT ACTGTTACGAAGCTTCTATCTCTGACATGGCTTCTGA CTCTAGATGTCCAACTCAAGGTGAAGCTTACTTGGAC AAGCAATCTGACACTCAATACGTTTGTAAGAGAACTT TGGTTGACAGAGGTTGGGGTAACGGTTGTGGTTTGTT CGGTAAGGGTTCTTTGGTTACTTGTGCTAAGTTCGCT TGTTCTAAGAAGATGACTGGTAAGTCTATCCAACCAG AAAACTTGGAATACAGA
63	Envelope domain 2 sequence B-D	GAAGCTTCTATCTCTGACATGGCTTCTGACTCTAGAT GTCCAACTCAAGGTGAAGCTTACTTGGACAAGCAATC TGACACTCAATACGTTTGTAAGAGAACTTTGGTTGAC AGAGGTTGGGGTAACGGTTGTGGTTTGTTCGGTAAGG GTTCTTTGGTTACTTGTGCTAAGTTCGCTTGTTCT
64	Envelope domain 1 glycan loop	ATGACTGGTAAGTCTATCCAACCAGAAAACTTGGAATACAG AATCATGTTGTCTGTTCACGGTTCTCAACACTCTGGTATGA TCGTTAACGACACTGGTCACGAAACTGACGAAAACAGAGCT AAGGTTGAAATCACTCCAAACTCTCCAAGAGCTGAAGCTAC TTTGGGTGGTTTCGGTTCTTTGGGTTTGGACTGTGAACCAA GAACTGGTTTGGACTTCTCTGACTTGTACTACTTGACTATG

Table 10. Polynucleotide sequences encoding WHcAg-ZIKV chimeric proteins with ZIKV E antigen shown in Table 2.

SEQ ID NO	WHcAg POLYNUCLEOTIDE SEQUENCE (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV E ANTIGEN
65	55	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTCAC TTGAAGTGTAGATTGAAGATGGACAAGTTGAGATTGAAGGG TGTTTCTTACTCTTTGTGTACTGCTGCTTTCACTTTCACTA AGATCCCAGCTGAAACTTTGCACGGTACTGTTACTGTTGAA GTTCAATACGCTGGTACTGACGGTCCATGTAAGGTTCCAGC TCAAATGGCTGTTGACATGCAAACTTTGACTCCAGTTGGTA GATTGATCACTGCTAACCCAGTTATCACTGAATCTACTGAA AACTCTAAGATGATGTTGGAATTGGACCCACCATTCGGTGA CTCTTACATCGTTATCGGTGTTGGTGAAAAGAAGATCACTC ACCACTGGCACAGATCTGGTTCTACTATCGGTAAGGCTTTC GAAGCTACTGTTAGAGGTGCTAAGAGAATGGCTGTTGGTGG TGGTGGTACTATCATCGTTAACCACGTTAACGACACTTGGG GTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGT TTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGTTTC TTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCAC CAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACTGTT ATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAG AAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTCAATCTC CAAGAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg POLYNUCLEOTIDE SEQUENCE (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV E ANTIGEN
66	56	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGCTTTCACTTTCACTAAG ATCCCAGCTGAAACTTTGCACGGTACTGTTACTGTTGAATT GCAATACGCTGGTACTGACGGTCCATGTAAGGTTCCAGCTC AAATGGCTGTTGACATGCAAACTTTGACTCCAGTTGGTAGA TTGATCACTGCTAACCCAGTTATCACTGAATCTACTGAAAA CTCTAAGATGATGTTGGAATTGGACCCACCATTCGGTGACT CTTACATCGTTATCGGTACTATCATCGTTAACCACGTTAAC GACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCA CTTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAAT TCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCA TACAGACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGA ACACACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTA GATCTCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGA TCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGC TAACTGT
67	57	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGCTTTCACTTTCACTAAG ATCCCAGCTGAAACTTTGCACGGTACTGTTACTGTTGAATT GCAATACGCTACTATCATCGTTAACCACGTTAACGACACTT GGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGTCT TGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGT TTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGAC CACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACT GTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCTCC AAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTCAAT CTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg POLYNUCLEOTIDE SEQUENCE (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV E ANTIGEN
68	58	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCCCATGTAAGGTTCCAGCT CAAATGGCTGTTGACATGCAAACTTTGACTCCAGTTGGTAG ATTGATCACTGCTAACCCAGTTATCACTACTATCATCGTTA ACCACGTTAACGACACTTGGGGTTTGAAGGTTAGACAATCT TTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACACAC TGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATCAGAA CTCCAGCTCCATACAGACCACCAAACGCTCCAATCTTGTCT ACTTTGCCAGAACACACTGTTATCAGAAGAAGAGGTGGTGC TAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCAA GAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCTCAA TCTCCATCTGCTAACTGT
69	59	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCAGATTGATCACTGCTAAC CCAGTTATCACTGAATCTACTGAAAACTCTAAGATGATGTT GGAATTGGACCCAACTATCATCGTTAACCACGTTAACGACA CTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTG TCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTT GGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACA GACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACAC ACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATC TCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTC AATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAAC TGT
70	60	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGGTGACTCTTACATCGTT ATCGGTGTTGGTGAAAAGAAGATCACTCACCACTGGCACAG AACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTGA AGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACT TTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGG TGTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACG CTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGA AGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAG AACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAA GAAGAAGATCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg POLYNUCLEOTIDE SEQUENCE (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV E ANTIGEN
71	61	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGGTGGTACTGACAGAGGT TGGGGTAACGGTTGTGGTTTGTTCGGTAAGGGTGGTACTAT CATCGTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTA GACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGT CAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTG GATCAGAACTCCAGCTCCATACAGACCACCAAACGCTCCAA TCTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGA GGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCC ATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAA GATCTCAATCTCCATCTGCTAACTGT
72	62	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTG TTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTG GTTGACACTGCTACTGCTTTGTACGAAGAAGAATTGACTGGTAGA GAACACTGTTCTCCACACCACACTGCTATCAGACAAGCTTTGGTT TGTTGGGACGAATTGACTAAGTTGATCGCTTGGATGTCTTCTAAC ATCGGTGGTACTACTACTGTTTCTAACATGGCTGAAGTTAGATCT TACTGTTACGAAGCTTCTATCTCTGACATGGCTTCTGACTCTAGA TGTCCAACTCAAGGTGAAGCTTACTTGGACAAGCAATCTGACACT CAATACGTTTGTAAGAGAACTTTGGTTGACAGAGGTTGGGGTAAC GGTTGTGGTTTGTTCGGTAAGGGTTCTTTGGTTACTTGTGCTAAG TTCGCTTGTTCTAAGAAGATGACTGGTAAGTCTATCCAACCAGAA AACTTGGAATACAGAGGTGGTACTATCATCGTTAACCACGTTAAC GACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTG TCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGTT TCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCACCA AACGCTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGA AGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACT CCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGA TCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg POLYNUCLEOTIDE SEQUENCE (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV E ANTIGEN
73	63	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGGTGGTGAAGCTTCTATC TCTGACATGGCTTCTGACTCTAGATGTCCAACTCAAGGTGA AGCTTACTTGGACAAGCAATCTGACACTCAATACGTTTGTA AGAGAACTTTGGTTGACAGAGGTTGGGGTAACGGTTGTGGT TTGTTCGGTAAGGGTTCTTTGGTTACTTGTGCTAAGTTCGC TTGTTCTGGTGGTACTATCATCGTTAACCACGTTAACGACA CTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTG TCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTT GGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACA GACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACAC ACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATC TCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTC AATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAAC TGT
74	64	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCA ATTGTTGAACTTCTTGCCATTGGACTTCTTCCCAGACTTGA ACGCTTTGGTTGACACTGCTACTGCTTTGTACGAAGAAGAA TTGACTGGTAGAGAACACTGTTCTCCACACCACACTGCTAT CAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTGA TCGCTTGGATGTCTTCTAACATCGGTGGTATGACTGGTAAG TCTATCCAACCAGAAAACTTGGAATACAGAATCATGTTGTC TGTTCACGGTTCTCAACACTCTGGTATGATCGTTAACGACA CTGGTCACGAAACTGACGAAAACAGAGCTAAGGTTGAAATC ACTCCAAACTCTCCAAGAGCTGAAGCTACTTTGGGTGGTTT CGGTTCTTTGGGTTTGGACTGTGAACCAAGAACTGGTTTGG ACTTCTCTGACTTGTACTACTTGACTATGGGTGGTACTATC ATCGTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTAG ACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTC AACACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGG ATCAGAACTCCAGCTCCATACAGACCACCAAACGCTCCAAT CTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGAG GTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCA TCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAG ATCTCAATCTCCATCTGCTAACTGT

Table 11. Polynucleotide sequences that encode the peptide and protein sequences of ZIKV NS1 antigen shown in Table 3.

SEQ ID NO	ZIKV NS1 ANTIGEN	POLYNUCLEOTIDE SEQUENCE OF ZIKV NS1 ANTIGEN
75	NS1 Beta 1-2	GACGTTGGTTGTTCTGTTGACTTCTCTAAGAAGGAAACTAGATGTGGTAC T
76	NS1 Beta 3-4	GACAGATACAAGTACCACCCAGACTCTCCAAGAAGATTGGCTGCTGCTGT TAAGCAAGCTTGGGAAGACGGTATCTGTGGTATCTCTTCTGTTTCTAGA
77	NS1 Alpha 2-Beta 5	ATGGAAAACATCATGTGGAGATCTGTTGAAGGTGAATTGAACGCTATCTT GGAAGAAAACGGTGTTCAATTGACTGTTGTTGTTGGTTCTGTT
78	NS1 Beta 4-5-6	TGTGGTATCTCTTCTGTTTCTAGAATGGAAAACATCATGTGGAGATCTGT TGAAGGTGAATTGAACGCTATCTTGGAAGAAAACGGTGTTCAATTGACTG TTGTTGTTGGTTCTGTTAAGAACCCAATGTGGAGAGGTCCACAAAGATTG CCAGTTCCAGTTAACGAATTGCCACACGGTTGGAAGGCTTGGGGTAAGTC TTACTTCGTTAGAGCTGCTAAGACTAACAACTCTTTCGTTGTTGACGGTG ACACTTTGAAGGAATGTGTT
79	NS1 Inter. Loop-Beta 6	AAGAACCCAATGTGGAGAGGTCCACAAAGATTGCCAGTTCCAGTTAACGA ATTGCCACACGGTTGGAAGGCTTGGGGTAAGTCTTACTTCGTTAGAGCTG CTAAGACTAACAACTCTTTCGTTGTTGACGGT
80	NS1 Beta 7-8-9	GACACTTTGAAGGAATGTCCATTGAAGCACAGAGCTTGGAACTCTTTCTT GGTTGAAGACCACGGTTTCGGTGTTTTCCACACTTCTGTTTGGTTGAAGG TTAGAGAAGACTACTCTTTGGAA
81	NS1 Beta 10-11-12-13	TGTGACCCAGCTGTTATCGGTACTGCTGTTAAGGGTAAGGAAGCTGTTCA CTCTGACTTGGGTTACTGGATCGAATCTGAAAAGAACGACACTTGGAGAT TGAAGAGAGCTCACTTGATCGAAATGAAGACTTGT
82	NS1 Beta 12-13	GGTTACTGGATCGAATCTGAAAAGAACGACACTTGGAGATTGAAGAGAGC TCACTTGATC
83	NS1 Spaghetti Loop-Beta 14	AGAGCTCACTTGATCGAAATGAAGACTTGTGAATGGCCAAAGTCTCACAC TTTGTGGACTGACGGTATCGAAGAATCTGACTTGATCATCCCAAAGTCTT TGGCTGGTCCATTGTCTCACCACAACACTAGAGAAGGTTACAGAACTCAA ATGAAGGGTCCATGGCACTCTGAAGAATTGGAAATCAGA
84	NS1 Beta 14-15-16-17	TTGGAAATCAGATTCGAAGAATGTCCAGGTACTAAGGTTCACGTTGAAGA AACTTGTGGTACTAGAGGTCCATCTTTGAGATCTACTACTGCTTCTGGTA GAGTTATCGAAGAATGGTGTTGTAGAGAATGTACTATGCCACCATTGTCT TTCAGAGCTAAG
85	NS1 Beta 15-16-17-18	TGTCCAGGTACTAAGGTTCACGTTGAAGAAACTTGTGGTACTAGAGGTCC ATCTTTGAGATCTACTACTGCTTCTGGTAGAGTTATCGAAGAATGGTGTT GTAGAGAATGTACTATGCCACCATTGTCTTTCAGAGCTAAGGACGGTTGT
86	NS1 Beta 14-15-16-17-18-19-C-term.	ATGAAGGGTCCATGGCACTCTGAAGAATTGGAAATCAGATTCGAAGAATG TCCAGGTACTAAGGTTCACGTTGAAGAAACTTGTGGTACTAGAGGTCCAT CTTTGAGATCTACTACTGCTTCTGGTAGAGTTATCGAAGAATGGTGTTGT AGAGAATGTACTATGCCACCATTGTCTTTCAGAGCTAAGGACGGTTGTTG GTACGGTATGGAAATCAGACCAAGAAAGGAACCAGAATCTAACTTGGTTA GATCTATGGTTACTGCT

Table 12. Polynucleotide sequences encoding WHcAg-ZIKV chimeric proteins with ZIKV NS1 antigen shown in Table 4.

SEQ ID NO	WHcAg (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV NS1 ANTIGEN
87	75	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGACGTTGGTTGT TCTGTTGACTTCTCTAAGAAGGAAACTAGATGTGGTACTGGTGGTGGTGGT ACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTAGACAA TCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAA GAATTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGA CCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGA AGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCT CCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCA TCTGCTAACTGT
88	76	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGACAGATACAAG TACCACCCAGACTCTCCAAGAAGATTGGCTGCTGCTGTTAAGCAAGCTTGG GAAGACGGTATCTGTGGTATCTCTTCTGTTTCTAGAGGTGGTGGTGGTACT ATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTAGACAATCT TTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAA TTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCA CCAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGA AGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCA AGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCT GCTAACTGT
89	77	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTATGGAAAACATC ATGTGGAGATCTGTTGAAGGTGAATTGAACGCTATCTTGGAAGAAAACGGT GTTCAATTGACTGTTGTTGTTGGTTCTGTTGGTGGTGGTGGTACTATCATC GTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGG TTCCACTTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTG GTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAAC GCTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGAGGT GGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCAAGAAGA AGAAGATCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAAC TGT

SEQ ID NO	WHcAg (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV NS1 ANTIGEN
90	78	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTTGTGGTATCTCT TCTGTTTCTAGAATGGAAAACATCATGTGGAGATCTGTTGAAGGTGAATTG AACGCTATCTTGGAAGAAAACGGTGTTCAATTGACTGTTGTTGTTGGTTCT GTTAAGAACCCAATGTGGAGAGGTCCACAAAGATTGCCAGTTCCAGTTAAC GAATTGCCACACGGTTGGAAGGCTTGGGGTAAGTCTTACTTCGTTAGAGCT GCTAAGACTAACAACTCTTTCGTTGTTGACGGTGACACTTTGAAGGAATGT GGTGGTGGTGGTACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTG AAGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAA CACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCA GCTCCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACAC ACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGA AGAACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGA TCTCAATCTCCATCTGCTAACTGT
91	79	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTAAGAACCCAATG TGGAGAGGTCCACAAAGATTGCCAGTTCCAGTTAACGAATTGCCACACGGT TGGAAGGCTTGGGGTAAGTCTTACTTCGTTAGAGCTGCTAAGACTAACAAC TCTTTCGTTGTTGACGGTGGTGGTGGTGGTACTATCATCGTTAACCACGTT AACGACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGTCT TGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGT GTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACGCTCCAATCTTG TCTACTTTGCCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCT TCTAGATCTCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTCAA TCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT
92	80	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGACACTTTGAAG GAATGTCCATTGAAGCACAGAGCTTGGAACTCTTTCTTGGTTGAAGACCAC GGTTTCGGTGTTTTCCACACTTCTGTTTGGTTGAAGGTTAGAGAAGACTAC TCTTTGGAAGGTGGTGGTGGTACTATCATCGTTAACCACGTTAACGACACT TGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACT TTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATC AGAACTCCAGCTCCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTG CCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCT CCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGA AGAAGAAGATCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV NS1 ANTIGEN
93	81	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTTGTGACCCAGCT GTTATCGGTACTGCTGTTAAGGGTAAGGAAGCTGTTCACTCTGACTTGGGT TACTGGATCGAATCTGAAAAGAACGACACTTGGAGATTGAAGAGAGCTCAC TTGATCGAAATGAAGACTTGTGGTGGTGGTGGTACTATCATCGTTAACCAC GTTAACGACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTG TCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTC GGTGTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACGCTCCAATC TTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCTAGA GCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCT CAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT
94	82	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGGTTACTGGATC GAATCTGAAAAGAACGACACTTGGAGATTGAAGAGAGCTCACTTGATCGGT GGTGGTGGTACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAAG GTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACAC ACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCT CCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACT GTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGA ACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCT CAATCTCCATCTGCTAACTGT
95	83	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTAGAGCTCACTTG ATCGAAATGAAGACTTGTGAATGGCCAAAGTCTCACACTTTGTGGACTGAC GGTATCGAAGAATCTGACTTGATCATCCCAAAGTCTTTGGCTGGTCCATTG TCTCACCACAACACTAGAGAAGGTTACAGAACTCAAATGAAGGGTCCATGG CACTCTGAAGAATTGGAAATCAGAGGTGGTGGTGGTACTATCATCGTTAAC CACGTTAACGACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCAC TTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCT TTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACGCTCCA ATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCT AGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGA TCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg (SEQ ID NO: 54) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV NS1 ANTIGEN
96	84	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTTTGGAAATCAGA TTCGAAGAATGTCCAGGTACTAAGGTTCACGTTGAAGAAACTTGTGGTACT AGAGGTCCATCTTTGAGATCTACTACTGCTTCTGGTAGAGTTATCGAAGAA TGGTGTTGTAGAGAATGTACTATGCCACCATTGTCTTTCAGAGCTAAGGGT GGTGGTGGTACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAAG GTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACAC ACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCT CCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACT GTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGA ACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCT CAATCTCCATCTGCTAACTGT
97	85	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTTGTCCAGGTACT AAGGTTCACGTTGAAGAAACTTGTGGTACTAGAGGTCCATCTTTGAGATCT ACTACTGCTTCTGGTAGAGTTATCGAAGAATGGTGTTGTAGAGAATGTACT ATGCCACCATTGTCTTTCAGAGCTAAGGACGGTTGTGGTGGTGGTGGTACT ATCATCGTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTAGACAATCT TTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAA TTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCA CCAAACGCTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGA AGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCA AGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCT GCTAACTGT
98	86	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTATGAAGGGTCCA TGGCACTCTGAAGAATTGGAAATCAGATTCGAAGAATGTCCAGGTACTAAG GTTCACGTTGAAGAAACTTGTGGTACTAGAGGTCCATCTTTGAGATCTACT ACTGCTTCTGGTAGAGTTATCGAAGAATGGTGTTGTAGAGAATGTACTATG CCACCATTGTCTTTCAGAGCTAAGGACGGTTGTTGGTACGGTATGGAAATC AGACCAAGAAAGGAACCAGAATCTAACTTGGTTAGATCTATGGTTACTGCT GGTGGTGGTGGTACTATCATCGTTAACCACGTTAACGACACTTGGGGTTTG AAGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTTCGGTCAA CACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATCAGAACTCCA GCTCCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTGCCAGAACAC ACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGA AGAACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGAAGAAGAAGA TCTCAATCTCCATCTGCTAACTGT

Table 13. Polynucleotide sequences that encode the peptide and protein sequences of ZIKV prM/M antigen shown in Table 5.

