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(54) **NS2B AS MARKER FOR ZIKA VIRUS INFECTIONS**

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(57) **ABSTRACT**

The present invention relates to protein NS2b or fragment(s) thereof as biomarker or diagnostic marker for the diagnosis and/or prognosis of Zika virus infections. The present invention further relates to peptides and cyclic peptides, compositions and arrays and multimer compounds comprising them. The present invention further relates to a method for the diagnosis and/or prognosis of Zika virus infections, comprising the use of protein NS2b or fragment(s) thereof, or of the peptides, cyclic peptides, compositions and/or arrays in immunoassays. The present invention further relates to peptide-based compounds comprising at least one fragment of protein NS2b and at least one further component and to methods for the diagnosis and/or prognosis of Zika virus infections.

Specification includes a Sequence Listing.

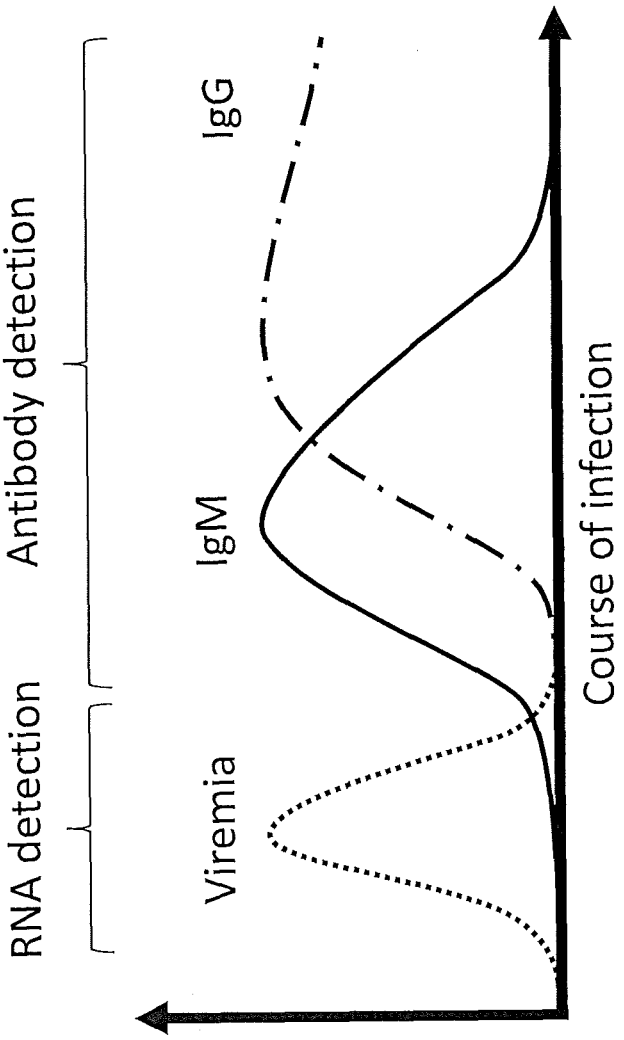

RNA detection
Antibody detection
Viremia
IgM
IgG
Course of infection

Figure 1

Figure 2

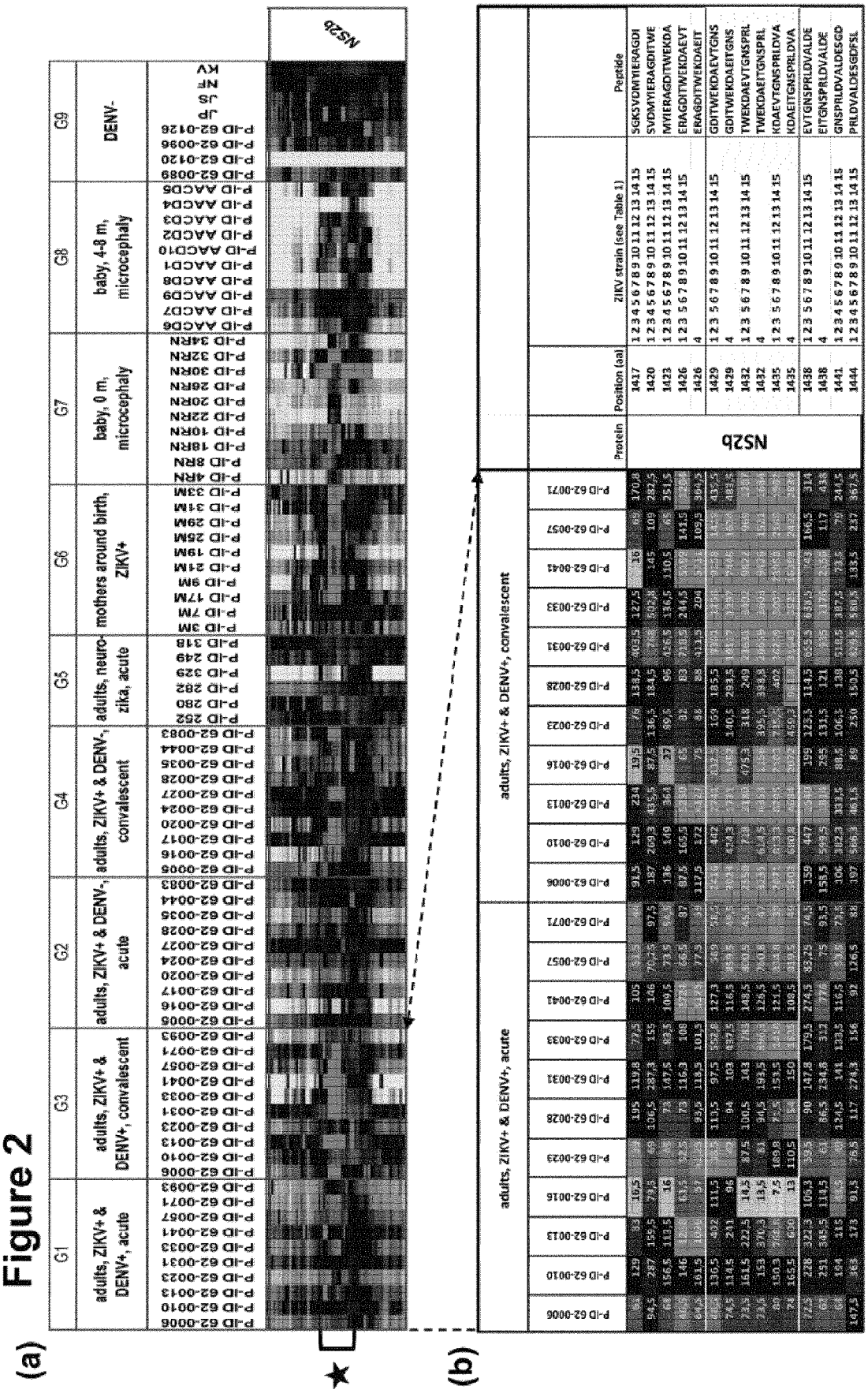
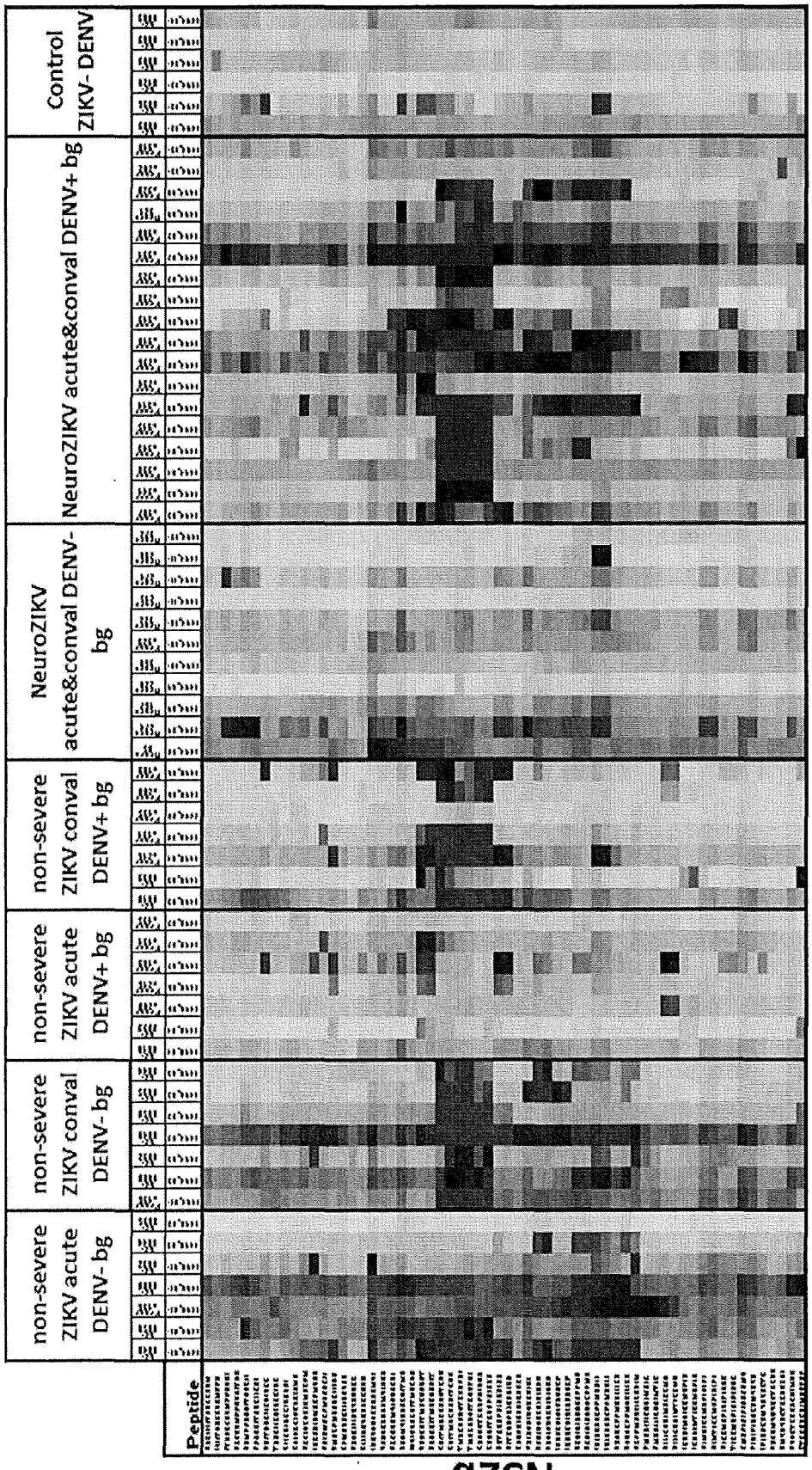