SEQ ID NO	ZIKV prM/M ANTIGEN	POLYNUCLEOTIDE SEQUENCE OF ZIKV prM/M ANTIGEN
99	prM Furin deficient	GCTGAAGTTACTAGAAGAGGTTCTGCTTACTACATGTACTTGGACAGAAACG ACGCTGGTGAAGCTATCTCTTTCCCAACTACTTTGGGTATGAACAAGTGTTA CATCCAAATCATGGACTTGGGTCACATGTGTGACGCTACTATGTCTTACGAA TGTCCAATGTTGGACGAAGGTGTTGAACCAGACGACGTTGACTGTTGGTGTA ACACTACTTCTACTTGGGTTGTTTACGGTACTTGTCACCACAAGAAGGGTGA AGCTGGTGGTTCTGGTGGTGCTGTTACTTTGCCATCTCACTCTACTAGAAAG TTGCAAACTAGATCTCAAACTTGGTTGGAATCTAGAGAATACACTAAGCACT TGATCAGAGTTGAAAACTGGATCTTCAGAAACCCAGGTTTCGCTTTGGCTGC TGCTGCTATCGCTTGGTTGTTGGGTTCTTCTACTTCTCAAAAGGTTATCTAC TTGGTTATGATCTTGTTGATCGCTCCAGCTTACTCT
100	M full length	GCTGTTACTTTGCCATCTCACTCTACTAGAAAGTTGCAAACTAGATCTCAAA CTTGGTTGGAATCTAGAGAATACACTAAGCACTTGATCAGAGTTGAAAACTG GATCTTCAGAAACCCAGGTTTCGCTTTGGCTGCTGCTGCTATCGCTTGGTTG TTGGGTTCTTCTACTTCTCAAAAGGTTATCTACTTGGTTATGATCTTGTTGA TCGCTCCAGCTTACTCT

Table 14. Polynucleotide sequences encoding WHcAg-ZIKV chimeric proteins with ZIKV prM/M antigen shown in Table 6.

SEQ ID NO	WHcAg (SEQ ID NO: 62) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV prM/M ANTIGEN
101	99	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGCTGAAGTTACT AGAAGAGGTTCTGCTTACTACATGTACTTGGACAGAAACGACGCTGGTGAA GCTATCTCTTTCCCAACTACTTTGGGTATGAACAAGTGTTACATCCAAATC ATGGACTTGGGTCACATGTGTGACGCTACTATGTCTTACGAATGTCCAATG TTGGACGAAGGTGTTGAACCAGACGACGTTGACTGTTGGTGTAACACTACT TCTACTTGGGTTGTTTACGGTACTTGTCACCACAAGAAGGGTGAAGCTGGT GGTTCTGGTGGTGCTGTTACTTTGCCATCTCACTCTACTAGAAAGTTGCAA ACTAGATCTCAAACTTGGTTGGAATCTAGAGAATACACTAAGCACTTGATC AGAGTTGAAAACTGGATCTTCAGAAACCCAGGTTTCGCTTTGGCTGCTGCT GCTATCGCTTGGTTGTTGGGTTCTTCTACTTCTCAAAAGGTTATCTACTTG GTTATGATCTTGTTGATCGCTCCAGCTTACTCTGGTGGTGGTGGTACTATC ATCGTTAACCACGTTAACGACACTTGGGGTTTGAAGGTTAGACAATCTTTG TGGTTCCACTTGTCTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTC TTGGTTTCTTTCGGTGTTTGGATCAGAACTCCAGCTCCATACAGACCACCA AACGCTCCAATCTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGA GGTGGTGCTAGAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCAAGA AGAAGAAGATCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCT AACTGT
102	100	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAAC TTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTGCT ACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCACAC CACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAAGTTG ATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGCTGTTACTTTG CCATCTCACTCTACTAGAAAGTTGCAAACTAGATCTCAAACTTGGTTGGAA TCTAGAGAATACACTAAGCACTTGATCAGAGTTGAAAACTGGATCTTCAGA AACCCAGGTTTCGCTTTGGCTGCTGCTGCTATCGCTTGGTTGTTGGGTTCT TCTACTTCTCAAAAGGTTATCTACTTGGTTATGATCTTGTTGATCGCTCCA GCTTACTCTGGTGGTGGTGGTACTATCATCGTTAACCACGTTAACGACACT TGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACT TTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATC AGAACTCCAGCTCCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTG CCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATCT CCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAAGA AGAAGAAGATCTCAATCTCCATCTGCTAACTGT

Table 15. Polynucleotide sequences that encode the peptide and protein sequences of ZIKV Capsid C antigen shown in Table 7.

SEQ ID NO	ZIKV C CAPSID ANTIGEN	POLYNUCLEOTIDE SEQUENCE OF ZIKV C ANTIGEN
103	C full length	ATGAAGAACCCAAAGAAGAAGTCTGGTGGTTTCAGAATCGTTAACATGTT GAAGAGAGGTGTTGCTAGAGTTTCTCCATTCGGTGGTTTGAAGAGATTGC CAGCTGGTTTGTTGTTGGGTCACGGTCCAATCAGAATGGTTTTGGCTATC TTGGCTTTCTTGAGATTCACTGCTATCAAGCCATCTTTGGGTTTGATCAA CAGATGGGGTTCTGTTGGTAAGAAGGAAGCTATGGAAACTATCAAGAAGT TCAAGAAGGACTTGGCTGCTATGTTGAGAATCATCAACGCTAGAAAGGAA AAGAAGAGAAGA
104	C alpha 2	GGTCACGGTCCAATCAGAATGGTTTTGGCTATCTTGGCTTTCTTGAGATT CACTGCTATCAAGCCATCTTTGGGT

Table 16. Polynucleotide sequences encoding WHcAg-ZIKV chimeric proteins with ZIKV C antigen shown in Table 8.

SEQ ID NO	WHcAg (SEQ ID NO: 62) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV C ANTIGEN
105	103	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAA CTTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTG CTACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCA CACCACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAA GTTGATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTATGAAGA ACCCAAAGAAGAAGTCTGGTGGTTTCAGAATCGTTAACATGTTGAAGAGA GGTGTTGCTAGAGTTTCTCCATTCGGTGGTTTGAAGAGATTGCCAGCTGG TTTGTTGTTGGGTCACGGTCCAATCAGAATGGTTTTGGCTATCTTGGCTT TCTTGAGATTCACTGCTATCAAGCCATCTTTGGGTTTGATCAACAGATGG GGTTCTGTTGGTAAGAAGGAAGCTATGGAAACTATCAAGAAGTTCAAGAA GGACTTGGCTGCTATGTTGAGAATCATCAACGCTAGAAAGGAAAAGAAGA GAAGAGGTGGTGGTGGTACTATCATCGTTAACCACGTTAACGACACTTGG GGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGTCTTGTTTGACTTT CGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTCGGTGTTTGGATCA GAACTCCAGCTCCATACAGACCACCAAACGCTCCAATCTTGTCTACTTTG CCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCTAGAGCTTCTAGATC TCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGATCTCAATCTCCAA GAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT

SEQ ID NO	WHcAg (SEQ ID NO: 62) PLUS SEQ ID NO:	POLYNUCLEOTIDE SEQUENCE OF CHIMERIC PROTEIN WITH ZIKV C ANTIGEN
106	104	ATGGACATCGACCCATACAAGGAATTCGGTTCTTCTTACCAATTGTTGAA CTTCTTGCCATTGGACTTCTTCCCAGACTTGAACGCTTTGGTTGACACTG CTACTGCTTTGTACGAAGAAGAATTGACTGGTAGAGAACACTGTTCTCCA CACCACACTGCTATCAGACAAGCTTTGGTTTGTTGGGACGAATTGACTAA GTTGATCGCTTGGATGTCTTCTAACATCGGTGGTGGTGGTACTGGTCACG GTCCAATCAGAATGGTTTTGGCTATCTTGGCTTTCTTGAGATTCACTGCT ATCAAGCCATCTTTGGGTGGTGGTGGTGGTACTATCATCGTTAACCACGT TAACGACACTTGGGGTTTGAAGGTTAGACAATCTTTGTGGTTCCACTTGT CTTGTTTGACTTTCGGTCAACACACTGTTCAAGAATTCTTGGTTTCTTTC GGTGTTTGGATCAGAACTCCAGCTCCATACAGACCACCAAACGCTCCAAT CTTGTCTACTTTGCCAGAACACACTGTTATCAGAAGAAGAGGTGGTGCTA GAGCTTCTAGATCTCCAAGAAGAAGAACTCCATCTCCAAGAAGAAGAAGA TCTCAATCTCCAAGAAGAAGAAGATCTCAATCTCCATCTGCTAACTGT

[0113] Methods of making polynucleotides of a predetermined sequence are well-known. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed. 1989) and F. Eckstein (ed.) Oligonucleotides and Analogues, 1st Ed. (Oxford University Press, New York, 1991). Solid-phase synthesis methods are preferred for both polyribonucleotides and polydeoxyribonucleotides (the well-known methods of synthesizing DNA are also useful for synthesizing RNA). Polyribonucleotides can also be prepared enzymatically. Non-naturally occurring nucleobases can be incorporated into the polynucleotide, as well. See, e.g., U.S. Patent No. 7,223,833; [25, 26].

Vectors

[0114] In some embodiments, vectors are used to express the polynucleotides described herein. Expression vectors generally include expression control sequences selected for a type of host cell to be used for protein expression. In some embodiments, the expression vector is a yeast expression vector. Various expression vectors are known in the art, including but not limited to pD912 or pD902 for secretory or cytosolic production of VLPs respectively (ATUM (https://www.atum.bio/)). Components and structure of an exemplary expression vector is depicted in Figure 2.

VLP production/purification

[0115] A number of appropriate yeast strains for protein expression exist, including but not limited to Komagataella phaffii Kurtzman (ATCC® 76273™) or Komagataella pastoris (ATCC® 76274™). Using in silico analysis we have designed codon optimized DNA constructs

expressing the Zika Virus antigens conserved between different strains (Figure 3A and 3B). VLPs are produced by recombinant constructs using the promoter from the Pichia alcohol oxidase 1 (AOX1) gene to drive production of the recombinant protein according to ATUM (https://www.atum.bio/) with further optimization. VLPs are purified by, for example, precipitation, ultracentrifugation, chromatography, tangential flow filtration (TFF) or ultrafiltration methods or combination of such methods (Figure 4) [16]. VLPs are quantified for purity and antigenicity using biochemical and immune assays such as Western blotting, dot blot, ELISA (Figures 6 and 7), gel electrophoresis (SDS-PAGE or native Agarose gel, combined with Coomassie Blue staining), and electron microscopy (Figure 5) [16].

Antigenic and Immunogenic Characterization of VLPs

A. Antigenicity

[0116] Prior to immunogenicity testing, VLPs comprising a chimeric peptide as described herein are characterized for expression, particle assembly, and ability to bind a peptide-specific antibody. Capture enzyme-linked immunosorbent assays (ELISAs), dot blot or Western blot are utilized and designed to assess three VLP properties according to methods known in the art [16] (Figures 6 and 7): 1) protein expression of the WHcAg polypeptide by use of an antibody that is specific for the WHcAg (e.g. Santa Cruz Biotechnology, antibody Hep B cAg Antibody (13A9): sc-23946); 2) particle assembly using an antibody specific for a conformational epitope on WHcAg; and 3) display of the epitope of a Zika peptide of the disclosure by use of Zika peptide-reactive antibodies (*e.g.*, ATCC BEI Resources NR-50414 Monoclonal Anti-Zika Virus Envelope (E) Protein, Clone ZV-2). Constructs that are positive for all three properties are selected for further purification (*e.g.*, Ultracentrifugation, Ultrafiltration, Chromatography). In brief, expression, particle assembly, and antibody binding are assayed by ELISA, dot blot, and Western blotting. SDS-PAGE and Agarose electrophoresis, along with electron microscopy (Figure 5), are used to assess the purity and assembly of VLPs. VLPs can be tested for non-cross-reactivity using *in vitro* Antibody-Dependent Enhancement Assay according to the literature [27].

B. Immunogenicity

[0117] VLP-based vaccine antigenicity is assessed in different adjuvant formulations in animal model such as immunocompetent mouse model (e.g. BALB/c) or immunodeficient mouse model (e.g. AG129 and A129). The immune response to VLPs is assessed in mice models for Zika Virus infection according to the literature [27, 28]. In addition to anti-insert, anti-peptide-protein and anti-WHcAg antibody endpoint titers, antibody specificity, isotype

distribution, antibody persistence and antibody avidity are monitored. VLPs immune stimulation can be tested for inducing non-cross-reactivity antibody analyzing serum samples of VLP immunized mice by dot blot analysis (Figure 9) or *in vitro* for Antibody-Dependent Enhancement Assay according to the literature [27]. Immune sera are compared to the activity of a reference antibody by ELISA and neutralization assays known in the art [16, 28]. Immune responses are tested *in vivo* in various mammalian species (*e.g.*, rodents such as rats and mice, nonhuman primates (NHP), and/or humans).

Compositions

[0118] The invention includes compositions that comprise a chimeric peptide or VLP described herein or a polynucleotide encoding the chimeric peptide. In some embodiments, the composition is an antigenic composition. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. The term "carrier" refers to a vehicle within which the VLP, vector, chimeric peptide or polynucleotide encoding the chimeric peptide is administered to a mammalian subject. The term carrier encompasses diluents, excipients, adjuvants and combinations thereof. Pharmaceutically acceptable carriers are well known in the art (see, *e.g.*, Remington's Pharmaceutical Sciences by Martin, 1975).

[0119] Exemplary "diluents" include sterile liquids such as sterile water, saline solutions, and buffers (*e.g.*, phosphate, tris, borate, succinate, or histidine). Exemplary "excipients" are inert substances that may enhance vaccine stability and include but are not limited to polymers (*e.g.*, polyethylene glycol), carbohydrates (*e.g.*, starch, glucose, lactose, sucrose, or cellulose), and alcohols (*e.g.*, glycerol, sorbitol, or xylitol).

[0120] Adjuvants are broadly separated into two classes based upon their primary mechanism of action: vaccine delivery systems (*e.g.*, emulsions, microparticles, immune stimulating complexes (ISCOMS), or liposomes) that target associated antigens to antigen presenting cells (APC); and immunostimulatory adjuvants (*e.g.*, LPS, MPL, or CpG) that directly activate innate immune responses. Different types of adjuvants can be combined to enhance their immunostimulatory activity (e.g. AS04 (GSK) is composed of MPL mixed with an aluminum salt).

A. Traditional and Molecular Adjuvants

[0121] Although adjuvants are not required when using the WHcAg delivery system disclosed herein, some embodiments of the present invention employ adjuvant formulations. Adjuvants are a class of immunomodulatory molecules and compositions able to augment vaccine

effectiveness and safety by: 1) enhancing immunogenicity and increasing the duration of protection; 2) broadening the induction of the immune response; 3) reducing vaccine dosage and vaccination cost (antigen sparing); 4) accelerating the immune response; 5) stimulating a stronger immunological memory; 6) improving efficacy in weak responder patients such as neonates, the elderly and immunocompromised individuals [12]. In addition, some adjuvants formulation may also increase VLP-based vaccine stability and play an important role in VLPs delivery. Adjuvant formulations for this disclosure includes the classical aluminum-based adjuvants, and novel classes of adjuvants such as liposomes (e.g., CAF01), agonists of pathogen recognition receptors (e.g. Immune stimulating complexes (ISCOMs), Lipid A analogs (MPL, RC-529, and GLA), double stranded RNA analogs (e.g.Poly I:C and Poly ICLC), cytidine monophosphate guanosine oligodeoxynucleotide (e.g. CpG, CpG ODN), flagellin, imidazoquinoline (Imiquimod and Resiquimod), polymeric particles (e.g. Chitosan), emulsions (e.g. squalene oil-based), cytokines (e.g. Interleukin-12), bacterial toxins (e.g Cholera Toxin (CT) or Escherichia coli enterotoxin (LT)), Quil A and other saponins known in the art, and the plant polysaccharide inulin [12]. Specifically, immunization in saline effectively elicits immune response against the vaccine preparation antigen/s. However, formulation in non-inflammatory agents such as IFA (mineral oil), Montanide ISA 720 (squalene), and aluminum phosphate (AIP04), or immunomodulatory agents or adjuvants enhance vaccine immunogenicity. Additionally, administration of WHcAg results in the production of multiple IgG isotypes, regardless of which if any adjuvant is employed. The WHcAg VLPs have shown superior stability as compared to recombinant protein from subunit vaccines in the particularly harsh mucosal environment. This characteristic is quite advantageous for developing vaccines for mucosal administration such as the oral, nasal, rectal and vaginal route. Inclusion of a CpG motif also enhances the primary response. Moreover, use of an inflammatory adjuvant such as the Ribi formulation is not more beneficial than is the use of non-inflammatory adjuvants, indicating that the benefits of the adjuvants result from a depot effect rather than from non-specific inflammation. Thus, the core platform is used with no adjuvant or with non-inflammatory adjuvants depending upon the application and the quantity of antibody desired. In some embodiments of the present disclosure, IFA is used in murine studies, whereas alum or squalene is used in human studies. In instances where it is desirable to deliver hybrid WHcAg particles in a single dose in saline, a molecular adjuvant is employed. A number of molecular adjuvants are employed to bridge the gap between innate and adaptive immunity by providing a co-stimulus to target B cells or other APCs.

B. Other Molecular Adjuvants

[0122] Genes encoding the murine CD40L (both 655 and 470 nucleic acid versions) have been used successfully to express these ligands at the C-terminus of WHcAg (See, *e.g.*, WO 2005/011571). Moreover, immunization of mice with hybrid WHcAg-CD40L particles results in the production of higher anti-core antibody titers than does the immunization of mice with WHcAg particles. However, lower than desirable yields of purified particles have been obtained. Therefore, mosaic particles containing less than 100% CD40L-fused polypeptides are produced to overcome this problem. The other molecular adjuvants inserted within the WHcAg, including the C3d fragment, BAFF and LAG-3, have a tendency to become internalized when inserted at the C-terminus. Therefore tandem repeats of molecular adjuvants are used to resist internalization. Alternatively, various mutations within the so-called hinge region of WHcAg, between the assembly domain and the DNA/RNA-binding region of the core particle are made to prevent internalization of C-terminal sequences. However, internalization represents a problem for those molecular adjuvants such as CD40L, C3d, BAFF and LAG-3, which function at the APC/B cell membrane. In contrast, internalization of molecular adjuvants such as CpG ODN is not an issue as these types of adjuvants function at the level of cytosolic receptors.

[0123] Another type of molecular adjuvant or immune enhancer is the inclusion within hybrid core particles of a $CD4^+$ T cell epitope, preferably a "universal" $CD4^+$ T cell epitope that is recognized by a large proportion of $CD4^+$ T cells (such as by more than 50%, preferably more than 60%, more preferably more than 70%, most preferably greater than 80%), of $CD4^+$ T cells. In one embodiment, universal $CD4^+$ T cell epitopes bind to a variety of human MHC class II molecules and are able to stimulate T helper cells. In another embodiment, universal $CD4^+$ T cell epitopes are preferably derived from antigens to which the human population is frequently exposed either by natural infection or vaccination [29]. A number of such universal $CD4^+$ T cell epitopes have been described including, but not limited to: Tetanus Toxin (TT) residues 632-651; TT residues 950-969; TT residues 947-967, TT residues 830-843, TT residues 1084-1099, TT residues 1174-1189 [30]; Diphtheria Toxin (DT) residues 271-290; DT residues 321-340; DT residues 331-350; DT residues 411-430; DT residues 351-370; DT residues 431-450 [31]; Plasmodium falciparum circumsporozoite (CSP) residues 321-345 and CSP residues 378-395 [32]; Hepatitis B antigen (HBsAg) residues 19-33 [33]; Influenza hemagglutinin residues 307-319; Influenza matrix residues 17-31 [34]; and measles virus fusion protein (MVF) residues 288-302 [35].

Methods of Inducing an Immune Response

[0124] The invention includes methods for eliciting an immune response in a subject in need thereof, comprising administering to the subject an effective amount of an antigenic composition comprising one or more of the peptides, proteins, or VLP described herein. Also provided are methods for eliciting an immune response in a subject in need thereof, comprising administering to the subject an effective amount of an antigenic composition comprising a polynucleotide encoding a chimeric peptide described herein, wherein said chimeric polypeptide expressed *in vivo* assembles as a hybrid VLP. Unless otherwise indicated, the antigenic composition is an immunogenic composition.

[0125] The immune response raised by the methods of the present disclosure generally includes an antibody response, preferably a neutralizing antibody response, antibody dependent cell-mediated cytotoxicity (ADCC), antibody cell-mediated phagocytosis (ADCP), complement dependent cytotoxicity (CDC), and T cell-mediated response such as $CD4^+$, $CD8^+$. The immune response generated by the chimeric peptides, proteins, or VLPs as disclosed herein generates an immune response that recognizes, and preferably ameliorates and/or neutralizes, Zika virus. Methods for assessing antibody responses after administration of an antigenic composition (immunization or vaccination) are known in the art and/or described herein. In some embodiments, the immune response comprises a T cell-mediated response (*e.g.*, peptide-specific response such as a proliferative response or a cytokine response). In preferred embodiments, the immune response comprises both a B cell and a T cell response. Antigenic compositions can be administered in a number of suitable ways, such as intramuscular injection, subcutaneous injection, intradermal administration and mucosal administration such as oral or intranasal. Additional modes of administration include but are not limited to intranasal administration, intra-vaginal, intra-rectal, and oral administration. A combination of different routes of administration in the immunized subject, for example intramuscular and intranasal administration at the same time, is also contemplated by the disclosure.

[0126] Antigenic compositions may be used to treat both children and adults, including pregnant women. Thus a subject may be less than 1 year old, 1-5 years old, 5-15 years old, 15-55 years old, or at least 55 years old. Preferred subjects for receiving the vaccines are the elderly (*e.g.*, >55 years old, >60 years old, preferably >65 years old), and the young (*e.g.*, <6 years old, 1-5 years old, preferably less than 1 year old). Additional subjects for receiving the vaccines or compositions of the disclosure include naïve (versus previously infected) subjects, currently infected subjects, or immunocompromised subjects.

[0127] Administration can involve a single dose or a multiple dose schedule. Multiple doses may be used in a primary immunization schedule and/or in a booster immunization schedule. In a multiple dose schedule the various doses may be given by the same or different routes, *e.g.*, a parenteral prime and mucosal boost, or a mucosal prime and parenteral boost. Administration of more than one dose (typically two doses) is particularly useful in immunologically naive subjects or subjects of a hyporesponsive population (*e.g.*, diabetics, or subjects with chronic kidney disease (*e.g.*, dialysis patients)). Multiple doses will typically be administered at least 1 week apart (*e.g.*, about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, or about 16 weeks). Preferably multiple doses are administered from one, two, three, four or five months apart. Antigenic compositions of the present disclosure may be administered to patients at substantially the same time as (*e.g.*, during the same medical consultation or visit to a healthcare professional) other vaccines.

[0128] In general, the amount of protein in each dose of the antigenic composition is selected as an amount effective to induce an immune response in the subject, without causing significant, adverse side effects in the subject. Preferably the immune response elicited includes: neutralizing antibody response; antibody dependent cell-mediated cytotoxicity (ADCC); antibody cell-mediated phagocytosis (ADCP); complement dependent cytotoxicity (CDC); T cell-mediated response such as $CD4^+$, $CD8^+$, or a protective antibody response. Protective in this context does not necessarily require that the subject is completely protected against infection. A protective response is achieved when the subject is protected from developing symptoms of disease, especially severe disease associated with the pathogen corresponding to the heterologous antigen. As described above, the immune response generated by the chimeric peptides or VLP as disclosed herein generates an immune response that recognizes, and preferably ameliorates and/or neutralizes, Zika virus.

[0129] The WHcAg-ZIKV chimera vaccine administration and formulation may be optimized to induce mucosal immune protection for preventing sexual transmission. The invention contemplates mucosal route administration such as nasal, vaginal, rectal or oral. The vaccine formulation can be optimized using adjuvant/s formulation for stimulation of mucosal immune response such as IgA and induction of mucosa-associated lymphoid tissues (MALTs). Adjuvants for mucosal immunization considered for WHcAg-ZIKV chimera vaccine include but are not limited to polymeric particles (*e.g.*, Chitosan), cholera toxin (CT), and imidazoquinoline (Imiquimod and Resiquimod).

[0130] The WHcAg-ZIKV chimera vaccine formulation and administration may be designed to achieve a broader immune response for protection against multiple transmission routes: mosquito transmission, blood transfusion, maternal transmission, sexual transmission, organ transplant and other possible routes.

[0131] The amount of antigen (*e.g.*, VLP) can vary depending upon which antigenic composition is employed. Generally, it is expected that each human dose will comprise 0.1-2000 µg of protein (*e.g.*, chimeric peptide), such as from about 1 µg to about 2000 µg, for example, from about 1 µg to about 1500 µg, or from about 1 µg to about 1000 µg, or from about 1 µg to about 500 µg, or from about 1 µg to about 100 µg. In some embodiments, the amount of the protein is within any range having a lower limit of 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240 or 250 µg, and an independently selected upper limit of 2000, 1950, 1900, 1850, 1800, 1750, 1700, 1650, 1600, 1550, 1500, 1450, 1400, 1350, 1300 or 1250, 1200, 1150, 1100, 1050, 1000, 950, 900, 850, 800, 750, 700, 650, 600, 550, 500, 450, 400, 350, 300 or 250 µg, provided that the lower limit is less than the upper limit. Generally a human dose will be in a volume of from 0.1 ml to 1 ml, preferably from 0.25 ml to 0.5 ml. The amount utilized in an antigenic composition is selected based on the subject population. An optimal amount for a particular composition can be ascertained by standard studies involving observation of antibody titers and other responses (*e.g.*, antigen-induced cytokine secretion) in subjects. Following an initial vaccination, subjects can receive a boost in about 4-12 weeks.

Articles of Manufacture and Kits

[0132] The invention additionally includes articles of manufacture and kits comprising a peptide, a chimeric peptide or protein, a fusion protein, or VLP described herein (Figures 12-14. In some embodiments, the kits further comprise a solid support (*e.g.*, referring to Figure12, the solid support can be a plate ***1***, a test strip ***3***, or a microbead ***4***). Kits or articles also comprise, in some variations, a capture antibody ***5*** and/or a detection antibody ***6***. In some embodiments, the kits further comprise instructions for measuring peptide-specific antibodies. In some embodiments, the antibodies are present in serum from a blood sample of a subject immunized with an antigenic composition comprising the VLP.

[0133] Chimeric WHcAg-ZIKV VLP are designed for capturing anti-ZIKV and include but are not limited to specific and selected amino acids sequence(s) from ZIKV viral protein E, NS1, prM/M or C (see Tables 1, 3, 5, and 7). Such recombinant amino acid sequences are inserted

at a location between two amino acids in the region of amino acids 77 to 82 of the WHcAg protein (GenBank accession number NP_671816). See Table 2, 4, 6, and 8.

[0134] As used herein, the term "instructions" refers to directions or protocols for using reagents contained in the kit for measuring antibody titer. In some embodiments, the instructions further comprise the statement of intended use required by the U.S. Food and Drug Administration (FDA) in labeling *in vitro* diagnostic products. The FDA classifies *in vitro* diagnostics as medical devices and required that they be approved through the 510(k) procedure. Information required in an application under 510(k) includes: 1) The *in vitro* diagnostic product name, including the trade or proprietary name, the common or usual name, and the classification name of the device; 2) The intended use of the product; 3) The establishment registration number, if applicable, of the owner or operator submitting the 510(k) submission; the class in which the *in vitro* diagnostic product was placed under section 513 of the FD&C Act, if known, its appropriate panel, or, if the owner or operator determines that the device has not been classified under such section, a statement of that determination and the basis for the determination that the *in vitro* diagnostic product is not so classified; 4) Proposed labels, labeling and advertisements sufficient to describe the *in vitro* diagnostic product, its intended use, and directions for use, including photographs or engineering drawings, where applicable; 5) A statement indicating that the device is similar to and/or different from other *in vitro* diagnostic products of comparable type in commercial distribution in the U.S., accompanied by data to support the statement; 6) A 510(k) summary of the safety and effectiveness data upon which the substantial equivalence determination is based; or a statement that the 510(k) safety and effectiveness information supporting the FDA finding of substantial equivalence will be made available to any person within 30 days of a written request; 7) A statement that the submitter believes, to the best of their knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted; and 8) Any additional information regarding the *in vitro* diagnostic product requested that is necessary for the FDA to make a substantial equivalency determination.

[0135] As described herein, the invention also includes methods for screening anti-Zika virus antibodies comprising: a) measuring binding of an antibody or fragment thereof to a VLP as described herein; and b) measuring binding of the antibody or fragment thereof to a Woodchuck Hepatitis core Antigen protein (WHcAg) VLP devoid of a peptide as disclosed herein; and c) determining that the antibody or fragment thereof is specific or selective for a peptide of the disclosure when the antibody or fragment thereof binds to the chimeric VLP but not the WHcAg VLP devoid of a peptide of the disclosure. In some embodiments, the VLP is attached to a solid

support. In further embodiments, the solid support is a microbead, an assay plate, a test strip, or a filter as depicted in Figures 12 and 13. Methods for (i) screening anti-Zika virus antibodies; (ii) detecting or measuring antibodies to Zika virus in a biological sample; or (iii) detecting a Zika virus infection may all be performed using a solid support as shown in Figures 12, 13, and 14. In various embodiments, antigen-antibody complex formation and detection may be performed by attaching a VLP as described herein directly to a solid support (such as, *e.g.*, a plate ***1***, a test strip ***3***, or a microbead ***4***) and then contacting the VLP ***7*** with a test sample putatively containing an anti-Zika virus antibody ***6*** (see Figure 12). Alternatively, or in addition, a VLP of the disclosure may be indirectly attached to a solid support by first attaching an anti-VLP antibody ***5*** to the solid support and then contacting the VLP ***7*** with the anti-VLP antibody to form a complex (see Figure 12). A test sample putatively containing an anti-Zika virus antibody ***6*** is then applied, creating a "sandwich" complex between the anti-VLP antibody, the VLP, and the antibody from the test sample having an affinity for the VLP. Regardless of the method chosen, detection of binding of an antibody from the test sample to the VLP is indicative of a Zika virus antibody being present in the sample.

[0136] Sandwich ELISA is used for detection of Zika Virus antibody in patients. The sandwich ELISA test for human Immunoglobulin G (IgG) is useful for the detection of circulating long-lived, neutralizing anti-Zika virus antibody. The Immunoglobulin M (IgM) sandwich ELISA is very effective for the early onset of the infection when IgM response peaks. Using the ELISA format, the wells of microtitre plates are coated with either Goat anti-human IgG or IgM, followed by incubation with subject serum containing anti-ZIKV antibodies in case of viral infection. After incubation, VLPs carrying Zika Virus peptide sequence are added to the well, unbound antigen is washed out, and Horseradish Peroxidase (HRP) conjugated anti-Zika Virus monoclonal antibody (revealing monoclonal antibody) is added. The bound conjugate is detected after addition of substrate solution such as TMB or enhanced chemiluminescence (ECL) reagent. The TMB reaction is terminated using stop solution and the degree of substrate hydrolysis is measured using spectrophotometry plate reader. Alternatively, the ECL signal can be detected using a plate reader with luminometer detector right after the ECL substrate addition.

[0137] Early and accurate diagnosis of Zika Virus is very important, especially in the field. The Lateral Flow Immuno Assay (LFIA) is able to detect anti Zika Virus antibodies in sera from clinically proven patients, as well as in healthy control subjects. The LFIA is used to detect subject serum antibody against Zika Virus antigen. Colloidal gold particle labelled goat anti human IgG/IgM (*e.g.*, 1.0 mg/L) is used as the detector reagent. Recombinant VLP protein (*e.g.*, 1.0 mg/L) is captured in the strip by anti-WHcAg antibody or absorbed directly to the

support. Rabbit anti-goat IgG (1.0 mg/L) are immobilized in test and control lines, respectively, on a nitrocellulose membrane, acting as the capture reagents (Figures 13 and 14).

EXAMPLES

[0138] As described herein, the present disclosure is related to chimeric VLPs containing and displaying epitopes and antigen from ZIKV. The disclosure also provides methods for creation and production of such chimeric VLPs to their applications, including but not limited to vaccines, diagnostics, clinical studies, assay development and antibody discovery. The recombinant and chimeric WHcAg VLP function as a carrier for highly immunogenic and optimized amino acids sequence(s) from the Domain III of the E protein (E DIII) or other immunogenic sequences from E protein of ZIKV. In addition, chimeric WHcAg VLP may include specific and selected amino acids sequence(s) from ZIKV viral protein NS1, prM/M or C (see Tables 1, 3, 5, and 7). Such recombinant amino acid sequences are inserted at a location between amino acids 77 and 82 of the WHcAg protein (GenBank accession number NP_671816). See Tables 2, 4, 6, and 8.

[0139] The disclosure also provides optimized production and purification of recombinant WHcAg chimeric VLPs in Yeast cellular system: *Komagataella phaffii* Kurtzman (ATCC® 76273™). The WHcAg chimera constructs are subcloned in pD912 vector from ATUM (formerly DNA2.0) (www.atum.bio) with secretion alpha-factor signal (SS_Alphafactor) linked to the N terminus of the WHcAg chimera sequence (Figure 2). Alternatively, the WHcAg chimeric construct is inserted in pD902 vector without a secretion signal for cytosolic protein expression and accumulation. Vector is linearized and used for creating high expressing yeast clones by transformation or electroporation in yeast cells. Yeast clones are selected using Zeocin resistance marker in semi-solid culture (YPD Agar). WHcAg chimera protein expression induction is obtained by optimized culture and using methanol supplementation. The secreted VLPs are purified from the yeast culture media by biochemical methods such as precipitation, ultracentrifugation, ultrafiltration, chromatography, tangential flow filtration (TFF) or a combination of such methods. VLPs that are expressed and accumulated in the yeast cytosol (not secreted) are purified by cell lysis methods (physical and chemical) followed by precipitation, ultracentrifugation, ultrafiltration, chromatography, tangential flow filtration (TFF) or a combination of such methods.

[0140] Cimica *et al.* [16] have developed a VLP vaccine for Respiratory Syncytial Virus (RSV) at TechnoVax Inc. (Tarrytown, NY). RSV-like particle (RS-VLP) vaccine was assembled with human metapneumovirus (hMPV) matrix protein as the structural particle scaffold, and RSV fusion glycoprotein (F) as the main immunogen. Structural vaccinology was applied for

increasing and optimizing F protein immunogenicity; multiple F constructs were generated and tested in antigenically different conformations. The immunization with RS-VLP vaccine adjuvanted with the squalene-based emulsion afforded full protection and was safe in the mouse model of RSV disease [16]. The present disclosure utilized an alternative approach for the creation and production of ZIK-VLP. VLPs can be produce in large scale fermentation of *Pichia pastoris* culture form selected clones. VLP purification is performed using state of the art methods such as: precipitation, ultracentrifugation, Tangential Flow Filtration (TFF), ultrafiltration and chromatography. Purity and quality of ZIK-VLPs chimera is tested by immunoassays and electron microscopy.