(a)
(b)
NS2b
G1
adults, ZIKV+ & DENV+, acute
G3
adults, ZIKV+ & DENV+, convalescent
G2
adults, ZIKV+ & DENV-, acute
G4
adults, ZIKV+ & DENV-, convalescent
G5
adults, neuro-zika, acute
G6
mothers around birth, ZIKV+
G7
baby, 0 m, microcephaly
G8
baby, 4-8 m, microcephaly
G9
DENV-
Protein
Position (aa)
ZIKV strain (see Table 1)
Peptide
SGKSVDMYIERAGDI
SVDMYIERAGDITWE
MYIERAGDITWEKDA
ERAGDITWEKDAEVT
ERAGDITWEKDAEIT
GDITWEKDAEVTGNS
GDITWEKDAEITGNS
TWEKDAEVTGNSPRL
TWEKDAEITGNSPRL
KDAEVTGNSPRLDVA
KDAEITGNSPRLDVA
EVTGNSPRLDVALDE
EITGNSPRLDVALDE
GNSPRLDVALDESGD
PRLDVALDESGDFSL

Figure 3


NS2b
Peptide
non-severe ZIKV acute DENV- bg
non-severe ZIKV conval DENV- bg
non-severe ZIKV acute DENV+ bg
non-severe ZIKV conval DENV+ bg
NeuroZIKV acute&conval DENV- bg
NeuroZIKV acute&conval DENV+ bg
Control ZIKV- DENV-

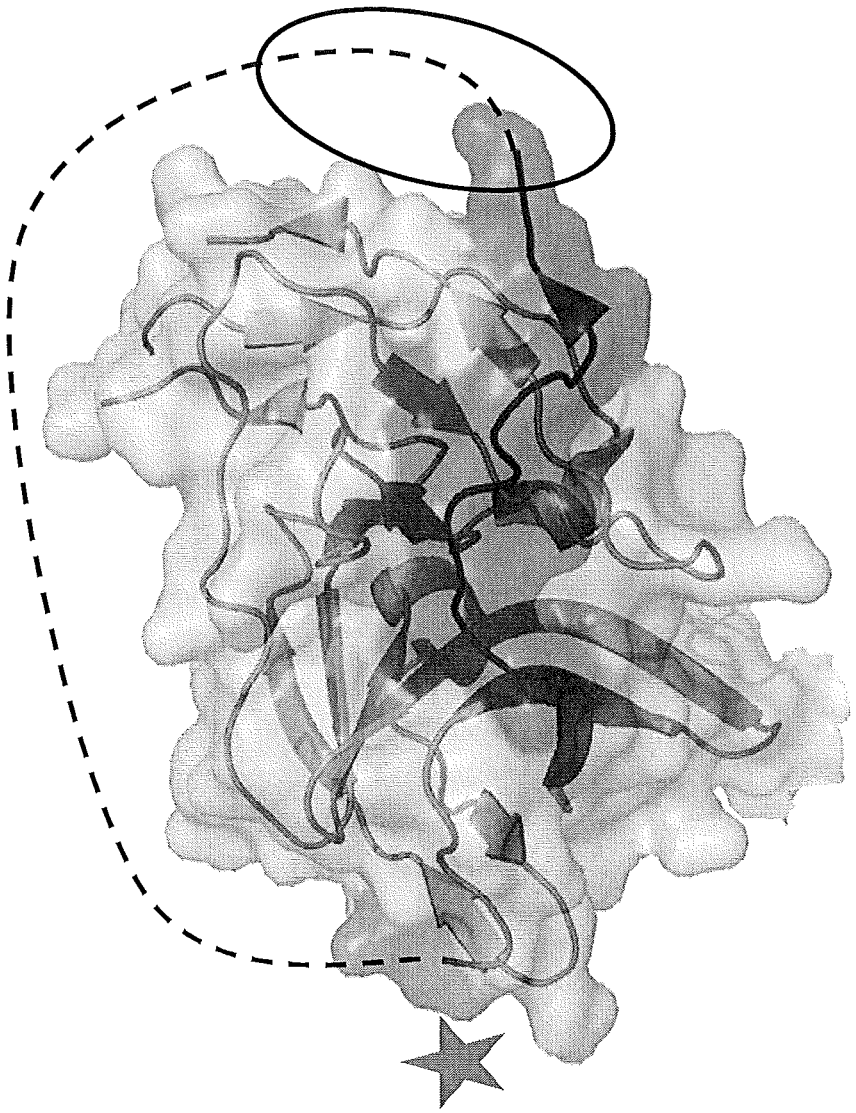


Figure 4

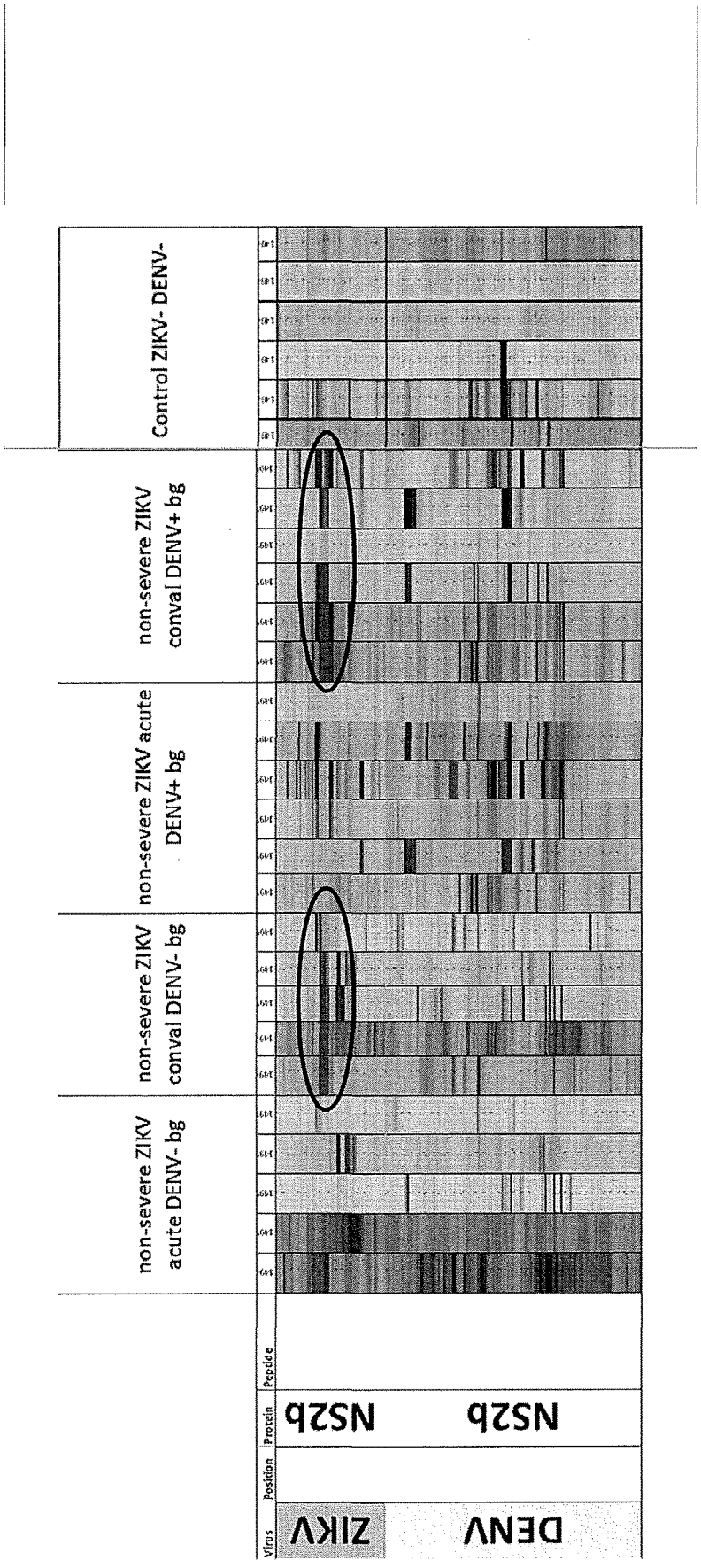

Virus
Position
Protein
Peptide
ZIKV
DENV
NS2b
NS2b
non-severe ZIKV acute DENV- bg
non-severe ZIKV conval DENV- bg
non-severe ZIKV acute DENV+ bg
non-severe ZIKV conval DENV+ bg
Control ZIKV- DENV-