Example 1

Early development of a ZIKV VLP candidate

[0141] The ZIKV Envelope (E) protein is a primary target for vaccine development because it displays epitopes able to induce neutralizing and protective antibody in the host [36]. The E protein comprises the majority of the flavivirus surface and plays multiple roles in viral infection: host receptor recognition and binding, membrane fusion, viral release from endosomal compartment, virion assembly, and egress. The ZIKV shell is assembled with 180 copies of the E protein and comprises the majority of the virion surface [23, 37]. The E protein of any flavivirus including ZIKV shows a highly conserved structure that is divided into three domains: Domain I (DI) consisting of a central beta-barrel domain; Domain II (DII) important for dimerization and virion assembly; and Domain III (DIII) characterized by an immunoglobulin-like segment. Noticeably, the distal part of the DII contains a Fusion Loop domain with very high amino acid sequence identity between flavivirus.

[0142] Several studies in flavivirus including ZIKV have demonstrated that the E protein DIII (EDIII) is a primary antigenic target of specific neutralizing antibodies [38, 39]. In particular, it was shown that structural domains inside DIII can induce highly neutralizing and protective antibodies in a mouse model [38]. The ZIKV Fusion Loop domain in DII can induce highly neutralizing antibodies [18] that are able to cross react with other flavivirus. Cross-reacting antibodies, however, have been demonstrated to induce antibody-dependent enhancement (ADE) of ZIKV infection in patients with a history of DENV infection [40]. The Fusion Loop domain is implicated in ADE effects of ZIKV infection [41]. For these reasons, the present disclosure describes the use of a ZIK-VLP vaccine using EDIII selected epitopes as immunogen targets for neutralizing ZIKV.

[0143] Structural vaccinology was utilized for selecting specific epitopes and antigens from ZIKV EDIII (Figure 3 and Table 5). Antigenic sequences from Zika Virus Envelope (E) protein were identified using the Cn3D software from NIH (https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml) for structural analysis, and the CLC Sequence Viewer Qiagen (https://www.qiagenbioinformatics.com/products/clc-sequence-viewer/) for analysis of acid sequence conservation and specificity between flavivirus. The sequencing and structural data was obtained from US National Library of Medicine National Institutes of Health (https://www.ncbi.nlm.nih.gov/pubmed). Using recombinant DNA technology, such E DIII epitopes were included in the Woodchuck Hepatitis core Antigen (WHcAg) scaffolding system for delivery of the epitopes (Figure 3).

	CONSTRUCTS GENERATED	TESTED
1	WHcAg (MOCK CONTROL FOR IMMUNIZATION)	YES
2	WHcAg CHIMERA E PROTEIN DOMAIN III FULL LENGTH	YES
3	WHcAg CHIMERA E PROTEIN DOMAIN III, A-B LOOP	YES
4	WHcAg CHIMERA E PROTEIN DOMAIN III, CX-C-D-DX LOOP	YES
5	WHcAg CHIMERA E PROTEIN DOMAIN III, DX-E LOOP	YES
6	WHcAg CHIMERA E PROTEIN DOMAIN III, F-G LOOP	YES
7	E PROTEIN (POSITIVE CONTROL FOR IMMUNIZATION)	YES

Table 17. DNA Constructs for production of ZIKV-VLPs

[0144] Such a system has been used successfully for vaccine candidates: human HBV surface protein (HBsAg) or HBV core antigen protein (HBcAg) are currently in clinical trials for influenza virus and the malaria parasite (Plasmodium falciparum) [42]. Although the HB-VLP system is a very efficient platform for antigen delivery to APCs and B cells [43], such technology has two limitations: i) HBV proteins may not assemble properly because the steric hindrance of the carried antigen; ii) preexisting immunity against HBV may reduce greatly the immunization efficiency. For these reasons, we will adopt the WHcAg scaffolding system [21] that was successfully applied for developing VLP-based vaccines for RSV [44], and malaria parasite [45]. The WHcAg has the ability to function as a carrier for a selected epitope/antigen peptide (*e.g.*, 5-100 amino acids) for inducing a very specific antibody response. Applying structural vaccinology, we have designed ZIKV DIII-optimized antigens comprising either full length DIII domain, or selected DIII structural domains comprising the A-B loop, C-D loop, D-E loop and F-G loop (Table 1 and 2, Figure 3). Using recombinant expression technology, DNA constructs for ZIK-VLP expression in *Pichia pastoris* were developed, and the potential vaccine candidates are tested for efficacy and safety in an A129, AG129 and C57BL/6 treated with anti-IFNAR1 antibodies mouse model for ZIKV infection.

Example 2

Production of ZIK-VLP Using the Pichia Expression System

[0145] Appropriate *Pichia* yeast strains for protein expression are available from, *e.g.*, ATCC (*e.g.*, *Komagataella phaffii* Kurtzman ATCC 76274™ or *Komagataella pastoris* ATCC® 76274™). Using in silico analysis, codon-optimized DNA constructs expressing the ZIKA EDIII antigens conserved between different strains were designed (Figures 2, 3A, and 3B). Constructs using the promoter from the Pichia alcohol oxidase 1 (AOX1) gene were developed to drive production of the recombinant protein (ATUM.bio). Purification of VLPs by ultracentrifugation and ultrafiltration methods and assays for quantification, purity and immunogenicity of the VLPs has been established [16]. Importantly, VLPs morphology and purity was assessed using Electron Microscopy analysis (Figure 5). Antigenicity of VLPs was tested using Western blotting and dot blot methods using different commercially available and tested commercially available monoclonal antibodies against EDIII domain such as ZV-2 (ATCC BEI Resources NR-50414 Monoclonal Anti-Zika Virus Envelope (E) Protein) and ZV-54 (Millipore Sigma MABF2046, Anti-Zika Virus Antibody) (Figures 6 and 7).

Example 3

Immunizing animals: Mouse Study

[0146] Safety is determined in the context of pregnant female BALB/c mice and in the context of 5 week old male and female mice. In both cases (n=10/concentration), three different concentrations (10µg, 25µg and 50µg) of WHcAg-ZIKV chimera VLP are injected intramuscularly. As negative controls, PBS and WHcAg VLPs without Zika virus antigen are injected. To evaluate safety in the context of a prime-boost strategy, an independent set of animals (n=10/concentration) is injected at 3 weeks post the initial vaccination event with the same concentration of VLP as used in the prime vaccination. The animals are weighed daily and their morphological features and behavior (eating, drinking, mobility, social behavior) are recorded in comparison with the negative control group. Terminally sacrificed animals are necropsied to assess gross toxicity at the level of the internal organs including the spleen and the liver. The spleen tissue is banked for B-cell assays. Inflammatory load is evaluated in these animals at the end of the study. Following a terminal bleed, serum is obtained and utilized to quantify inflammatory mediators in circulation following the prime alone and the prime-boost strategy. The Aushon Multiplex Platform (Ciraplex, Aushon Biosystems) or Luminex system is used to simultaneously quantify the levels of inflammatory mediators. Such assays allow an analysis of multiple cytokines and chemokines in serum and tissue in vaccinated animals.

[0147] Animal studies towards characterizing the WHcAg-ZIKV chimera VLP vaccine are performed using three lethal models for ZIKV infection: i) the A129 mouse model [46]; ii) the AG129 mouse model [47]; and iii) the C57BL/6 immunocompetent mouse model treated with Anti-IFNAR1 antibody [27] (Figure 15). The challenge experiments are carried out according to Rossi et al in AG126 mouse model: 3 week old mice are the most susceptible to ZIKV infection while 5 week old mice showed signs of disease but recovered [46]. The 5 week old mice continued to maintain detectable viral load in the serum that could be compared with the 3 week old mice. Typical vaccination strategies require at least 2-3 weeks duration for the host to mount an immune response. For the three week old mice, this requires vaccination to be carried out immediately after birth. There are uncertainties regarding robustness of the immune system in a newborn animal. To address these concerns, in the current study, the 5 week old animal are challenged with a prime immunization at week 1 after birth and a boost at week 4 after birth, followed by challenge in week 5. The A129 is an immunocompromised animal model that could be unable to recapitulate the immunization response. For this reason, the immunocompetent mouse model BALB/c treated with Anti-IFNAR1 antibody is included before ZIKV challenge. The comparison between the two models is relevant to improve immunization strategies including vaccine dosage and formulation according to [27]. The challenge experiments are conducted using Zika Virus FSS13025 Cambodia strain [46], the Puerto Rico strain (PRVABC59) and other strains available at ATCC BEI-Resources (www.beiresources.org). Standardized assays for the quantification of this strain by plaque assay and quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) have been developed. The prime-only, prime-boost vaccinated animals (at the maximum tolerated concentration of VLP with no apparent toxic outcomes) are challenged after vaccination by intra peritoneal challenge with a Zika virus strain (*e.g.*, PRVABC59) with 1×10^4 plaque-forming units (PFU). The infected animals are monitored continuously for one week. If there is no protection or suboptimal protection, the animals will show symptoms of disease. The animals are monitored for signs of illness including weight loss, hunched posture and ruffled fur and for signs of severe disease including tremors, lethargy and anorexia. The mortality rate of vaccinated animals versus unvaccinated controls is quantified. At the end of the study period, survivors are sacrificed and samples collected for follow up studies. All sacrificed animals are terminally bled and serum collected. The serum is subjected to analysis of inflammatory mediators. In addition, the circulating viral load (infectious viral titers and genomic copy numbers) is quantified in all experimental and control groups. The neutralization antibody titers are determined using the serum samples by plaque reduction neutralization assay (PRNT assay). $PRNT_{50}$ and $PRNT_{80}$

titer values will be obtained by the method described in the art [16, 48]. Necropsy is conducted on all animals and spleen isolated for B cell activity studies (described below). General gross morphological examination of other internal organs including the liver is conducted. As flaviviruses, in general, demonstrate a tropism to the liver, the viral load in the liver +/- VLP is quantified.

[0148] A group of 5 mice were immunized twice (prime and boost) with the placebo control (WHcAg CTRL VLPs devoid Zika antigen) and the Zika vaccine candidate (WHcAg CD loop VLPs) by intramuscular injection. The VLPs dosage was 10 µg adjuvanted with squalene-based oil-in-water nano-emulsion AddaVax (InvivoGen). Boost immunization was performed 14 days after prime immunization. After 28 days the prime immunization, animals were conditioned for Zika Virus infection using the anti-IFNAR1 antibody according to the literature protocol [27], see Figure 16. Viral infection was performed by intraperitoneal injection 1 day after anti-IFNAR1 antibody treatment, using 10,000 plaque forming units (PFU) of Zika Virus Puerto Rico strain PRVABC59 (ATCC, BEI Resources NR-50240). Serum viremia was analyzed 3 days viral post-injection using quantitative Real-Time PCR (qRT-PCR), with the instrument for Bio-Rad CFX96 Touch™, and the kit Bio-Rad iTaq Universal SYBR Green kit (Catalog #172-5151), following the manufacturer's instructions. Specific Zika PCR primers used were according to the protocol of Lanciotti, R. et al. [49]: Forward oligo 5' CCGCTGCCCAACACAAG 3'; and Reverse oligo 5' CCACTAACGTTCTTTTGCAGACAT 3'. Quantification of viral copy number per microliter (µl) was obtained by standard curve approach using the Zika Virus (strain PRVABC59) genomic RNA standard (ATCC, BEI Resources NR-50244). Figure 17 shows that ZIKV copy number was decreased in the mice receiving the Zika vaccine candidate (WHcAg CD loop VLPs).

[0149] Safety is determined in the context of pregnant female BALB/c mice and in the context of 5 week old male and female mice. In both cases (n=10/concentration), three different concentrations (10µg, 25µg and 50µg) of VLP are injected intramuscularly. As negative controls, PBS and WHcAg VLPs without Zika virus antigen are injected. To evaluate safety in the context of a prime-boost strategy, an independent set of animals (n=10/concentration) is injected at 3 weeks post the initial vaccination event with the same concentration of VLP as used in the prime vaccination. The animals are weighed daily and their morphological features and behavior (eating, drinking, mobility, social behavior) are recorded in comparison with the negative control group. Terminally sacrificed animals are further necropsied to assess gross toxicity at the level of the internal organs including the spleen and the liver. The spleen tissue is banked for B-cell assays. Inflammatory load is evaluated in these animals at the end of the study. Following a terminal bleed, serum is obtained and utilized to quantify inflammatory mediators in circulation

following the prime alone and the prime-boost strategy. The Aushon Multiplex Platform (Ciraplex, Aushon Biosystems) or Luminex system is used to simultaneously quantify the levels of inflammatory mediators. Such assays allow an analysis of multiple cytokines and chemokines in serum and tissue in vaccinated animals. Zika-VLP vaccine candidates are tested in a murine model for protection against fetal transmission, assessing fetal viability, morphology and viremia. The well-established model for trans-placental transmission using the A129 mouse is employed. In this model, infecting dams at embryonic day six (E6) results in placental insufficiency and fetal demise, while dams infected at midstage E9 show cranial dimension reduction. Importantly, infection at E6 results in 100% nonviable fetuses, while infection at E9 results in 90% fetal viability, 5 days after infection in both groups (see Figure 16).

[0150] Mouse models will be useful for identify specific neutralizing antibody against Zika Virus according to the literature [38].

Example 4

Cross-reactivity for Zika Virus antibody and Antigen Dependent Enhancement test

[0151] In vitro Assays for testing Antigen Dependent Enhancement (ADE) in ZIK-VLP chimera vaccinated mice are performed for testing vaccine specificity. Mouse serum from immunized animals with ZIK-VLP chimera vaccine is tested in a standard *in vitro* assay using U937 (ATCC® CRL-1593.2™) and K562 (ATCC® CCL-243™) lymphocyte cell-lines from ATCC according to methods known in the art. Briefly, serial dilutions of heat- inactivated sera from BALB/c mice is incubated with DENV strains for each serotypes 1 to 4, for 1 hour at 37°C. As a positive control for ADE the pan-Flavivirus antibody, clone D1-4G2-4-15 (ATCC BEI Resources, NR-50327) is also included. Serum from animals immunized with WHcAg VLPs will be used as a negative control.

[0152] The cells are incubated with the serum-virus mixture for 2 hours at 37°C with multiplicity of infection (MOI) 3, and are washed in order to remove free viral particles. Viral titer in the culture supernatant is measured according to the art [27, 50] with standard quantitative Real-Time-PCR (qRT-PCR) after 4 days, to allow for viral replication.

Example 5

Zika Virus Diagnostic

[0153] **Antibody-sandwich ELISA**. Antibody-sandwich ELISA is perhaps the most useful of the immunosorbent assays for detecting antigen/antibody because it is between 2 and 5 times more sensitive than the direct/indirect ELISA in which antigen is directly bound to the solid

phase. Two sets of sandwich ELISAs will be developed to 1) detect the presence of long-lasting, neutralizing anti-Zika virus antibodies (IgG), and 2) enable early detection of anti-Zika IgM in clinical samples.

[0154] To detect ZIKV antigens in sandwich ELISA format, the wells of microtitre plates are coated with antibody against the scaffolding system WHcAg in order to capture different types of WHcAg-ZIKV chimera VLPs. The ELISA plates are incubated with subject serum (human or mouse) containing anti-ZIKV antibodies. The bound conjugate is detected after addition of specific secondary antibody against IgG or IgM labeled with Horseradish Peroxidase (HRP). The detection of antibody against Zika Virus antigen is performed using HRP substrates such as TMB or ECL and a microplate reader instrument. A positive control using antibody generated against Zika Virus is included in the test, while negative controls include: WHcAg VLPs without any Zika antigen or not immunized serum against Zika. The sandwich ELISA test for human IgG is useful for the detection of circulating long-lived, neutralizing anti-Zika virus IgG. The IgM sandwich ELISA will be very effective for the early onset of the infection when IgM response peaks.

[0155] **Rapid Diagnostic Detection using Lateral Flow Immunoassay (LFIA) system**. Early and accurate diagnosis of Zika Virus is very important, especially on the field. The LFIA (Figure 13) will be used to detect anti Zika Virus antibodies in sera from clinically proven patients, as well as in healthy control subjects (Figure 14). The lateral flow immunoassay (LFIA) is developed to detect subject serum antibody against Zika Virus Envelope and NS1 antigen. Colloidal gold particle labelled goat anti human IgG/IgM (1.0 mg/L) is used as the detector reagent. Recombinant WHcAg-ZIKV chimera VLP protein (1.0 mg/L) and rabbit anti-goat IgG (1.0 mg/L) were immobilized in test and control lines, respectively, on a nitrocellulose membrane, acting as the capture reagents. Alternatively recombinant WHcAg-ZIKV chimera VLPs can be captured on the support by immobilized antibody able to bind the WHcAg scaffolding protein.

Example 6

Developing a formulation of VLPs

[0156] Zika VLP vaccine is manufactured according cGMP guidelines and formulated following standard FDA guidelines. The vaccine is free from adventitious agents and toxic chemicals. Formulations will include diluents, stabilizers, adjuvants and preservatives [12, 51]. The studies disclosed herein include formulation optimization in order to increase vaccine efficacy and safety.

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WHAT IS CLAIMED IS:

1. An isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11.

2. The peptide of claim 1 comprising or consisting of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 2-11.

3. An isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 22-33.

4. The peptide of claim 3 comprising or consisting of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 22-33.

5. An isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 46-47.

6. The peptide of claim 5 comprising or consisting of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 46-47.

7. An isolated peptide comprising or consisting of an amino acid sequence that is at least 80% identical to a sequence as set out in any one or more of SEQ ID NOs: 50-51.

8. The peptide of claim 7 comprising or consisting of an amino acid sequence that is 100% identical to a sequence as set out in any one or more of SEQ ID NOs: 50-51.

9. A chimeric peptide comprising the peptide of any one of claims 1-8 linked to a heterologous peptide having an amino acid sequence that is at least 90% identical to a Woodchuck Hepatitis core Antigen protein (WHcAg) comprising or consisting of an amino acid sequence as set out in SEQ ID NO: 1, or comprising or consisting of at least one fragment of SEQ ID NO: 1.