Figure 5

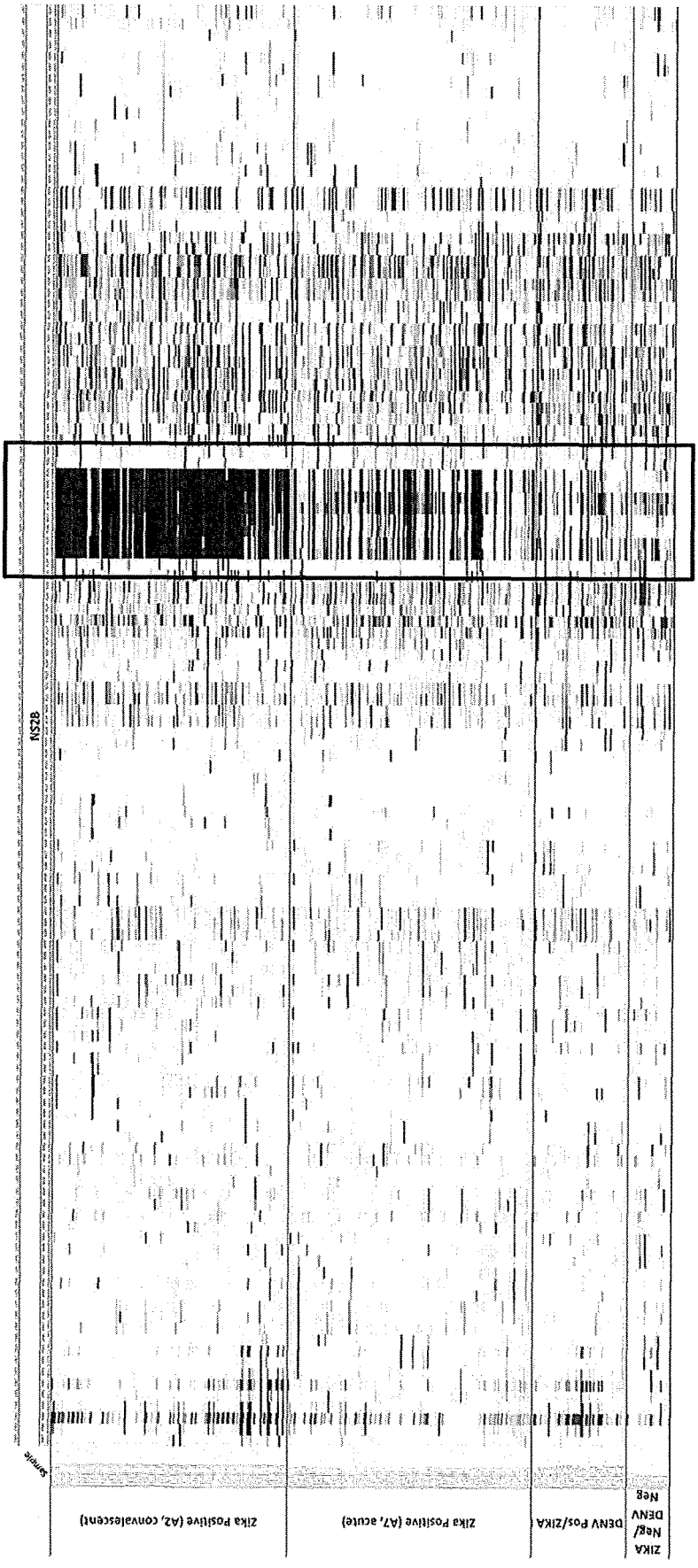

Zika Positive (A2, convalescent)
Zika Positive (A7, acute)
DENV Pos/ZIKA
ZIKA Neg/ DENV Neg
NS2B
Smoke