10. A chimeric peptide comprising the peptide of any one of claims 1-8 linked to a heterologous peptide having an amino acid sequence that is at least 90% identical to a Woodchuck Hepatitis core Antigen protein (WHcAg) comprising or consisting of an amino acid sequence as set out in SEQ ID NO: 1, wherein the peptide of any one of claims 1-8 is inserted between amino acids 77 and 82 of SEQ ID NO: 1.

11. The chimeric peptide according to claim 9 or 10, further including at least one peptide linker of 1-10 amino acids linking the sequence that is at least 80% identical to any one or more of SEQ ID NOs: 2-11 or 22-33, 46-47 and 50-51 to the sequence that is at least 90% identical to a WHcAg protein.

12. The chimeric peptide according to claim 9 or 10 comprising or consisting of an amino acid sequence at least 90% or at least 95% identical to any one of SEQ ID NOs: 12-21, 34-45, 48-49, and 52-53.

13. A polynucleotide comprising a nucleotide sequence encoding the chimeric peptide of any one of claims 9-12.

14. The polynucleotide according to claim 13, comprising a nucleotide sequence at least 90% identical to or at least 95% identical to any one of SEQ ID NOs: 65-74, 87-98, 101-102, and 105-106.

15. A vector comprising the polynucleotide of claim 13 or 14.

16. An expression vector comprising the polynucleotide of claim 13 or 14 operably linked to an expression control sequence.

17. A recombinant host cell comprising the vector of claim 15 or the expression vector of claim 16.

18. The recombinant host cell of claim 17, wherein the host cell is:

(i) a eukaryotic cell selected from the group consisting of mammalian, yeast, insect, plant, amphibian and avian cells; or

(ii) a prokaryotic cell.

19. A virus like particle (VLP) comprising or consisting essentially of the chimeric peptide of any one of claims 9-12.

20. An article comprising the chimeric peptide according to any one of claims 9-12, or the VLP according to claim 19, attached to a solid support.

21. The article of claim 20, wherein the solid support is selected from the group consisting of a microbead, an assay plate, a test strip, or a filter.

22. An antigenic composition comprising the VLP of claim 19, wherein the VLP is present in the composition at a concentration of about 0.1-2000 µg/ml, in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant.

23. An antigenic composition comprising the VLP of claim 19 in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant.

24. The antigenic composition of claim 23 comprising first and second VLP according to claim 19, wherein the first and second VLP comprise different sequences independently

selected from amino acid sequences at least 80% identical to SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51.

25. The antigenic composition of claim 23 comprising first, second, and third VLP according to claim 19, wherein the first, second, and third VLP comprise different sequences independently selected from amino acid sequences at least 80% identical to SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51.

26. The antigenic composition of claim 23 comprising first, second, third, fourth, fifth, sixth, and seventh VLP according to claim 19, wherein the first, second, third, fourth, fifth, sixth, and seventh VLP comprise different sequences independently selected from SEQ ID NOs: 2-11, 22-33, 46-47, or 50-51.

27. A composition comprising the vector of claim 15 or claim 16 in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant.

28. The composition of claim 27, wherein the adjuvant is a polymeric particle, cholera toxin, or imidazoquinoline.

29. A kit comprising the VLP of claim 19 or the article of claim 20 or claim 21 packaged with at least one reagent selected from an enzyme substrate, a detection antibody, and a blocking buffer.

30. A vaccine comprising the antigenic composition of any one of claims 22-26 or 28, and an adjuvant.

31. An antigenic composition comprising the VLP of claim 19 in a pharmaceutically acceptable carrier, diluent, stabilizer, preservative, or adjuvant, wherein the composition is capable of generating an immune response including antibody generation or a protective immune response in a mammalian subject.

32. The antigenic composition of claim 31, wherein the antibody response generated by the composition is improved relative to that achieved with Zika virus or Zika envelope protein.

33. The antigenic composition of claim 31 or claim 32, wherein the antibody response is a neutralizing antibody response or a protective antibody response.

34. A method of producing an immune response to a Zika virus in a subject, comprising administering to the subject an effective amount of the antigenic composition of claims 22-26 or 31-33, or the vaccine of claim 30, thereby producing an immune response to a Zika virus in the subject.

35. A method of treating a Zika virus infection in a subject in need thereof, comprising administering to the subject an effective amount of the antigenic composition of claims 22-26 or 31-33, thereby treating a Zika virus infection in the subject.

36. A method of preventing a disease or disorder caused by a Zika virus infection in a subject, comprising administering to the subject an effective amount of the vaccine of claim 30, thereby preventing a disease or disorder caused by a Zika virus infection in the subject.

37. A method of protecting a subject from developing one or more symptoms of Zika virus infection, comprising administering to the subject an effective amount of the vaccine of claim 30, thereby protecting the subject from developing one or more symptoms of Zika virus infection.

38. A method of immunizing a mammalian subject against a Zika virus infection comprising administering to the subject an effective amount of the antigenic composition of claims 22-26 or 31-33, or the vaccine of claim 30, thereby immunizing the subject against a Zika virus infection.

39. A method of protecting a subject from sexual transmission of Zika virus, comprising administering to the subject an effective amount of the antigenic composition of claims 22-26 or 31-33, or the vaccine of claim 30, thereby protecting the subject from sexual transmission of Zika virus.

40. The method of claim 39, wherein the administering is mucosal administration.

41. The method of claim 30, wherein the mucosal administration is nasal, vaginal, rectal, or oral.

42. A method of detecting or measuring antibodies to Zika virus in a biological sample comprising:

a) contacting the VLP of claim 19 with a biological sample under conditions suitable for the formation of an antigen-antibody complex; and

b) measuring or detecting antibodies to Zika virus by detecting or measuring an antigen-antibody complex formed between antibodies in the biological sample and the VLP.

43. A method of detecting a Zika virus infection comprising the steps of:

a) contacting the VLP of claim 19 with a biological sample from a mammalian subject under conditions suitable for the formation of an antigen-antibody complex; and

b) detecting the antigen-antibody complex formed between the VLP and antibodies in the biological sample, thereby detecting the Zika virus infection.

44. The method of claim 43, further comprising the step of detecting the Zika virus in the biological sample, wherein presence of the Zika virus indicates a current Zika virus infection.

45. A method for screening anti-Zika virus antibodies comprising:

a) measuring binding of an antibody or fragment thereof to the VLP of claim 19; and

b) measuring binding of the antibody or fragment thereof to a Woodchuck Hepatitis core Antigen protein (WHcAg) VLP; and

c) determining that the antibody or fragment thereof is an anti-Zika antibody when the antibody or fragment thereof binds to the VLP but not the WHcAg.

46. The method of any one of claims 42-45, wherein the virus like particle is attached to a solid support.

47. The method of claim 46, wherein the solid support is a microbead, an assay plate, a test strip, or a filter.

48. A method of producing a virus like particle (VLP) comprising introducing into a host cell the vector of claim 15 under conditions such that the cell produces the VLP.

49. The method of claim 48, wherein the host cell is:

(a) a eukaryotic cell selected from the group consisting of mammalian, fungal, insect, plant, amphibian and avian cells; or

(b) a prokaryotic cell.

50. The method of claim 49, wherein the host cell is a yeast cell.

51. The method of claim 50, wherein the yeast cell is a *Pichia pastoris* cell.

52. The method of any one of claims 48-51, wherein the vector is introduced into the host cell via transformation, transfection, transduction, or electroporation.

53. The method of any one of claims 48-52, wherein the cells are cultured at temperatures ranging from 25°C to 37°C.

54. The method of any one of claims 48-53, wherein the VLP is purified from the host cell or a culture media of the host cell.

55. The method of claim 54, wherein the VLP is purified via precipitation, ultracentrifugation, density gradient ultracentrifugation, ultrafiltration, chromatography, tangential flow filtration (TFF), or a combination thereof.

56. A virus like particle (VLP) produced by the method of any one of claims 48-55.

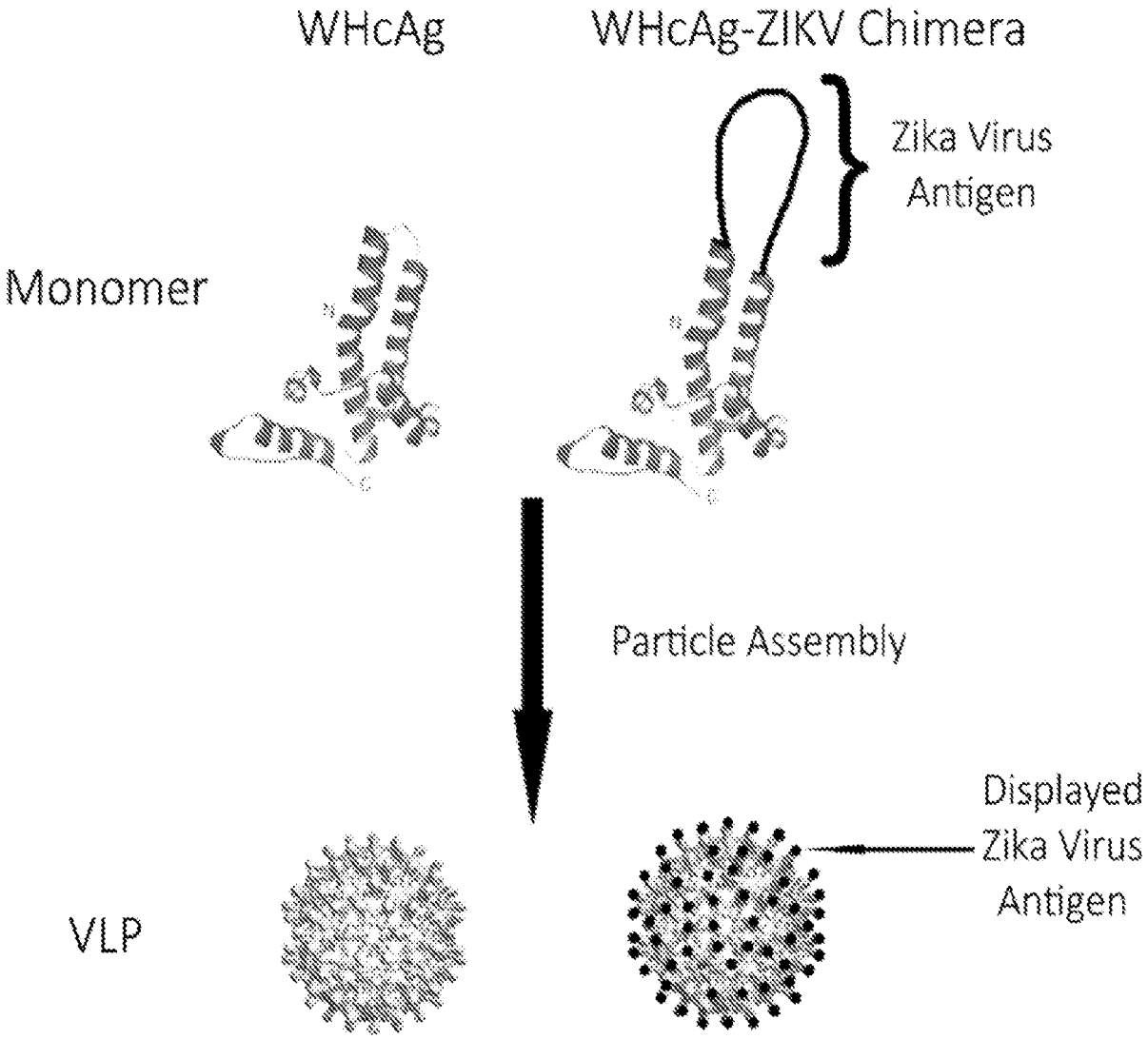

WHcAg
WHcAg-ZIKV Chimera
Zika Virus Antigen
Monomer
Particle Assembly
Displayed Zika Virus Antigen
VLP

FIG. 1

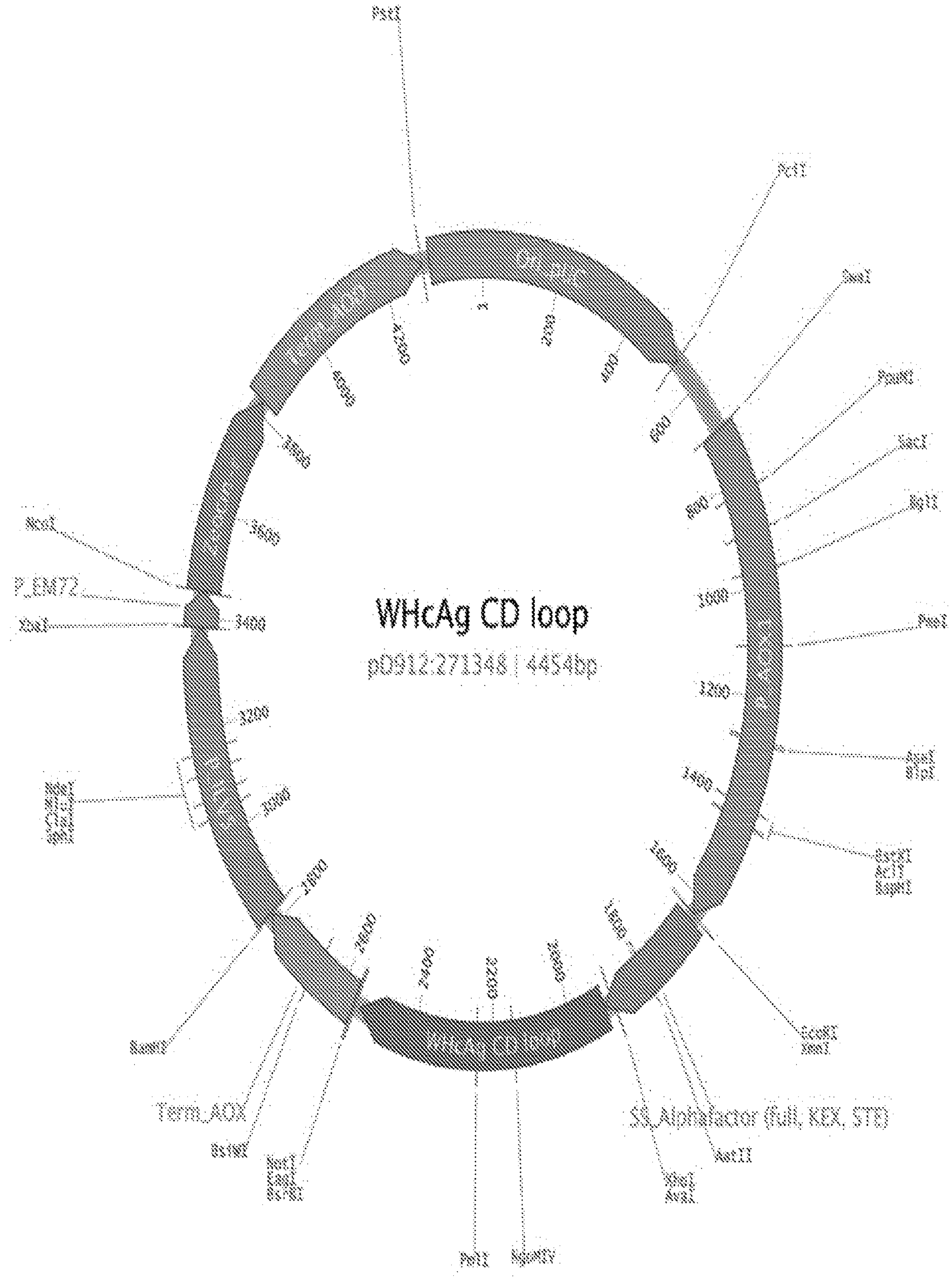
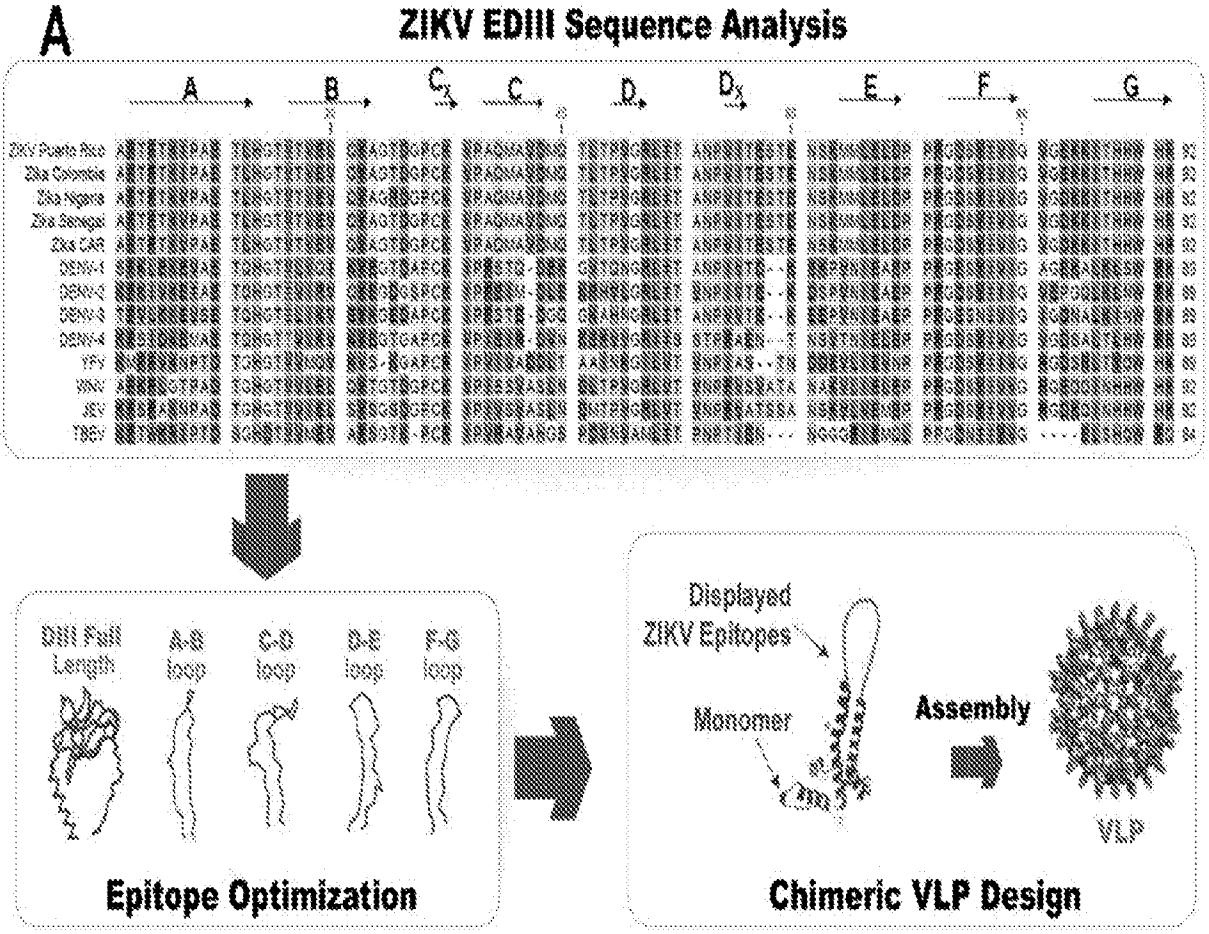

WHcAg CD loop
pD912:271348 | 4454bp
P_EM72
Term_AOX
SS_Alphafactor (full, KEX, STE)
PstI
XbaI
NcoI
PmeI
SacI

FIG. 2


A
ZIKV EDIII Sequence Analysis
A
B
Cx
C
D
Dx
E
F
G
DIII Full Length
A-B loop
C-D loop
D-E loop
F-G loop
Epitope Optimization
Displayed ZIKV Epitopes
Monomer
Assembly
VLP
Chimeric VLP Design

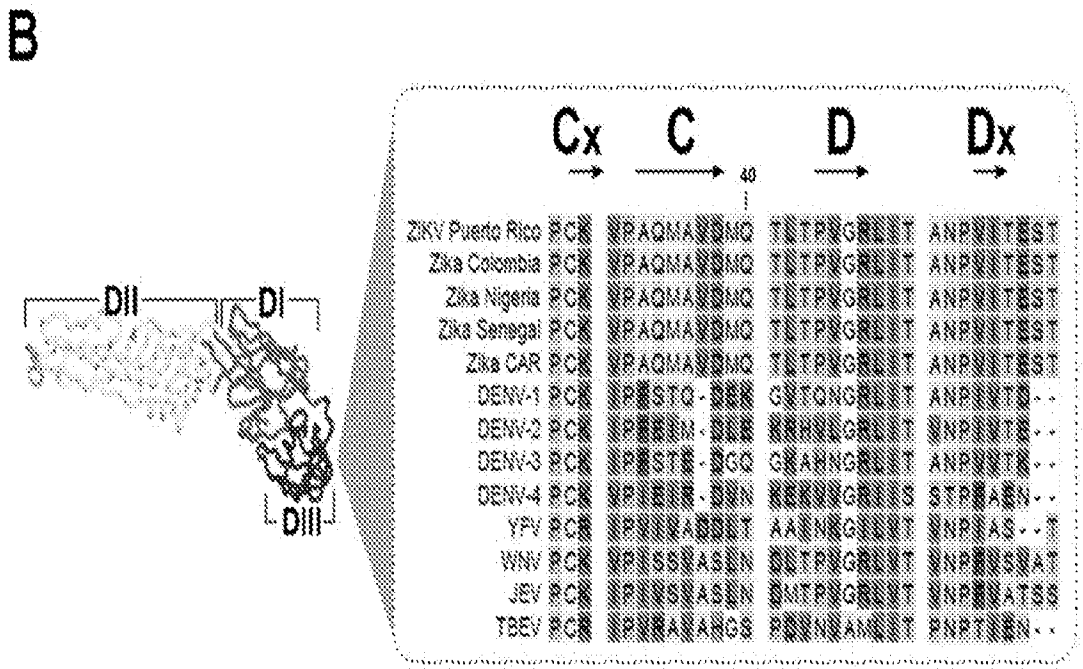

B
DII
DI
DIII
Cx
C
D
Dx
40
ZIKV Puerto Rico
Zika Colombia
Zika Nigeria
Zika Senegal
Zika CAR
DENV-1
DENV-2
DENV-3
DENV-4
YFV
WNV
JEV
TBEV

FIG. 3

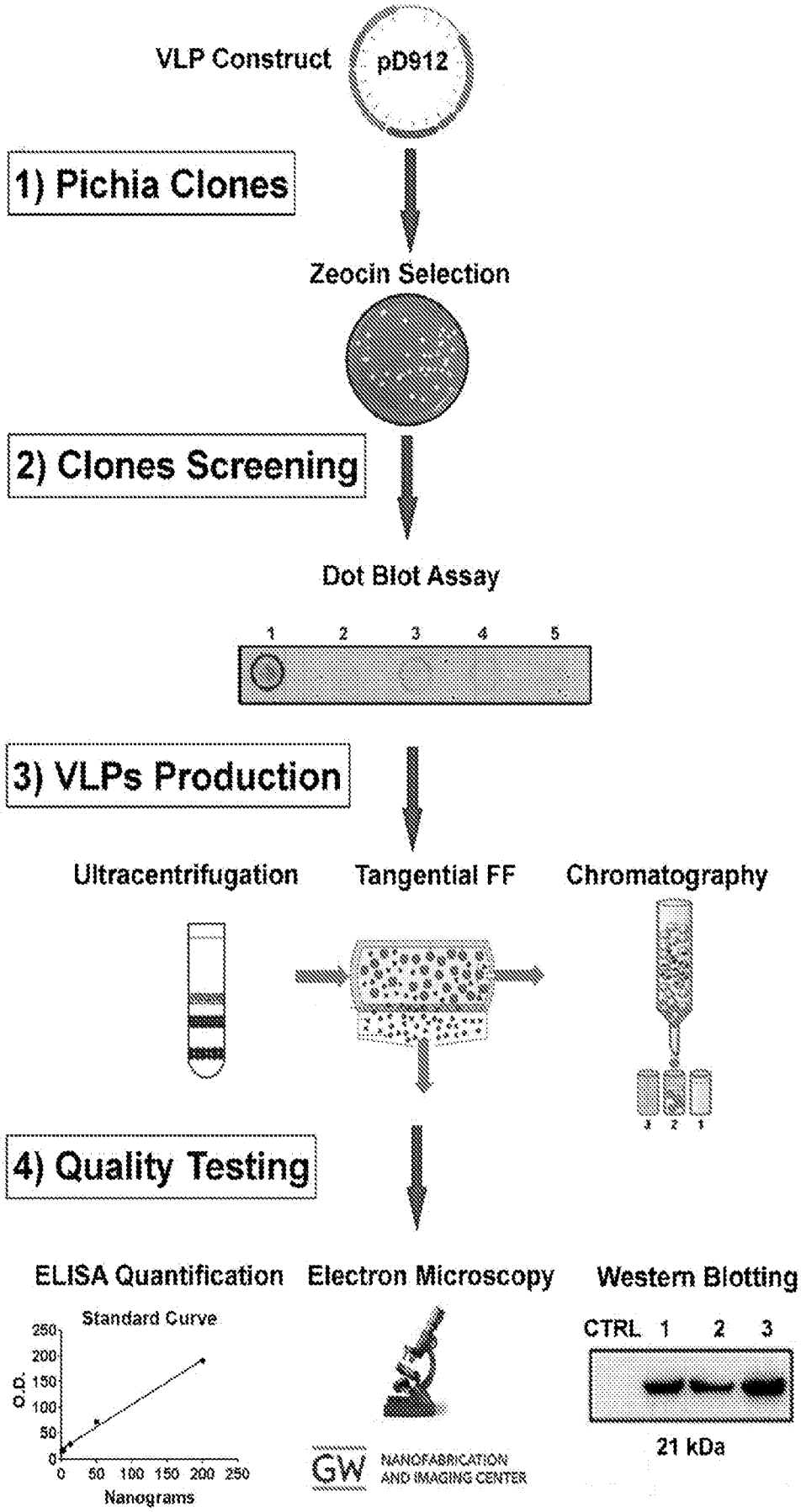

VLP Construct
pD912
1) Pichia Clones
Zeocin Selection
2) Clones Screening
Dot Blot Assay
1 2 3 4 5
3) VLPs Production
Ultracentrifugation
Tangential FF
Chromatography
4) Quality Testing
ELISA Quantification
Standard Curve
O.D.
250
200
150
100
50
0
0 50 100 150 200 250
Nanograms
Electron Microscopy
GW NANOFABRICATION AND IMAGING CENTER
Western Blotting
CTRL 1 2 3
21 kDa

FIG. 4

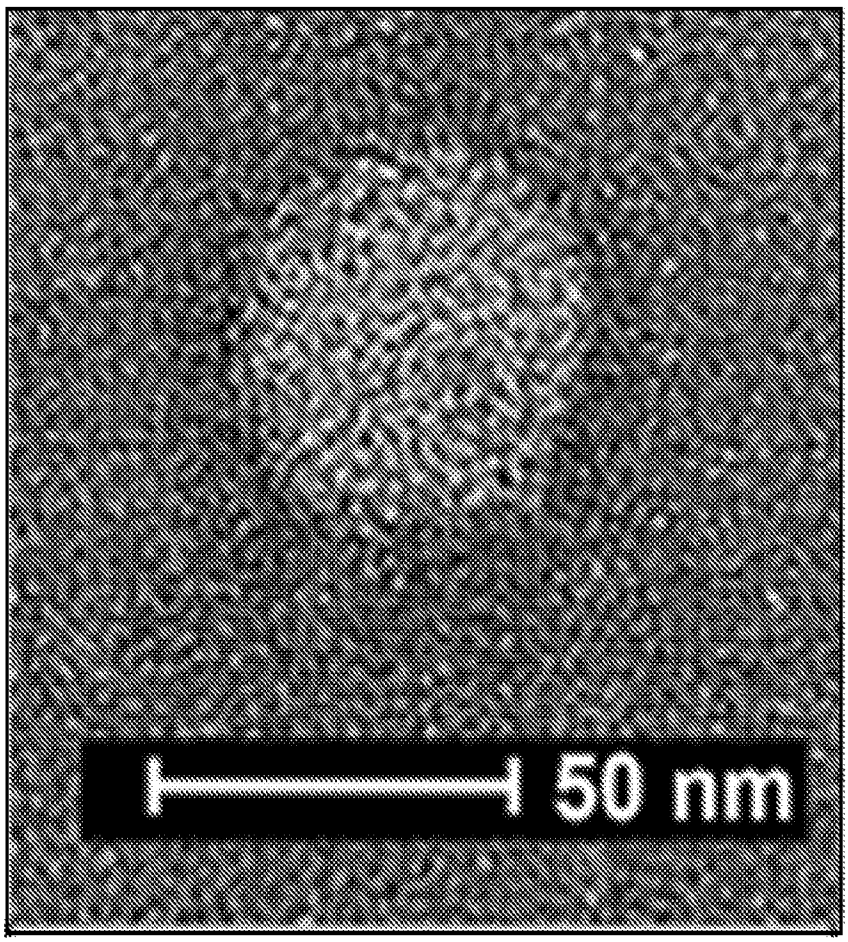


FIG. 5

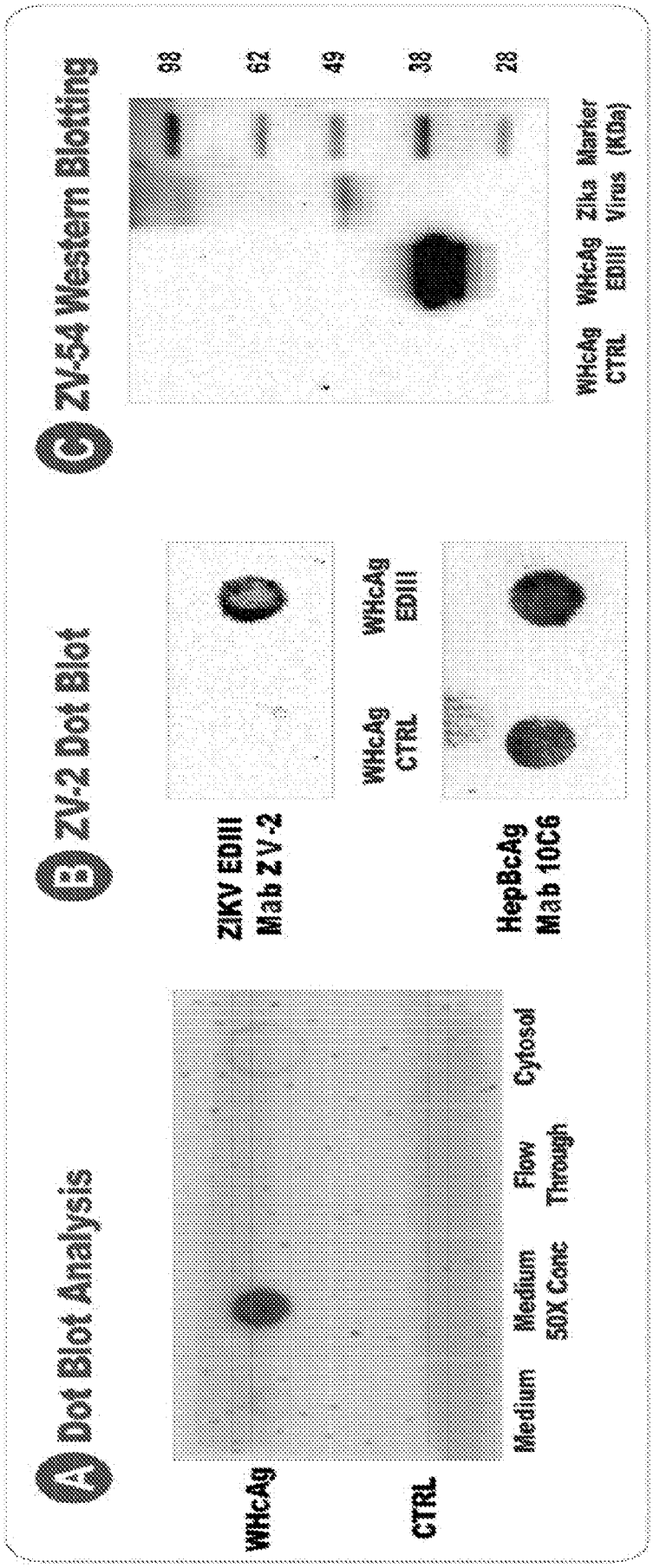

A Dot Blot Analysis
WHcAg
CTRL
Medium
Medium 50X Conc
Flow Through
Cytosol
B ZV-2 Dot Blot
ZIKV EDIII Mab ZV-2
WHcAg CTRL
WHcAg EDIII
HepBcAg Mab 10C6
C ZV-54 Western Blotting
98
62
49
38
28
WHcAg CTRL
WHcAg EDIII
Zika Virus
Marker (KDa)

FIG. 6

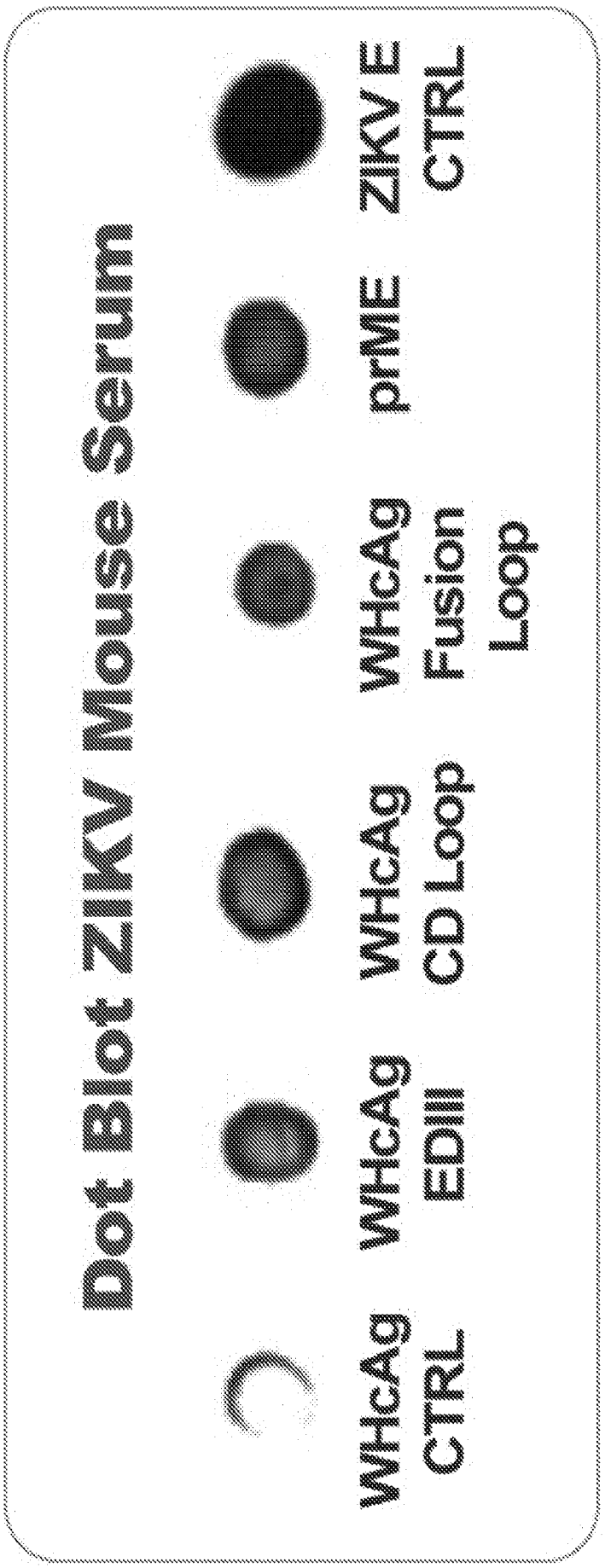

Dot Blot ZIKV Mouse Serum
WHcAg CTRL
WHcAg EDIII
WHcAg CD Loop
WHcAg Fusion Loop
prME
ZIKV E CTRL