Figure 6

Figure 7A

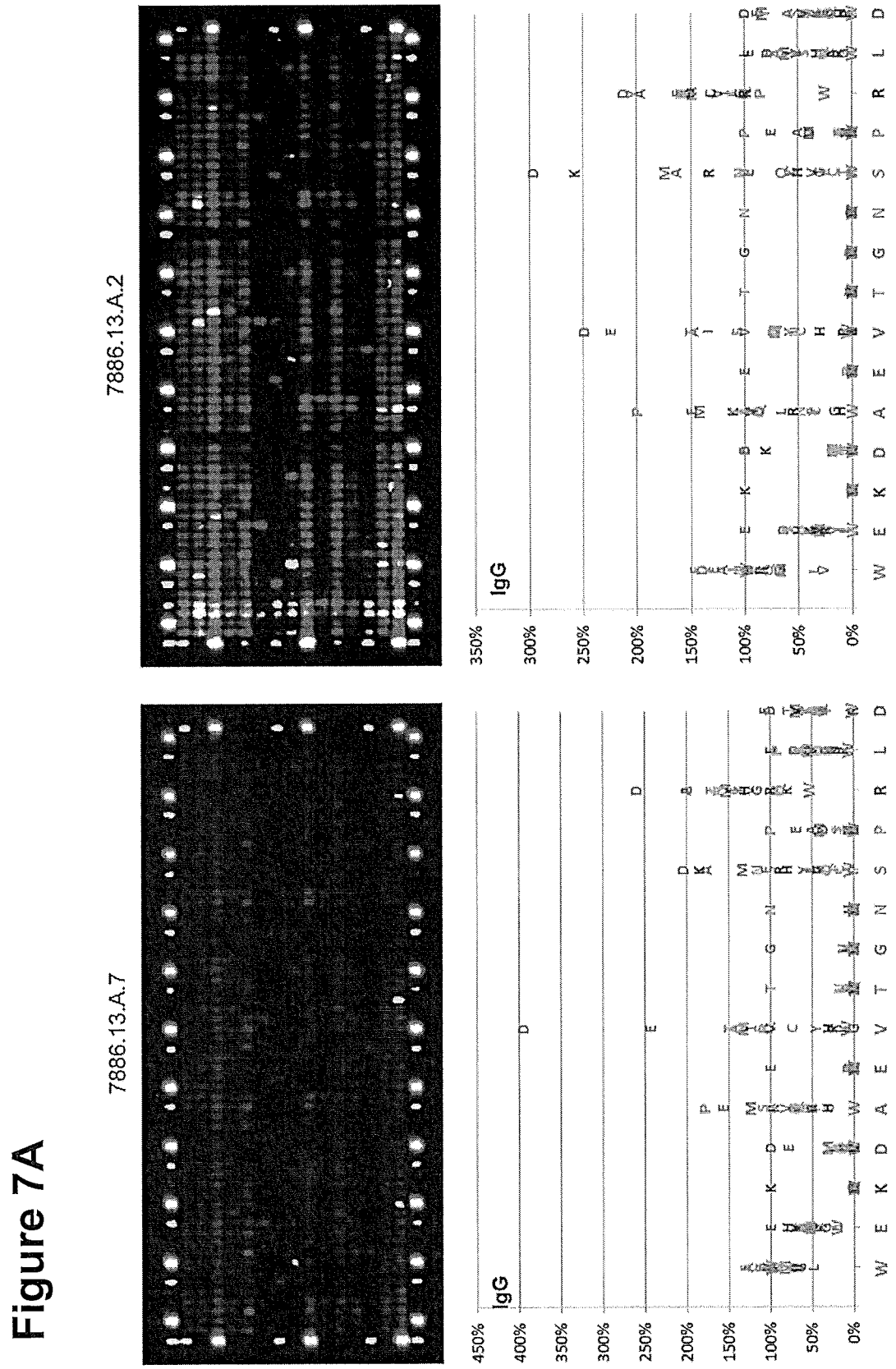

7886.13.A.7
7886.13.A.2
IgG
IgG
450%
400%
350%
300%
250%
200%
150%
100%
50%
0%
W E K D A E E V T G N S P R L D

Figure 7B

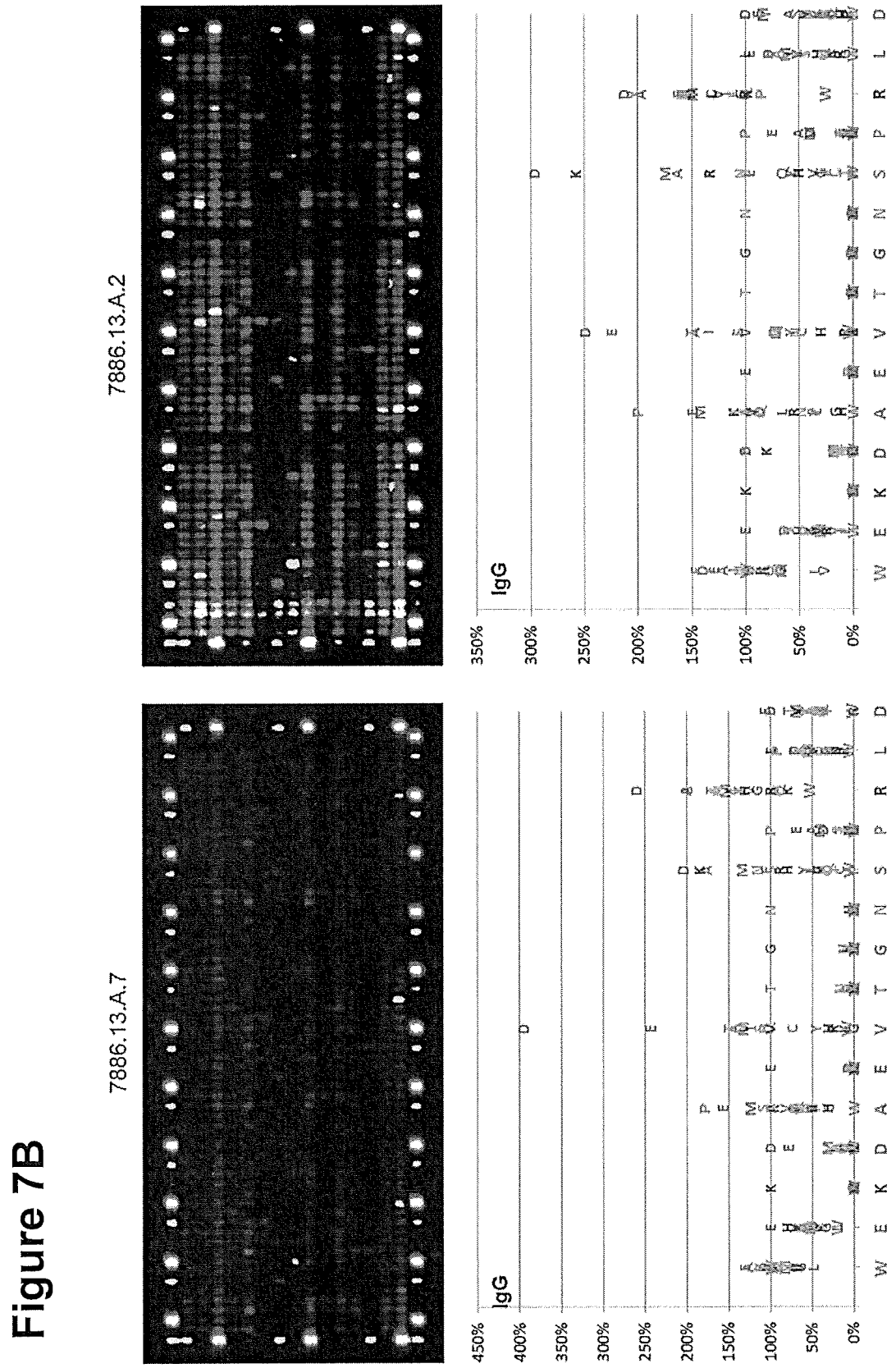

7886.13.A.7
7886.13.A.2
IgG
IgG
450%
400%
350%
300%
250%
200%
150%
100%
50%
0%
W E K D A E V T G N S P R L D

Figure 8A

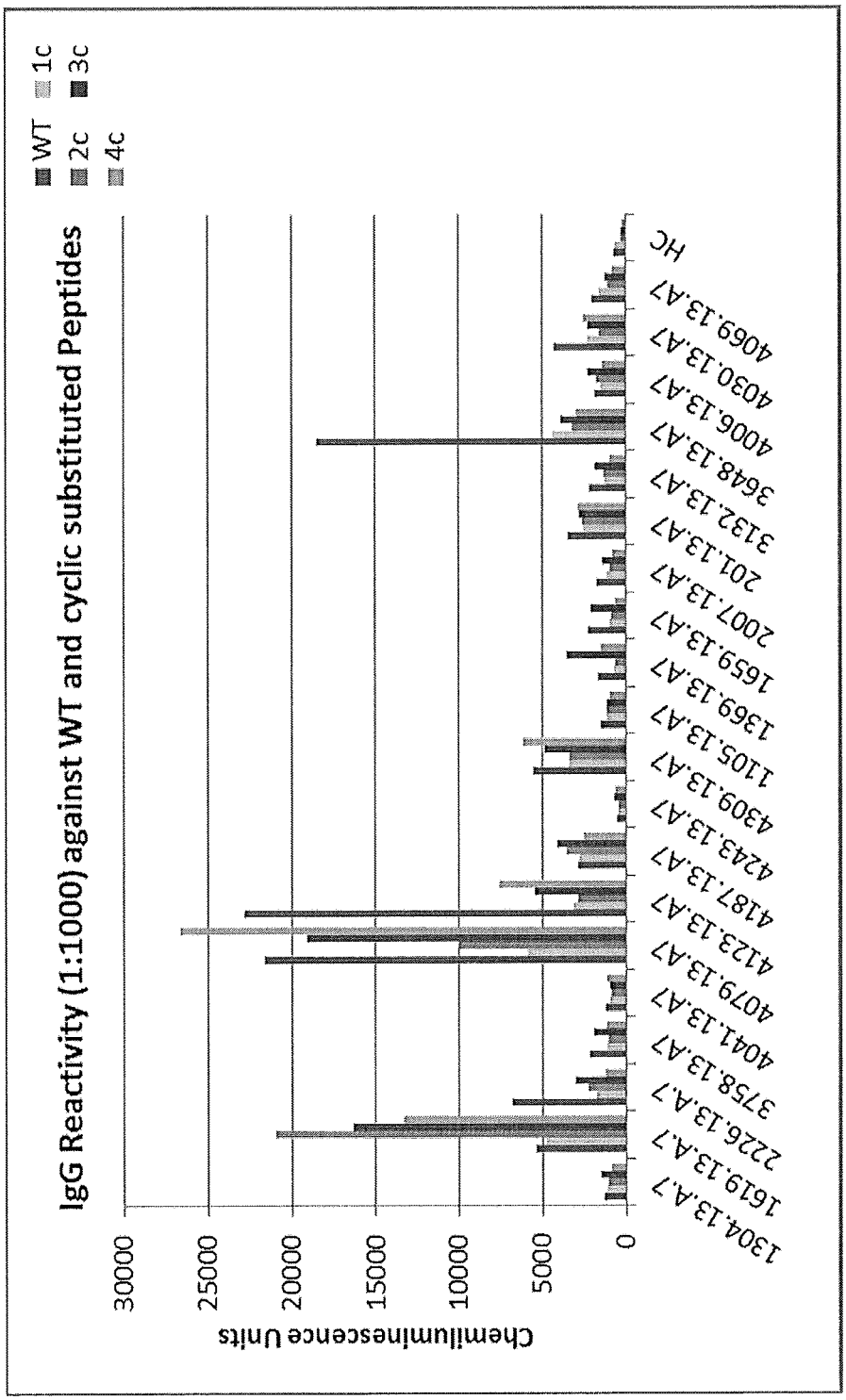
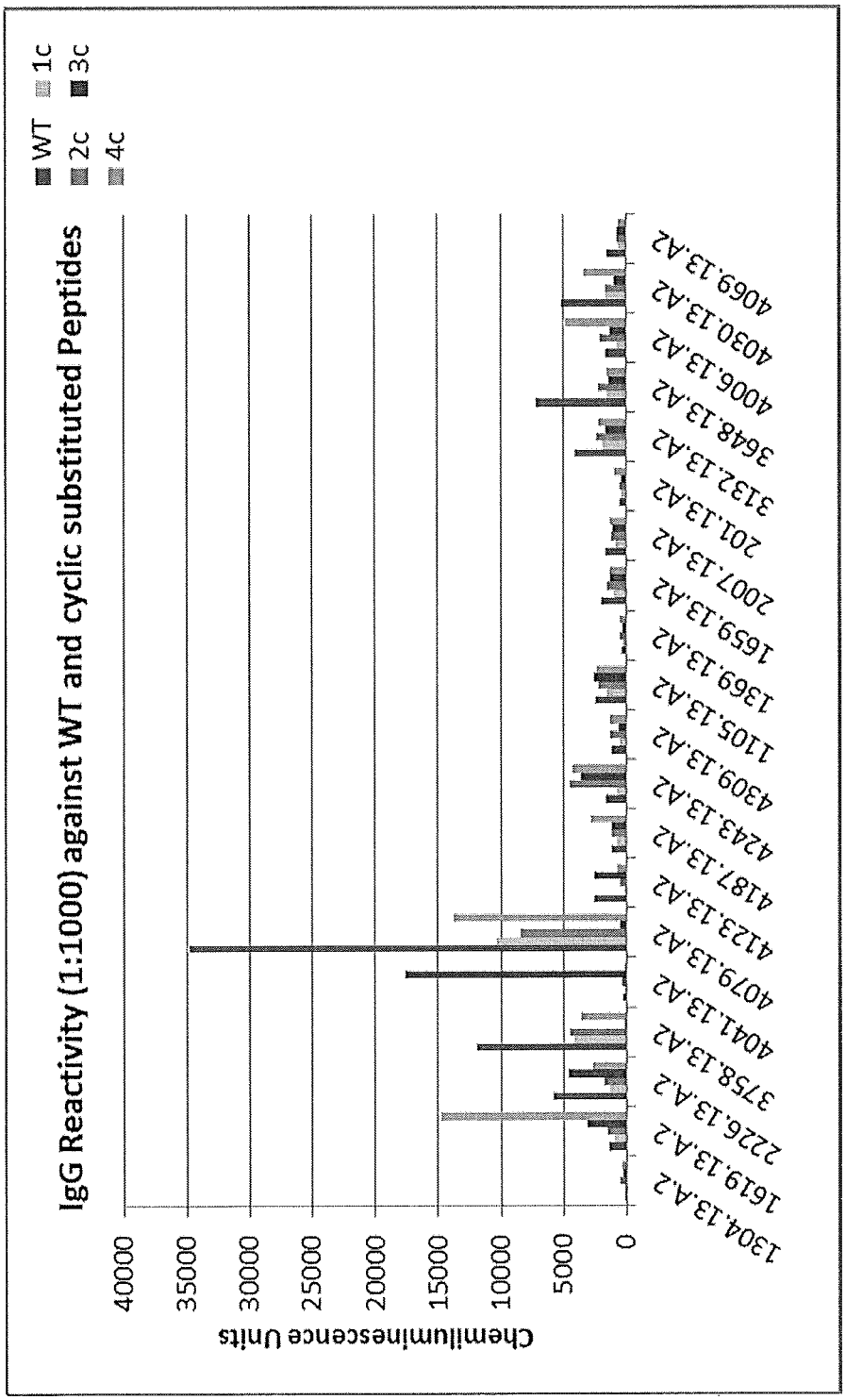

IgG Reactivity (1:1000) against WT and cyclic substituted Peptides
WT
1c
2c
3c
4c
Chemiluminescence Units
30000
25000
20000
15000
10000
5000
0
1304.13.A.7
1619.13.A.7
2226.13.A.7
3758.13.A.7
4041.13.A.7
4079.13.A.7
4123.13.A.7
4187.13.A.7
4243.13.A.7
4309.13.A.7
1105.13.A.7
1369.13.A.7
1659.13.A.7
2007.13.A.7
201.13.A.7
3132.13.A.7
3648.13.A.7
4006.13.A.7
4030.13.A.7
4069.13.A.7
HC

Figure 8B


IgG Reactivity (1:1000) against WT and cyclic substituted Peptides
WT
1c
2c
3c
4c
Chemiluminescence Units
40000
35000
30000
25000
20000
15000
10000
5000
0
1304.13.A.2
1619.13.A.2
2226.13.A.2
3758.13.A.2
4041.13.A.2
4079.13.A.2
4123.13.A.2
4187.13.A.2
4243.13.A.2
4309.13.A.2
1105.13.A.2
1369.13.A.2
1659.13.A.2
2007.13.A.2
201.13.A.2
3132.13.A.2
3648.13.A.2
4006.13.A.2
4030.13.A.2
4069.13.A.2

NS2B AS MARKER FOR ZIKA VIRUS INFECTIONS

[0001] The present invention relates to protein NS2b or fragment(s) thereof as biomarker or diagnostic marker for the diagnosis and/or prognosis of Zika virus infections. The present invention further relates to peptides and cyclic peptides, compositions and arrays and multimer compounds comprising them. The present invention further relates to a method for the diagnosis and/or prognosis of Zika virus infections, comprising the use of protein NS2b or fragment (s) thereof, or of the peptides, cyclic peptides, compositions and/or arrays in immunoassays. The present invention further relates to peptide-based compounds comprising at least one fragment of protein NS2b and at least one further component and to methods for the diagnosis and/or prognosis of Zika virus infections.