FIG. 7

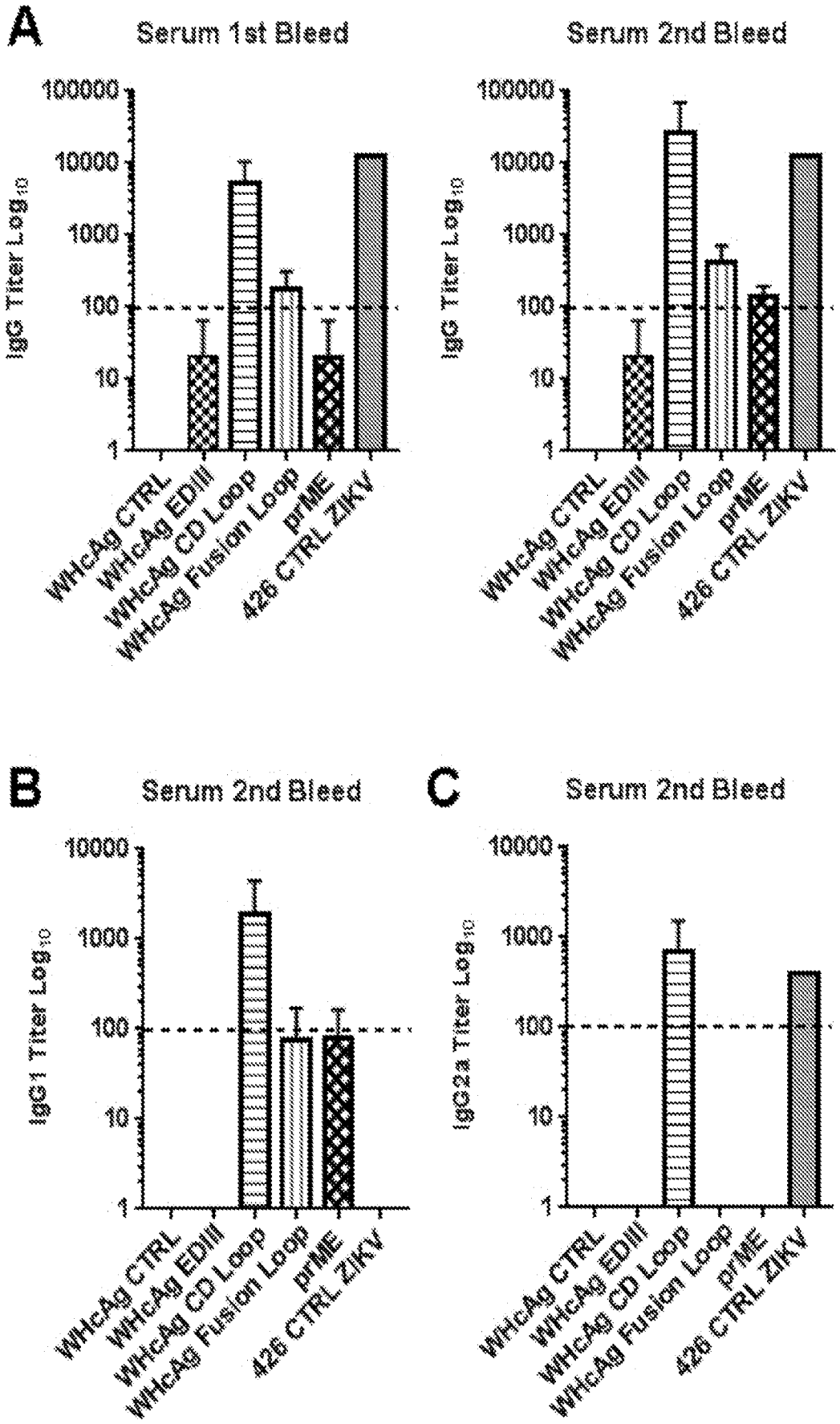

A
Serum 1st Bleed
Serum 2nd Bleed
IgG Titer Log10
100000
10000
1000
100
10
1
WHcAg CTRL
WHcAg EDIII
WHcAg CD Loop
WHcAg Fusion Loop
prME
426 CTRL ZIKV
B
Serum 2nd Bleed
IgG1 Titer Log10
C
Serum 2nd Bleed
IgG2a Titer Log10

FIG. 8

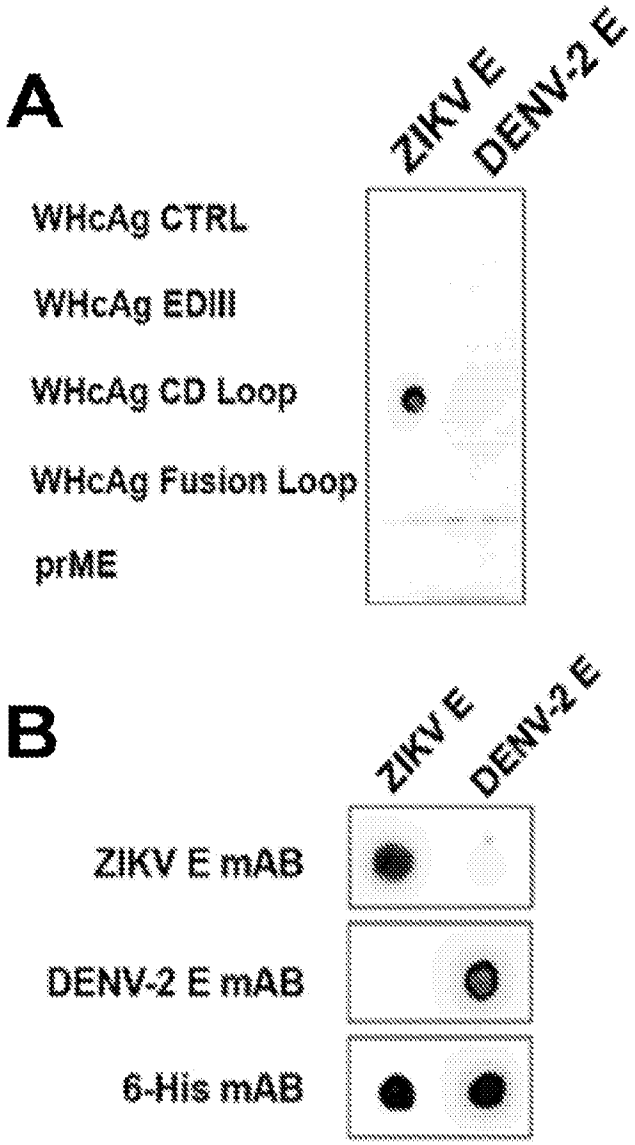

A
ZIKV E
DENV-2 E
WHcAg CTRL
WHcAg EDIII
WHcAg CD Loop
WHcAg Fusion Loop
prME
B
ZIKV E
DENV-2 E
ZIKV E mAB
DENV-2 E mAB
6-His mAB

FIG. 9

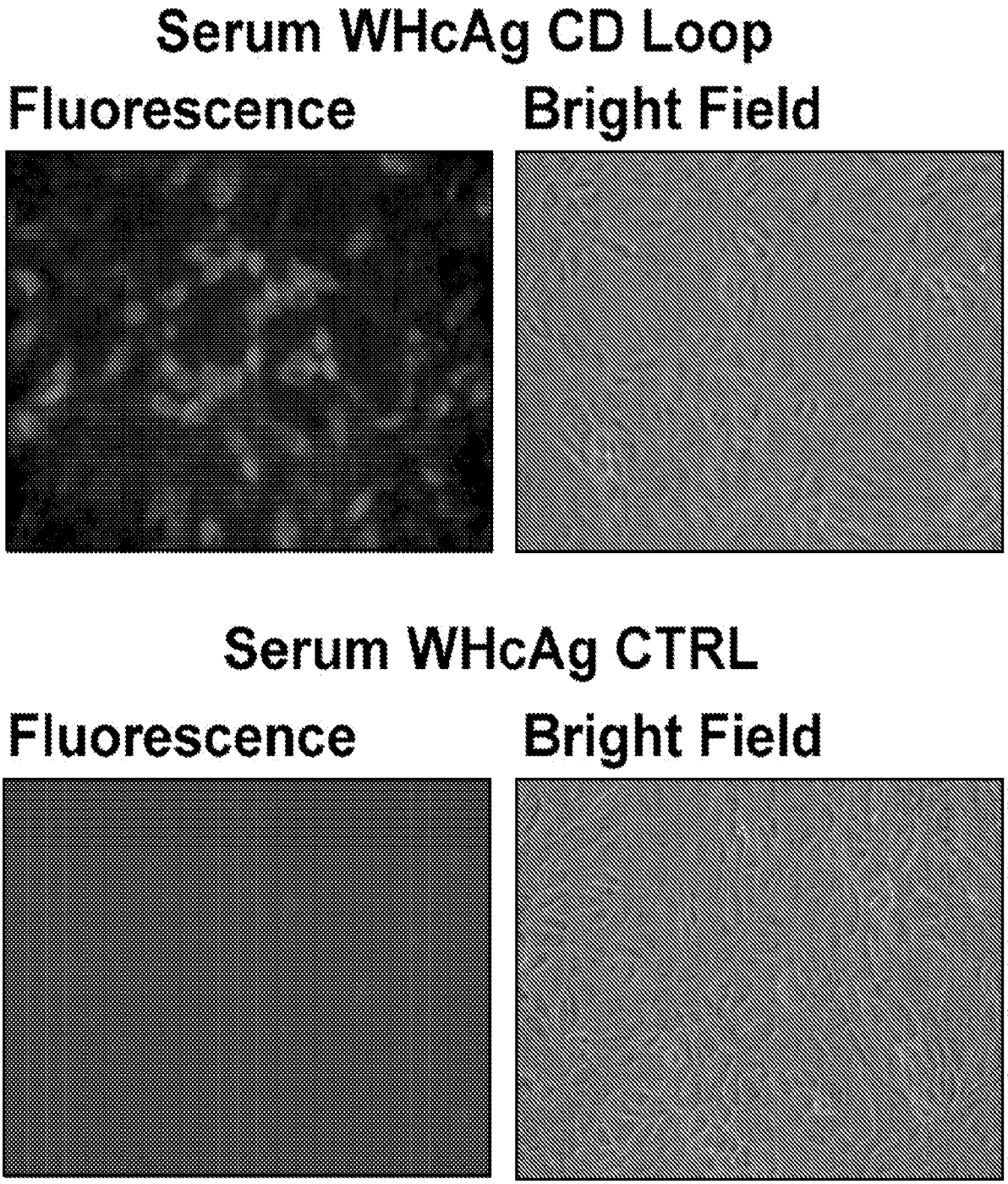

Serum WHcAg CD Loop
Fluorescence
Bright Field
Serum WHcAg CTRL
Fluorescence
Bright Field

FIG. 10

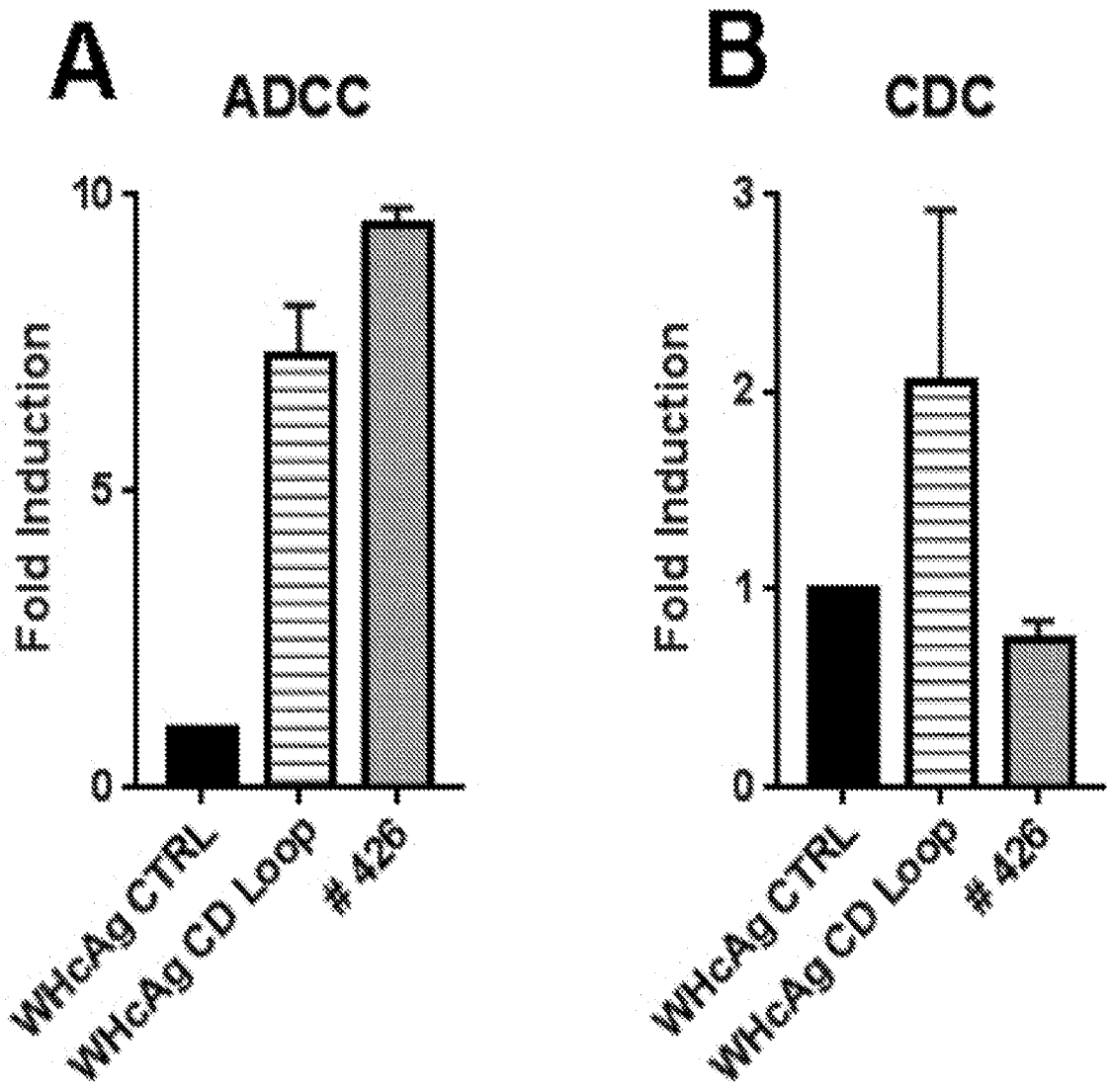

A
ADCC
Fold Induction
10
5
0
WHcAg CTRL
WHcAg CD Loop
426
B
CDC
Fold Induction
3
2
1
0
WHcAg CTRL
WHcAg CD Loop
426

FIG. 11

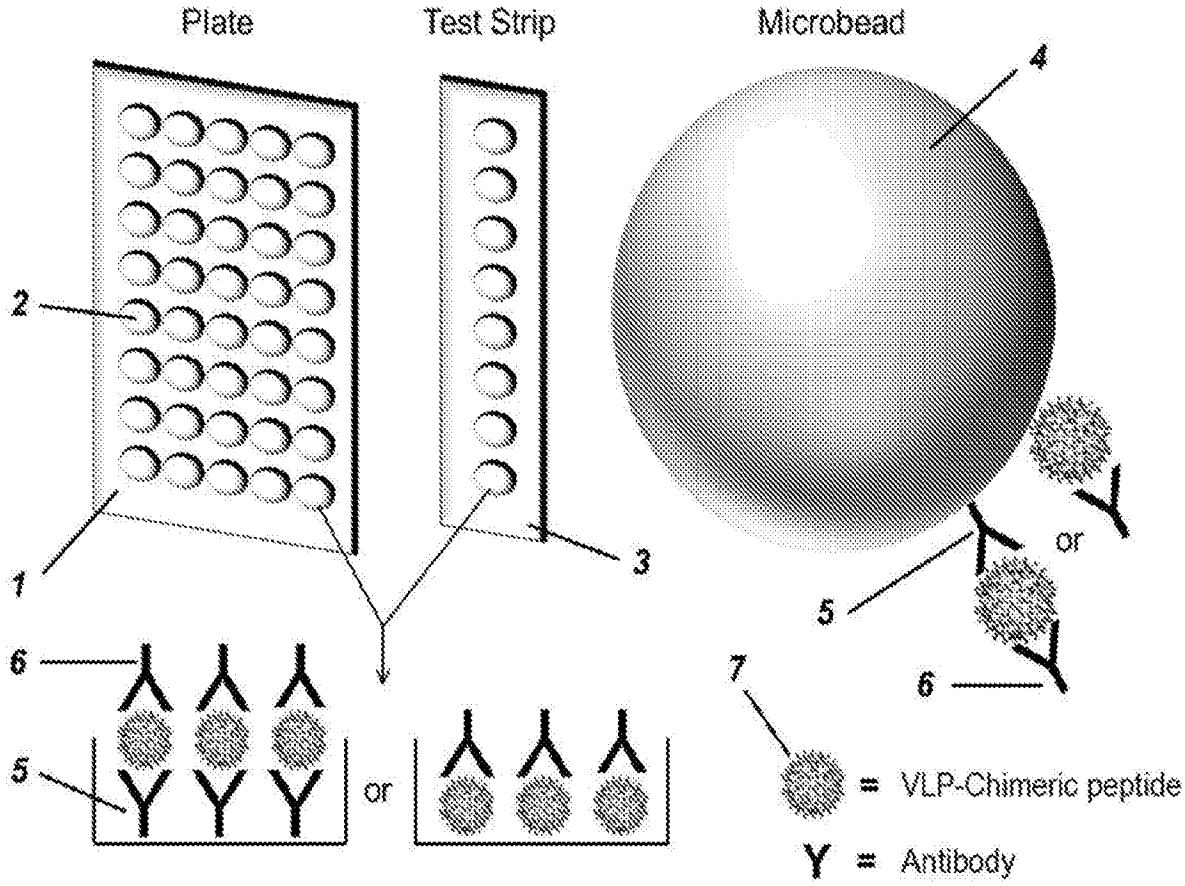


Plate
Test Strip
Microbead
1
2
3
4
5
6
7
or
or
= VLP-Chimeric peptide
Y = Antibody