BACKGROUND OF THE INVENTION

[0002] Given the recent outbreak of Zika virus (ZIKV) in the Americas in 2015 and the association with microcephaly cases (Lover 2016; de Araújo et al., 2016) and neurological disorders, such as Guillain-Barre syndrome, ZIKV was recently placed as a public health emergency of international concern by the World Health Organization (WHO) (Lessler et al., 2016).

[0003] ZIKV is a flavivirus of the family Flaviviridae, transmitted by *Aedes* sp. Mosquitoes (Chakraborty et al., 2016), and closely related to dengue virus (DENV), West Nile virus (WNV) and Japanese encephalitis virus (JEV) and yellow fever virus (YFV) (Lazear et al., 2016).

[0004] Up to date there is no prophylactic treatment or vaccine available against ZIKV and disease control is limited to vector eradication strategies. The presumptive diagnosis is typically clinical, while confirmatory laboratory tests include classic virus isolation on cell culture and viral RNA detection by reverse transcriptase PCR (RT-PCR) in serum, saliva and/or urine samples within the first 5 or 6 days of infection (Dawes et al., 2016; Waggoner et al., 2016). ZIKV serology is usually performed by Enzyme-Linked Immunosorbent Assay (ELISA), using full-length viral proteins or linear peptides. However, due to high cross-reactivity of IgM and IgG antibodies between ZIKV and other related flavivirus, especially in endemic areas where co-circulation exists, confirmatory tests are necessary. Currently, confirmation testing is performed by plaque reduction neutralization test (PRNT), according to previous published protocols (Johnson et al., 2000; Martin et al., 2000; Kuno 2003; Maeda and Maeda, 2013).

[0005] To the present there are only very few commercially available serology kits for ZIKV antibody detection, moreover the specificity of these kits remains elusive given the high cross reactivity already reported among individuals who experienced consecutive flavivirus infections (PMID: 28094237 and PMID: 27982355). Based on that, PRNT remains as the "gold standard" for anti-flavivirus differentiation (Kuno 2003). PRNT, however, is a high cost technique that requires highly specialized laboratories and special regulation due to live virus manipulation. Although new protocols using recombinant viruses (Johnson et al., 2009) or reporter virus particles (Maeda and Maeda, 2013) have been developed, these are still not available for ZIKV routine diagnosis (Musso and Gubler, 216).

[0006] Thus, there is an urgent need for a low-cost unequivocal serological diagnostic method for ZIKV able to overcome the high cross reactivity with other flaviviruses, such as DENV, and yellow fever (especially on vaccinated individuals) (Stettler et al., 2016).

[0007] There is a need in the art of improved means and methods for diagnosing Zika virus infections.

SUMMARY OF THE INVENTION

[0008] According to the present invention this object is solved by protein NS2b as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

[0009] According to the present invention this object is solved by at least one fragment of protein NS2b as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

[0010] According to the present invention this object is solved by at least one mutated fragment of protein NS2b as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

[0011] According to the present invention this object is solved by a peptide comprising an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

[0012] According to the present invention this object is solved by a cyclic peptide comprising

[0013] an amino acid sequence selected from SEQ ID Nos. 1 to 10, or

[0014] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

[0015] According to the present invention this object is solved by a composition or an array comprising

[0016] (a) at least one peptide comprising

[0017] an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

[0018] an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60%, more preferably more than about 70 or 80 or 90% sequence identity, or

[0019] (b) at least one peptide comprising

[0020] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

and/or

[0021] (c) at least one cyclic peptide according to the present invention.

[0022] According to the present invention this object is solved by a multimer compound comprising at least two of component(s) (a) to (d):

[0023] (a) a peptide comprising

[0024] an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

[0025] an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about

60%, more preferably more than about 70 or 80 or 90% sequence identity, or

[0026] (b) a peptide of the present invention,

[0027] (c) a cyclic peptide of the present invention, and/or

[0028] (d) a NS2b fragment, as defined herein,

wherein at least two of component(s) (a) to (d) are covalently coupled to each other or are connected via a linear or cyclic scaffold.

[0029] According to the present invention this object is solved by providing the peptide or the cyclic peptide according to the present invention or the composition or array of the present invention or the multimer compound of the present invention as biomarker or diagnostic marker for Zika virus infections.

[0030] According to the present invention this object is solved by peptide or the cyclic peptide according to the present invention or the composition or array of the present invention or the multimer compound of the present invention for use in a method of the diagnosis and/or prognosis of Zika virus infections.

[0031] According to the present invention this object is solved by a peptide-based compound comprising

[0032] (i) at least one peptide comprising

[0033] an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

[0034] an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60%, more preferably more than about 70 or 80 or 90% sequence identity, or

[0035] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

[0036] and/or at least one cyclic peptide according to the present invention,

[0037] (ii) at least one further component, preferably covalently coupled to the peptide and/or cyclic peptide (i).

[0038] According to the present invention this object is solved by providing a peptide-based compound of the present invention for use in a method of the diagnosis and/or prognosis of Zika virus infections.

[0039] According to the present invention this object is solved by an in vitro method for the diagnosis and/or prognosis of Zika virus infections, comprising the step of

(a) providing a sample of a patient to be tested,

(b) providing

[0040] (1) protein NS2b or at least one fragment thereof, as defined herein;

[0041] (2) at least one peptide-based compound of the present invention;

[0042] (3) at least one peptide of the present invention;

[0043] (4) at least one cyclic peptide of the present invention;

[0044] (5) a composition or an array of the present invention;

[0045] and/or

[0046] (6) a multimer compound of the present invention

(c) performing an immunoassay, comprising detecting an anti-Zika antibody response in said patient sample.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

[0047] Before the present invention is described in more detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific tennis used herein have the same meanings as commonly understood by one of ordinary skill in the art. For the purpose of the present invention, all references cited herein are incorporated by reference in their entireties.

[0048] Concentrations, amounts, and other numerical data may be expressed or presented herein in a range format. It is to be understood that such a range format is used merely for convenience and brevity and thus should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. As an illustration, a numerical range of "5 to 100" should be interpreted to include not only the explicitly recited values of 5 to 100, but also include individual values and sub-ranges within the indicated range. Thus, included in this numerical range are individual values such as 5, 6, 7, 8, 9, 10, 11, 12, 13 . . . 97, 98, 99, 100 and sub-ranges such as from 10 to 40, from 12 to 17 and 41 to 50, etc. This same principle applies to ranges reciting only one numerical value, such as "at least 8". Furthermore, such an interpretation should apply regardless of the breadth of the range or the characteristics being described. Also it is to be understood that ranges may differ depending on the institute/facility where the measurements are being performed, methodology of measurement, type of tissue, and technique of tissue collection.

[0049] Biomarker for Zika Virus

[0050] As discussed above, the present invention provides protein NS2b as biomarker or diagnostic marker for Zika virus infections.

[0051] In particular, the present invention provides protein NS2b as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

[0052] Furthermore, the present invention provides at least one fragment of protein NS2b as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

[0053] Furthermore, the present invention provides at least one mutated fragment of protein NS2b as biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

[0054] The ZIKV positive-sense RNA genome comprises a single ORF (Open Reading Frame) encoding an unique polyprotein that is cleaved into three structural proteins (Capsid (C), pre-membrane (prM) and envelope (E), which form the virus particle, and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5), which perform essential functions in genome replication, polyprotein cleavage and modulation of cellular processes to viral advantage (Kuno et al., 2007; Brown et al., 2016).

[0055] Several of the non-structural proteins function as enzymes for the virus. Among the NS proteins, the NS1

glycoprotein is a multifunctional virulence factor (Muller and Young, 2013; Watterson et al., 2016). Furthermore there is the serine protease NS3, whose function is to cleave the virus polyprotein at proper sites, and is required for ZIKV replication. NS3 requires a cofactor, NS2b. The crystal structure of the NS2b-NS3 complex was recently resolved by Zhang et al. (2016).

[0056] In a preferred embodiment, the least one fragment of NS2b is an amino acid sequence of the ZIKV polyproteome of positions 1429-1449 of Zika Uganda Strain MR766 NIID (SEQ ID NO. 1).

Said amino acid sequence of SEQ ID NO. 1 is GDITWEKDAEVTGNSPRLDVA

[0057] Said amino acid sequence of the ZIKV polyproteome was identified by the inventors as a major epitope. Said amino acid sequence refers, for example, to

[0058] positions 1429-1449 of Zika Uganda Strain MR766_NIID; or

[0059] positions 1425-1445 of Zika Uganda Strain MR766.

[0060] See Table 1 below.

[0061] It is identical in the Zika strains (except for one V→I exchange, namely the central valine in position 11), such as the 15 Zika strains used for identification. See Table 1 below.

[0062] When compared to the corresponding regions of NS2b of other flaviviruses (as identified from Uniprot), it can be seen that the epitope of Zika virus differs, such as, among others, in position 7 (lysine), and is highly suitable for a specific diagnosis of Zika infections.

Zika virus
SEQ ID NO. 1
GDITWEKDAEVTGNSPRLDVA

West Nile Virus
SEQ ID NO. 18
ADITWESDAEITGSSERVDVR

DENV-1
SEQ ID NO. 20
AEVSWEEEAEHSGASHNILVE

DENV-2
SEQ ID NO. 22
ADVKWEDQAEISGSSPILSIT

DENV-3
SEQ ID NO. 24
PDVTWEEEAEQTGVSHNLMIT

Yellow fever virus
SEQ ID NO. 26
GEVSWEEEAEISGSSARYDVA

DENV-4
SEQ ID NO. 28
ANVQWDEMADITGSSPIIEVK

TBEV
SEQ ID NO. 30
GCVEWYPELVNEGGEVSLRVR

JEV
SEQ ID NO. 32
ATDMWLERAADISWEMDAAIT.

[0063] The amino acid sequences of the full length NS2b of the flaviviruses are (as identified from Uniprot)

Zika virus
Uniprot Accession No. Q32ZE1; Zika Strain MR766
1369-1498 NS2b
SEQ ID NO. 17
SWPPSEVLTAVGLICALAGGFAKADIEMAGPMAAVGLLIVSYVVSGKSVDM

YIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEEDGPPMREIILKVV

LMAICGMNPIAIPFAAGAWYVYVKTGKR

West Nile virus (WNV)
Uniprot Accession No. P06935
1371-1501 NS2b
SEQ ID NO. 19
GWPATEVMTAVGLMFAIVGGLAELDIDSMAIPMTIAGLMFAAFVISGKSTD

MWIERTADITWESDAEITGSSERVDVRLDDDGNFQLMNDPGAPWKIWMLRM

ACLAISAYTPWAILPSVIGFWITLQYTKR

Dengue virus type 1 (DENV-1)
Uniprot Accession No. P17763
1346-1475 NS2b
SEQ ID NO. 21
SWPLNEGIMAVGIVSILLSSLLKNDVPLAGPLIAGGMLIACYVISGSSADL

SLEKAAEVSWEEEAEHSGASHNILVEVQDDGTMKIKDEERDDTLTILLKAT

LLAISGVYPMSIPATLFVWYFWQKKKQR

Dengue virus type 2 (DENV-2)
Uniprot Accession No. P29990
1346-1475 NS2b
SEQ ID NO. 23
SWPLNEAIMAVGMVSILASSLLKNDIPMTGPLVAGGPLTVCYVLTGRSADL

ELERAADVKWEDQAEISGSSPILSITISEDGSMSIKNEEEEQTLTILIRTG

LLVISGLFPVSIPITAAAWYLWEVKKQR

Dengue virus type 3 (DENV-3)
Uniprot Accession No. Q6YMS4
1344-4473 NS2b
SEQ ID NO. 25
SWPLNEGVMAVGLVSILASSLLRNDVPMAGPLVAGGLLIACYVITGTSADL

TVEKAPDVTWEEEAEQTGVSHNLMITVDDDGTMRIKDDETENILTVLLKTA

LLIVSGIFPYSIPATLLVWHTWQKQTQR

Yellow fever virus (YFV)
Uniprot Accession No. P03314
1355-1484 NS2b
SEQ ID NO. 27
SIPVNEALAAAGLVGVLAGLAFQEMENFLGPIAVGGLLMMLVSVAGRVDGL

ELKKLGEVSWEEEAEISGSSARYDVALSEQGEFKLLSEEKVPWDQVVMTSL

ALVGAALHPFALLLVLAGWLFHVRGARR

Dengue virus type 3 (DENV-4)
Uniprot Accession No. Q5UCB8
1345-1474 NS2b
SEQ ID NO. 29
SWPLNEGIMAVGLVSLLGSALLKNDVPLAGPMVAGGLLLAAYVMSGSSADL

SLEKAANVQWDEMADITGSSPIIEVKQDEDGSFSIRDVEETNMITLLVKLA

LITVSGLYPLAIPVTMTLWYMWQVKTQR

-continued

Tick-borne encephalitis virus (TBEV)
Uniprot Accession No. P14336
1359-1489 NS2b

SEQ ID NO. 31

SFSEPLTVVGVMLTLASGMMRHTSQEALCALAVASFLLLMLVLGTRKMQLV

AEWSGCVEWYPELVNEGGEVSLRVRQDAMGNFHLTELEKEERMMAFWLIAG

LAASAIHWSGILGVMGLWTLTEMLRSSRR

Japanese encephalitis virus (JEV)
Uniprot Accession No. P27395
1374-1504 NS2b

SEQ ID NO. 33

GWPATEFLSAVGLMFAIVGGLAELDIESMSIPFMLAGLMAVSYVVSGKATD

MWLERAADISWEMDAAITGSSRRLDVKLDDDGDFHLIDDPGVPWKVWVLRM

SCIGLAALTPWAIVPAAFGYWLTLKTTKR

[0064] Preferably, the fragment is a peptide having a length of at least 5 amino acids, or at least 9 amino acids, such as 5 to 130 amino acids, or 5 to 100 amino acids, or 5 to 50 amino acids, or 9 to 50 amino acids, e.g. about 15 to 21 amino acids or about 9 to 15 amino acids or about 9 to 21 amino acids.

[0065] Other lengths will be determined by the skilled artisan depending on the use.

[0066] In a preferred embodiment, the at least one fragment comprises or is or consists of a peptide having

[0067] an amino acid sequence selected from SEQ ID Nos. 1 to 10 or combinations thereof, or

[0068] an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60%, more preferably more than about 70 or 80 or 90% sequence identity.

SEQ ID NO. 2

GDITWEKDAEVTGNS;

SEQ ID NO. 3

GDITWEKDAEITGNS;

SEQ ID NO. 4

TWEKDAEVTGNSPRL;

SEQ ID NO. 5

TWEKDAEITGNSPRL;

SEQ ID NO. 6

KDAEVTGNSPRLDVA;

SEQ ID NO. 7

KDAEITGNSPRLDVA;

SEQ ID NO. 1

GDITWEKDAEVTGNSPRLDVA;

SEQ ID NO. 8

GDITWEKDAEITGNSPRLDVA;

SEQ ID NO. 9

WEKDAEVTGNSPRLD;

SEQ ID NO. 10

KDAEVTGNS.

[0069] In one embodiment, the NS2b fragments can comprise non-natural amino acids, D-amino acids, modified amino acids and/or amino acid derivatives, such as β-amino acids (β^3 and β^2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, N-methyl amino acids.

[0070] Examples are ornithine, citrulline, norleucine, acetyllysine, phosphotyrosine.

[0071] In a preferred embodiment, the NS2b fragment does not comprise an amino acid substitution in position K7.

[0072] An amino acid substitution in position K7 will result in a peptide/NS2b fragment which will no longer detect Zika virus infections, as described herein below.

[0073] In one embodiment, the NS2b fragment comprises or is or consists of a peptide having

[0074] an amino acid sequence selected from SEQ ID Nos. 1 to 10, or

[0075] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

[0076] Positions 9, 11, 15 and 17 in amino acid sequence of SEQ ID NO. 1: (underlined)

SEQ ID NO. 1

GDITWEKDAEVTGNSPRLDVA

[0077] The amino acid substitution(s) are preferably

[0078] A9P, A9E, A9M, A9S, A9T, A9K;

[0079] V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11S, I11M, I11L;

[0080] S15D, S15K, S15M, S15A, S15R, S15N; and/or

[0081] R17D, R17E, R17T

more preferably

[0082] A9P, A9E,

[0083] V11D, MD, V11E, I11E,

[0084] S15D, S15K, and/or

[0085] R17D, R17E.

[0086] The amino acid substitution is not any substitution in position K7.

[0087] For example, the NS2b fragment comprises or is or consists of a peptide having an amino acid sequence selected from SEQ ID Nos. 11 to 16.

SEQ ID NO. 11

WEKDPEDTGNSPRLD

SEQ ID NO. 12

WEKDAEDTGNSPRLD

SEQ ID NO. 13

WEKDPEDTGNKPRLD

SEQ ID NO. 14

KDPEDTGNS

SEQ ID NO. 15

KDAEDTGNS

SEQ ID NO. 16

KDPEDTGNK

[0088] In one embodiment, the NS2b fragment comprises or is or consists of a cyclic peptide having

[0089] an amino acid sequence selected from SEQ ID Nos. 1 to 10, or

[0090] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid

substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

[0091] The cyclization is preferably via

[0092] thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus, or

[0093] head-to-tail cyclization, or

[0094] disulfide formation via two additional cysteine residues at or near the C- and the N-terminus, or

[0095] lactam formation via a C- or N-terminal located additional basic amino acid, such as lysine or ornithine, and a C- or N-terminal located acidic amino acid, such as glutamic acid or aspartic acid, or

[0096] triazole formation via a C- or N-terminal located additional amino acid with an alkyne side chain and a C- or N-terminal located additional amino acid with an azide side chain.

[0097] More preferably, the cyclization is via thioether formation or head-to-tail cyclization.

[0098] Preferably,

[0099] (i) one fragment is used which comprises more than one of said peptides, or

[0100] (ii) more than one fragment is used each comprising or consisting of one peptide.

[0101] In one embodiment (i), wherein one fragment is used which comprises more than one of said peptides, the more than one of said peptides are covalently coupled to each other and/or are coupled to a linear or cyclic scaffold. The skilled artisan is able to generate such coupled peptides and/or scaffolds and knows appropriate linear and cyclic scaffolds.

[0102] In one embodiment, said NS2b protein or said at least one fragment comprises at least one further component (s).

[0103] Said at least one further component(s) is/are preferably selected from

[0104] label(s),

[0105] such as fluorescent label(s),

[0106] tag(s),

[0107] preferably for immobilization, such as biotin, 6×His, alkyn, azid, thiol,

[0108] linker or anchoring group(s)

[0109] preferably for attachment of the label(s), and/or tag(s),

[0110] such as PEG, cysteine, poly-GS-linker, e.g. GSGSG, or βAla-βAla-linker, or combinations thereof.

[0111] Said at least one further component(s) is/are preferably covalently coupled to said NS2b protein or said at least one fragment thereof

[0112] via a linker,

[0113] via an amino acid side chain, and/or

[0114] to the N- and/or C-terminus.

[0115] Said method is an in vitro, ex vivo or in vivo method.

[0116] In a preferred embodiment, the use of NS2b protein or at least one fragment thereof comprises the use in immunoassays.