FIG. 12

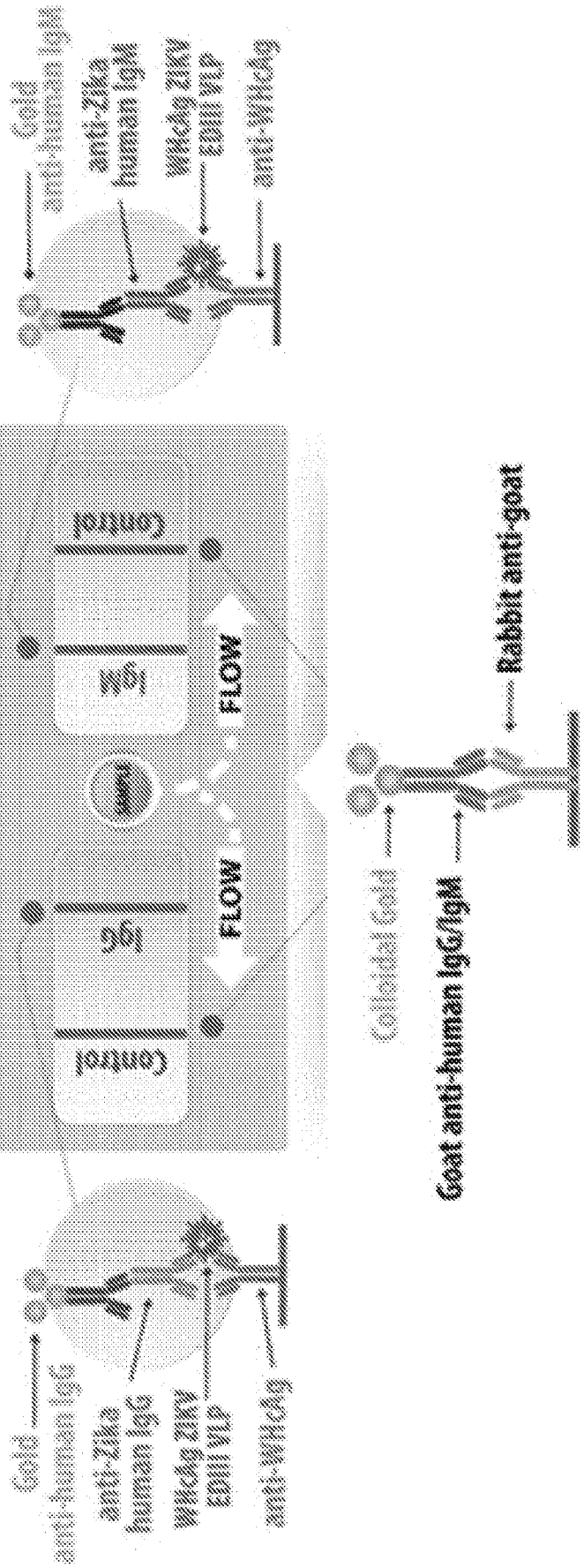

Gold
anti-human IgM
anti-Zika
human IgM
WNdg ZIKV
EDIII VLP
anti-WNdg
Control
IgM
FLOW
IgG
Control
FLOW
Colloidal Gold
Goat anti-human IgG/IgM
Rabbit anti-goat
Gold
anti-human IgG
anti-Zika
human IgG
WNdg ZIKV
EDIII VLP
anti-WNdg

FIG. 13

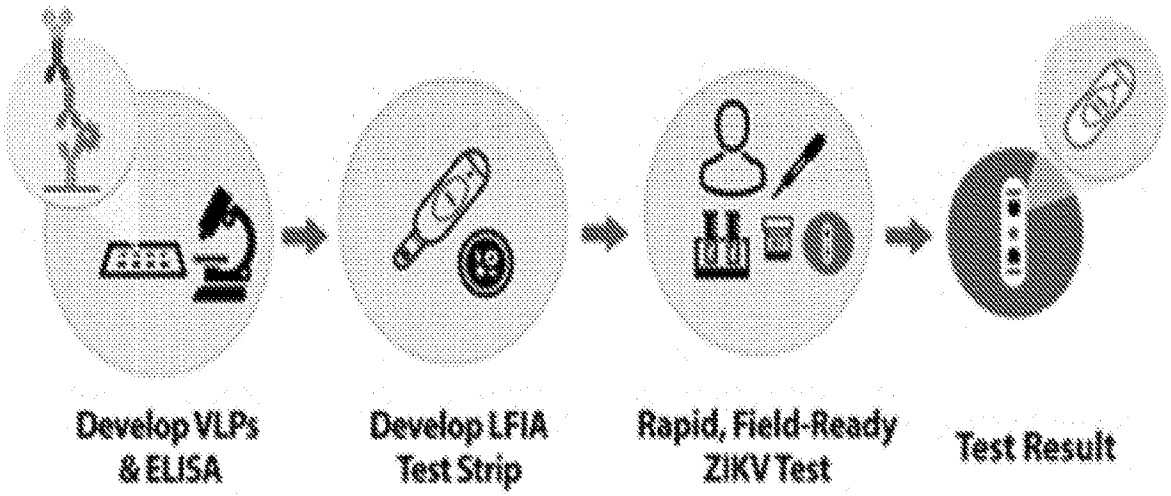

Develop VLPs & ELISA
Develop LFIA Test Strip
Rapid, Field-Ready ZIKV Test
Test Result

FIG. 14

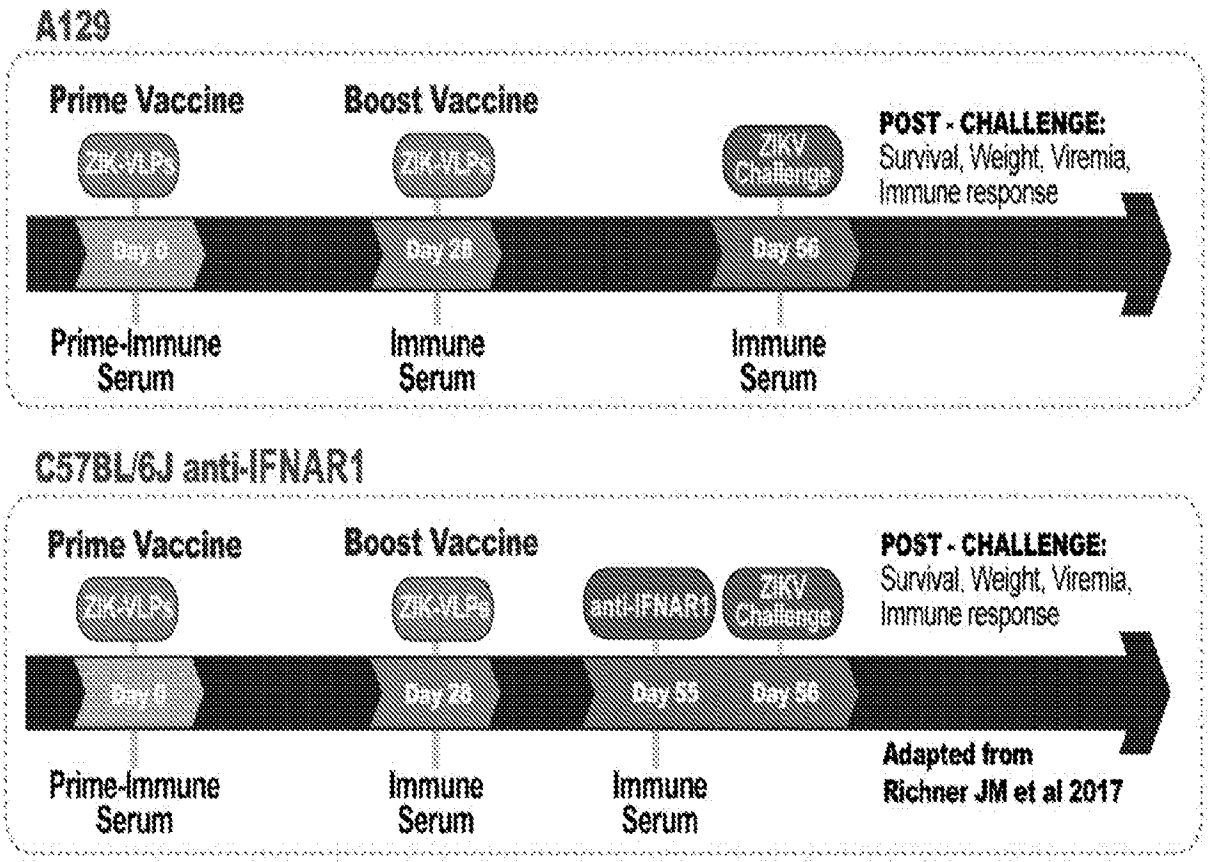

A129
Prime Vaccine
Boost Vaccine
ZIK-VLPs
ZIK-VLPs
ZIKV Challenge
POST - CHALLENGE:
Survival, Weight, Viremia, Immune response
Day 0
Day 28
Day 56
Prime-Immune Serum
Immune Serum
Immune Serum
C57BL/6J anti-IFNAR1
Prime Vaccine
Boost Vaccine
ZIK-VLPs
ZIK-VLPs
anti-IFNAR1
ZIKV Challenge
POST - CHALLENGE:
Survival, Weight, Viremia, Immune response
Day 0
Day 28
Day 53
Day 56
Prime-Immune Serum
Immune Serum
Immune Serum
Adapted from
Richner JM et al 2017

FIG. 15

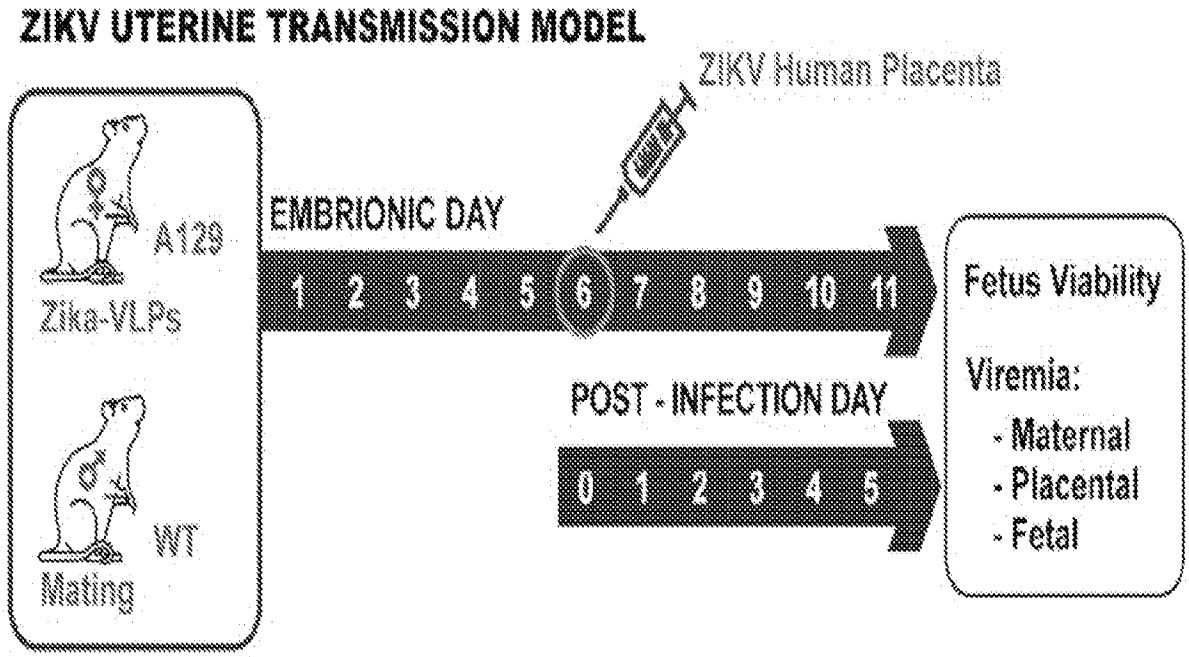

ZIKV UTERINE TRANSMISSION MODEL
ZIKV Human Placenta
A129
Zika-VLPs
WT
Mating
EMBRIONIC DAY
1 2 3 4 5 6 7 8 9 10 11
POST - INFECTION DAY
0 1 2 3 4 5
Fetus Viability
Viremia:
- Maternal
- Placental
- Fetal

FIG. 16

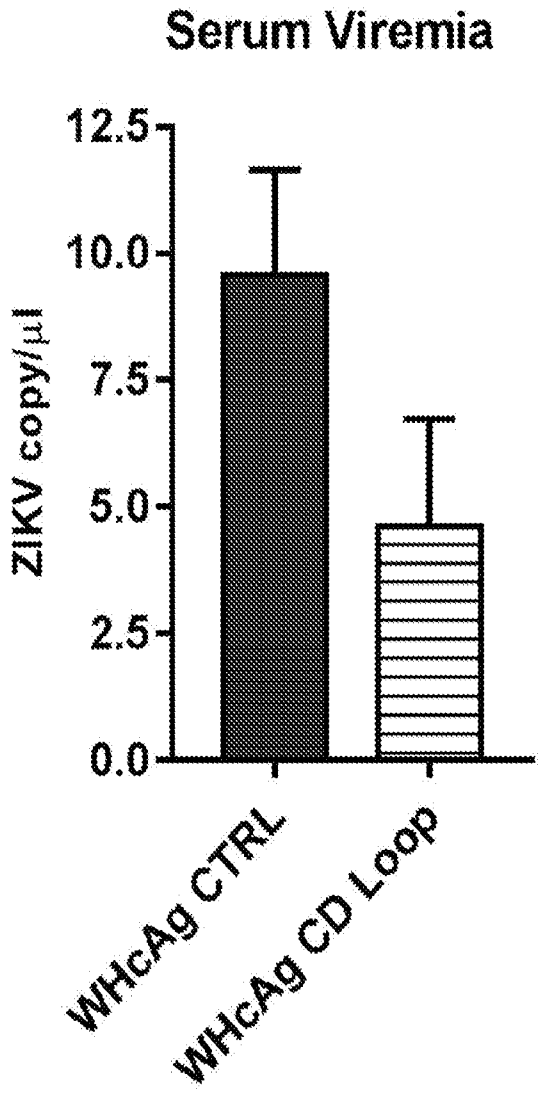

Serum Viremia
ZIKV copy/μl
12.5
10.0
7.5
5.0
2.5
0.0
WHcAg CTRL
WHcAg CD Loop

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/39079

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/12, A61K 39/165, C07K 14/005, C12N 7/00 (2018.01)
CPC - A61K 39/12, C12N 15/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/210127 A1 (TECHNOVAX INC.) 29 December 2016 (29.12.2016) Abstract; para [0003]; para [0173-0174]	1, 2, 9, 10
Y	UniProt Accession No. A0A1D8GUV4_ZIKV, Zika virus (strain Mr 766) (ZIKV), Polyprotein, 18 January 2017, [online]. [Retrieved on 8 September 2018]. Retrieved from the internet: <URL: https://www.uniprot.org/uniprot/A0A1D8GUV4> Entire document	1, 2, 9, 10
Y	US 2008/0220009 A1 (MILICH et al.) 11 September 2008 (11.09.2008) Abstract; Claim 32; para [0009-0010]	9, 10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2018	30 OCT 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/39079

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-56
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

----Please see continuation in first extra sheet ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 9(in part) and 10(in part), limited to SEQ ID NO: 2

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box No. III. Observations where unity of invention is lacking.

Group I+, Claims 1-10, directed to an isolated peptide comprising Zika virus antigenic epitopes. The zika peptide will be searched to the extent that the amino acid sequence encompasses SEQ ID NO: 2. It is believed that claims 1, 2, 9(in part) and 10(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that the zika peptide encompasses SEQ ID NO: 2. Additional zika peptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected zika peptide(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a zika peptide comprising amino acid sequence SEQ ID NO: 3 (claims 1, 2, 9(in part) and 10(in part)).

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Group I+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence encodes a unique peptide, and is considered a distinct technical feature.

No technical features are shared between the zika peptide amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori. Additionally, even if Group I+ were considered to share the technical features of including: an isolated peptide comprising or consisting of an amino acid sequence of a Zika virus antigenic epitope, and a chimeric peptide comprising said peptide linked to and inserted in a heterologous peptide of a Woodchuck Hepatitis core Antigen protein (WhcAg), these shared technical features are previously disclosed by WO 2016/210127 A1 to Technovax Inc (hereinafter 'Technovax') in view of US 2008/0220009 A1 to Milich et al., (hereinafter 'Milich').

Technovax teaches an isolated peptide comprising an amino acid sequence of a Zika virus antigenic epitope (Abstract - 'Described herein are flavivirus virus-like particles (VLPs) that display on their surfaces antigenic flavivirus proteins.'; para [0003] - 'The present invention relates to compositions comprising flavivirus (e.g., dengue or Zika) and/or alphavirus (e.g., chikungunya) virus-like particles (VLPs) and to methods of making and using these VLPs, including the creation and production of virus-like particle (VLP) based vaccines (e.g., for dengue, Zika, and/or chikungunya) as well as its use for diagnostic and therapeutic indications.'; para [0173] - 'We implemented our flavivivirus virus-like particle (VLP) vaccine platform technology to create immunoprotective countermeasures against Zika....The strategy to create flavivirus VLPs, and as an example Zika, is based on the simultaneous expression in mammalian cells of the structural proteins CprME together with a modified complex of the non-structural protein NS2B/NS3 protease to maximize assembly and production.'; para [0174] - 'These polypeptides suffice for the efficient self-assembly and release of particles into culture media.').

Technovax further teaches a chimeric peptide comprising said peptide linked to a heterologous peptide (para [0174] - 'In addition, this technology allows for the generation of combination vaccines via either blending distinct VLPs in a single formulation or by assembling chimeric VLPs following the co-expressing of E proteins from different pathogens or serotypes.'), yet does not expressly teach the heterologous peptide is Woodchuck Hepatitis core Antigen protein (WhcAg), or the Ziza epitope peptide is inserted in the WhcAg peptide.
Milich teaches use of Woodchuck Hepatitis core Antigen protein (WhcAg) heterologous antigenic peptide hybrid proteins as a vaccine (Claim 32 - 'An antigenic composition comprising a heterologous antigen inserted within the amino acid sequence set forth in SEQ ID NO:40, wherein said heterologous antigen is 50 or fewer amino acids in length and is inserted at a position chosen from amino acid residues 44...or 91 of SEQ ID NO:40, and wherein said heterologous antigen and said amino acid sequence assemble as a hybrid particle.'; para [0009] - 'compositions further comprising a modified woodchuck hepatitis virus core antigen comprising a heterologous antigen are provided. '). It would have been obvious to one of ordinary skill in the art that the Zika virus peptides of Technovax could be used to create hybrid polypeptides by fusion with Woodchuck Hepatitis core Antigen protein (WhcAg) of Milich since Milich teaches the hybrid protein elicits a higher antibody response (para [0010] - 'In further embodiments, the antibody response comprises at least three fold higher levels of antibody than that observed before administration of the at least one composition').

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, claims 11-56 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).