[0117] Immunoassays are known to the skilled artisan and comprise, for example,

[0118] enzyme-linked immunosorbent assay (ELISA),

[0119] bead-based immunoassay, such as Luminex,

[0120] plaque reduction neutralization test (PRNT),

[0121] lateral flow or strip tests,

[0122] encoded microparticle-based immunoassay,

[0123] magnetic bead-based immunoassay.

[0124] Preferably, detecting an anti-Zika antibody response in a sample of a patient is comprised.

[0125] Said patient sample is preferably blood. More preferably it is selected from serum, plasma and whole blood.

[0126] Substituted Peptides, Cyclic Peptides, Compositions and Arrays and Multimer Compounds and their Uses

[0127] As discussed above, the present invention provides a peptide comprising an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

[0128] Positions 9, 11, 15 and 17 in amino acid sequence of SEQ ID NO. 1: (underlined), as described above:

SEQ ID NO. 1

GDITWEKDAEVTGNSPRLDVA

[0129] The amino acid substitution(s) are preferably

[0130] A9P, A9E, A9M, A9S, A9T, A9K;

[0131] V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11S, I11M, I11L;

[0132] S15D, S 15K, S15M, S15A, S15R, S15N; and/or

[0133] R17D, R17E, R17T

more preferably

[0134] A9P, A9E,

[0135] V11D, MD, V11E, I11E,

[0136] S15D, S15K, and/or

[0137] R17D, R17E.

[0138] The amino acid substitution is not any substitution in position K7.

[0139] For example, the peptide comprises or is or consists of an amino acid sequence selected from SEQ ID Nos. 11 to 16.

SEQ ID NO. 11

WEKDPEDTGNSPRLD

SEQ ID NO. 12

WEKDAEDTGNSPRLD

SEQ ID NO. 13

WEKDPEDTGNKPRLD

SEQ ID NO. 14

KDPEDTGNS

SEQ ID NO. 15

KDAEDTGNS

SEQ ID NO. 16

KDPEDTGNK

[0140] In a preferred embodiment, the peptide of the present invention does not comprise an amino acid substitution in position K7.

[0141] An amino acid substitution in position K7 will result in a peptide/NS2b fragment which will no longer detect Zika virus infections, as described herein below.

[0142] As discussed above, the present invention provides a cyclic peptide comprising

[0143] an amino acid sequence selected from SEQ ID Nos. 1 to 10, or

[0144] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1.

[0145] In a preferred embodiment, the cyclization is via

[0146] thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

[0147] Or

[0148] head-to-tail cyclization.

[0149] The amino acid substitution(s) are preferably, as described above:

[0150] A9P, A9E, A9M, A9S, A9T, A9K;

[0151] V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11S, I11M, I11L;

[0152] S15D, S15K, S15M, S15A, S15R, S15N; and/or

[0153] R17D, R17E, R17T

more preferably

[0154] A9P, A9E,

[0155] V11D, MD, V11E, I11E,

[0156] S15D, S15K, and/or

[0157] R17D, R17E.

[0158] The amino acid substitution is not any substitution in position K7.

[0159] Preferred examples for cyclic peptides according to the present invention are:

[0160] The cyclic peptides shown comprise an additional cysteine residue at the C-terminus. The cyclization is via thioether formation with the N-terminus, which carried a bromoacetyl group as chemical handle for the thioether formation.

[0161] Further possibilities for cyclization are known to the skilled artisan. Cyclization is e.g. also possible via

disulfide formation via two additional cysteine residues at or near the C- and the N-terminus, or

lactam formation via a C- or N-terminal located additional basic amino acid, such as lysine or ornithine, and a C- or N-terminal located acidic amino acid, such as glutamic acid or aspartic acid, or

triazole formation via a C- or N-terminal located additional amino acid with an alkyne side chain and a C- or N-terminal located additional amino acid with an azide side chain.

[0162] Preferably, the peptide(s) or the cyclic peptide(s) of the present invention have a length of at least 5 amino acids, or at least 9 amino acids,

such as 5 to 130 amino acids, or 5 to 100 amino acids, or 5 to 50 amino acids, or 9 to 50 amino acids, e.g. about 15 to 21 amino acids or about 9 to 15 amino acids or about 9 to 21 amino acids.

[0163] Other lengths will be determined by the skilled artisan depending on the use.

[0164] Preferably, the peptide or the cyclic peptide comprise at least one further component(s), which is/are preferably selected from

[0165] label(s),

[0166] such as fluorescent label(s),

[0167] tag(s),

[0168] preferably for immobilization, such as biotin, 6×His, alkyn, azid, thiol,

[0169] linker or anchoring group(s)

[0170] preferably for attachment of the label(s), and/or tag(s),

[0171] such as PEG, cysteine, poly-GS-linker, e.g. GSGSG,

[0172] or combinations thereof,

wherein said at least one further component(s) is/are covalently coupled to said peptide via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

[0173] In one embodiment, the NS2b fragments can comprise non-natural amino acids, D-amino acids, modified amino acids and/or amino acid derivatives, such as β-amino acids (β^3 and β^2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, N-methyl amino acids.

[0174] Examples are ornithine, citrulline, norleucine, acetyllysine, phosphotyrosine.

[0175] As discussed above, the present invention provides a composition or an array comprising

[0176] (a) at least one peptide comprising

[0177] an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

[0178] an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60%, more preferably more than about 70 or 80 or 90% sequence identity, or

[0179] (b) at least one peptide comprising

[0180] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

and/or

[0181] (c) at least one cyclic peptide according to the present invention.

[0182] The preferred amino acid substitution(s) are as discussed above.

[0183] An "array" according to the present invention is preferably a peptide array or peptide microarray. Peptide (micro)arrays are known in the art. A peptide (micro)array is a number of peptides displayed or assembled on a solid surface, usually a glass or plastic chip or beads, silicone wafer.

[0184] A preferred surface for an array according to the invention is glass (chip).

[0185] An exemplary array is a bead-based array.

[0186] A composition according to the present invention is preferably a mixture of several of the peptides and/or cyclic

peptides. A composition can comprise excipient(s) and/or carrier, such as pharmaceutically active excipient(s) and/or carrier.

[0187] As discussed above, the present invention provides a multimer compound comprising at least two of component (s) (a) to (d):

[0188] (a) a peptide comprising

[0189] an amino acid sequence selected from SEQ ID NOs. 1 to 10, or

[0190] an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60%, more preferably more than about 70 or 80 or 90% sequence identity, or

[0191] (b) a peptide of the present invention,

[0192] (c) a cyclic peptide of the present invention, and/or

[0193] (d) a NS2b fragment, as defined herein.

[0194] The peptide of the present invention (b) is as defined herein above: a peptide comprising an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1. The preferred amino acid substitution(s) are as discussed above.

[0195] The at least two of component(s) (a) to (d) are covalently coupled to each other or are connected via a linear or cyclic scaffold.

[0196] The skilled artisan knows appropriate linear and cyclic scaffolds and is able to couple the component(s) with each other and/or to the scaffolds.

[0197] The at least two of component(s) (a) to (d) can be e.g. at least two components (a), or at least two components (b), or at least two components (c) and/or at least two components (d). The at least two of component(s) (a) to (d) can be e.g. three components (a), or three components (b), or five components (c) and/or four components (d).

[0198] The at least two of component(s) (a) to (d) can be e.g. component (a)+(b), or (a)+(b)+(c) etc.

[0199] As discussed above, the present invention provides the peptide or the cyclic peptide according to the present invention as biomarker or diagnostic marker for Zika virus infections.

[0200] As discussed above, the present invention provides the composition or array of the present invention as biomarker or diagnostic marker for Zika virus infections.

[0201] As discussed above, the present invention provides the multimer compound of the present invention as biomarker or diagnostic marker for Zika virus infections.

[0202] As discussed above, the present invention provides the peptide or the cyclic peptide according to the present invention for use in a method of the diagnosis and/or prognosis of Zika virus infections, wherein said method is an in vitro, ex vivo or in vivo method, as described herein.

[0203] As discussed above, the present invention provides the composition or array of the present invention for use in a method of the diagnosis and/or prognosis of Zika virus infections, wherein said method is an in vitro, ex vivo or in vivo method, as described herein.

[0204] As discussed above, the present invention provides the multimer compound of the present invention for use in a method of the diagnosis and/or prognosis of Zika virus infections, wherein said method is an in vitro, ex vivo or in vivo method, as described herein.

[0205] Biomarker Compounds and their Uses

[0206] As discussed above, the present invention provides a peptide-based compound comprising

[0207] (i) at least one peptide comprising an amino acid sequence selected from SEQ ID NOs. 1 to 10

SEQ ID NO. 2
GDITWEKDAEVTGNS;

SEQ ID NO. 3
GDITWEKDAEITGNS;

SEQ ID NO. 4
TWEKDAEVTGNSPRL;

SEQ ID NO. 5
TWEKDAEITGNSPRL;

SEQ ID NO. 6
KDAEVTGNSPRLDVA;

SEQ ID NO. 7
KDAEITGNSPRLDVA;

SEQ ID NO. 1
GDITWEKDAEVTGNSPRLDVA;

SEQ ID NO. 8
GDITWEKDAEITGNSPRLDVA;

SEQ ID NO. 9
WEKDAEVTGNSPRLD;

SEQ ID NO. 10
KDAEVTGNS.

[0208] or an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs. 1 to 10, preferably more than about 60%, more preferably more than about 70 or 80 or 90% sequence identity,

[0209] and/or

[0210] an amino acid sequence selected from SEQ ID Nos. 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO. 1,

[0211] and/or

[0212] at least one cyclic peptide according to the present invention,

[0213] and

[0214] (ii) at least one further component, preferably covalently coupled to the peptide and/or cyclic peptide (i).

[0215] Preferably, the peptide(s) or the cyclic peptide(s) of the present invention have a length of at least 5 amino acids, or at least 9 amino acids,

such as 5 to 130 amino acids, or 5 to 100 amino acids, or 5 to 50 amino acids, or 9 to 50 amino acids, e.g. about 15 to 21 amino acids or about 9 to 15 amino acids or about 9 to 21 amino acids.

[0216] In one embodiment, the NS2b fragments can comprise non-natural amino acids, D-amino acids, modified amino acids and/or amino acid derivatives, such as β-amino acids (β^3 and β^2), homo-amino acids, proline and pyruvic acid derivatives, 3-substituted alanine derivatives, glycine derivatives, ring-substituted phenylalanine and tyrosine derivatives, linear core amino acids, N-methyl amino acids.

[0217] Examples are ornithine, citrulline, norleucine, acetyllysine, phosphotyrosine.

[0218] Preferably, the at least one further component (ii) is selected from

[0219] label(s), such as fluorescent label(s),

[0220] tag(s), preferably for immobilization, such as biotin, 6×His, alkyn, azid, thiol,

[0221] linker or anchoring group(s) preferably for attachment of the label(s), and/or tag(s), such as PEG, cysteine, poly-GS-linker, e.g. GSGSG, or combinations thereof.

[0222] Said at least one further component(s) (ii) is/are preferably covalently coupled to said NS2b protein or said at least one fragment thereof via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

[0223] As discussed above, the present invention provides the peptide-based compound for use in a method of the diagnosis and/or prognosis of Zika virus infections.

[0224] Said method is an in vitro, ex vivo or in vivo method.

[0225] Preferably, the method comprises the use of immunoassays.

[0226] Immunoassays are known to the skilled artisan and comprise, for example,

[0227] enzyme-linked immunosorbent assay (ELISA),

[0228] bead-based immunoassay, such as Luminex,

[0229] plaque reduction neutralization test (PRNT),

[0230] lateral flow or strip tests

[0231] encoded microparticle-based immunoassay,

[0232] magnetic bead-based immunoassay.

[0233] Preferably, the method comprises detecting an anti-Zika antibody response in a sample of a patient.

[0234] Said patient sample is preferably blood. More preferably it is selected from serum, plasma and whole blood.

[0235] Methods for Diagnosis and/or Prognosis

[0236] As discussed above, the present invention provides an in vitro or ex vivo method for the diagnosis and/or prognosis of Zika virus infections.

[0237] Said method comprises the steps of

[0238] (a) providing a sample of a patient to be tested,

[0239] (b) providing

[0240] (1) protein NS2b or at least one fragment thereof, as defined herein;

[0241] (2) at least one peptide-based compound of the present invention;

[0242] (3) at least one peptide of the present invention;

[0243] (4) at least one cyclic peptide of the present invention;

[0244] (5) a composition or an array of the present invention;

[0245] and/or

[0246] (6) a multimer compound of the present invention;

[0247] (c) performing an immunoassay, comprising detecting an anti-Zika antibody response in said patient sample.

[0248] Immunoassays are known to the skilled artisan and comprise, for example,

[0249] enzyme-linked immunosorbent assay (ELISA),

[0250] bead-based immunoassay, such as Luminex,

[0251] plaque reduction neutralization test (PRNT),

[0252] lateral flow or strip tests

[0253] encoded microparticle-based immunoassay,

[0254] magnetic bead-based immunoassay.

[0255] Said patient sample is preferably blood. More preferably it is selected from serum, plasma and whole blood.

[0256] In a preferred embodiment, a negative control is used, which preferably excludes a Zika virus infection.

[0257] A preferred negative control is a peptide, a cyclic peptide or a peptide-based compound according to the present invention comprising an amino acid substitution in position K7 in reference to the amino acid sequence of SEQ ID NO. 1.

[0258] Examples for such negative controls are peptide(s), cyclic peptide(s) and/or peptide-based compound(s) comprising or consisting of an amino acid sequence selected from

SEQ ID NO. 34
WEXDAEVTGNSPRLD

SEQ ID NO. 35
XDAEVTGNS

SEQ ID NO. 36
WEXDPEDTGNSPRLD

SEQ ID NO. 37
WEXDAEDTGNSPRLD

SEQ ID NO. 38
WEXDPEDTGNKPRLD

SEQ ID NO. 39
XDPEDTGNS

SEQ ID NO. 40
XDAEDTGNS

SEQ ID NO. 41
XDPEDTGNK

[0259] wherein, in each sequence, X is any amino acid except K (lysine).

Further Description of Preferred Embodiments

[0260] In a first study, the inventors characterized the antibody profiles of Zika patients in a systematic manner. Therefore, they translated the proteomes of 15 different ZIKV strains (taken from the NCBI database, see Table 1 below) in the form of overlapping peptides as high-density peptide arrays and used 84 different patient and control sera to screen several thousands of potential antigens for pathogen induced antibodies.

TABLE 1

ZIKV strains contained in the NCBI database

#	Strain ID	Proteome length (in amino acids)	NCBI GeneBank Accession No.
1	ZikaUgandaStrainMR766-NIID	3423	LC002520.1
2	ZikaUgandaStrainMR766	3419	Q32ZE1
3	ZikaChinaStrainVE_Ganxian	3423	KU744693.1
4	ZikaPhilStrainCPC-0740	3423	KU681082.3
5	ZikaThaiStrainSV0127-14	3423	KU681081.3
6	ZikaChinaStrainZJ03	3423	KU761560.1 or KU820899.2
7	ZikaHaitiStrain1225/2014	3423	KU509998.3
8	ZikaBrazilStrainSPH2015	3423	KU321639.1

TABLE 1-continued

ZIKV strains contained in the NCBI database

#	Strain ID	Proteome length (in amino acids)	NCBI GeneBank Accession No.
9	ZikaBrazilStrainNatalRGN	3423	KU527068.1
10	ZikaDomRepStrainPD2	3423	KU853013.1
11	ZikaDomRepStrainPD1	3423	KU853012.1
12	ZikaChinaStrainGD01	3423	KU740184.2
13	ZikaBrazilStrainZKV2015	3423	KU497555.1
14	ZikaBrazilStrainSSABR1	3423	KU707826.1
15	ZikaPuertoRicoStrainPRVABC59	3423	KU501215.1

[0261] All strains carry the major epitope in polyproteome position 1429-1449, except strain #2, where it is located at position 1425-1445.

[0262] In addition, the inventors investigated the time-dependent antibody variation in patients (acute vs. convalescent stage). They screened patient sera from their collaboration partner in Brazil with different peptide arrays, containing the whole proteome of the ZIKV (first screening round). Analyses showed specific IgG responses towards NS2b, especially in convalescent samples. Almost no signals could be observed in control samples. Additional samples have been recently screened for specific reactivity towards three proteins (NS1, NS2a, NS2b).

[0263] In a first screening round of the first study, arrays with whole proteome content were used. The IgG antibody reactivity of Zika patients of different groups was screened against the whole ZIKV proteome (see FIG. **2**, only protein NS2b shown). The most prominent interaction was observed with the protein NS2b.

[0264] In a second screening round of the first study, specific arrays with NS1. NS2a. NS2b content were used. The IgG antibody reactivity showed clear signals in the NS2b protein. A major epitope was identified. See FIG. **3**. The major epitope is GDITWEKDAEVTGNSPRLDVA [SEQ ID NO. 1] or GDITWEKDAEITGNSPRLDVA (V->I) [SEQ ID NO. 8], maximum length 21 AA (ZIKV polyproteome position 14251445 of Zika Uganda Strain MR766 or position 1429-1449 of Zika Uganda Strain MR766-NIID).

[0265] The identified major epitope of the first study is only at the beginning, namely 13 amino acids, GDITWEKDAEVTG [SEQ ID NO. 2], part of an ordered 3D crystal structure, as shown in FIG. **4**. The missing 8 amino acids of this epitope (highlighted in the circle) are part of a probably unordered linear protrusion of 54 amino acid length (dotted line), connected to an amino acid (highlighted with a star).

[0266] In a second more extended study, the inventors further characterized the IgG and IgM antibody profiles of Zika patients in a more comprehensive manner Therefore, they translated the proteomes of 19 Zika virus strains taken from sequencing data from Nicaragua, Colombia, Guatemala, Honduras, Mexico and Panama 100 additional Zika virus proteomes from the USA with a higher sequence heterogeneity into 4,356 different overlapping Zika virus peptides printed in duplicate on high-density peptide arrays for a high-resolution proteome-wide epitope screening.

[0267] The inventors used plasma of 100 Zika virus patients with a confirmed dengue virus naïve status in the acute and convalescent phase (200 samples), plasma of 50 Zika virus patients with a confirmed dengue virus positive status in the acute and convalescent phase (100 samples), 50 plasma samples of dengue virus patients in the convalescent phase taken before the Zika virus outbreak as disease control and plasma of 25 individuals with a confirmed dengue virus and Zika virus negative status as healthy controls. The samples were pre-collected and purchased from the Nicaraguan Biorepository at the Sustainable Sciences Institute and tested for Dengue (DENV) and Zika (ZIKV) viremia by polymerase chain reaction. The multiplexed IgG and IgM analysis was enabled by highly specific fluorescently labeled anti-human IgG and anti-human IgM secondary antibodies with different fluorescence wavelengths for emission and detection.

[0268] As with the first study, all Zika virus strains carry the major epitope in the polyproteome at various positions. The inventors also investigated the time-dependent antibody profiles in the Zika virus patients by comparison of samples from the acute vs. the convalescent stage. The proteome-wide microarray analyses showed specific IgG responses towards NS2b, especially in convalescent, but also in the acute samples (see FIG. **6**, only protein NS2b shown). Almost no signals were observed in disease and healthy control samples. Interestingly, no specific IgM response towards NS2b was observed, neither in acute nor in convalescent samples.

[0269] The inventors could confirm the major epitope of the first study comprising the amino acid sequences WEKDAEVTGNSPRLD (SEQ ID NO. 9) or KDAEVTGNS (SEQ ID NO. 10).

[0270] In a second screening round of the second study, microarrays with peptide substitution scans of linear WT peptide WEKDAEVTGNSPRLD (SEQ ID NO. 9) and cyclic WT peptide KDAEVTGNS (SEQ ID NO. 10) were used to determine essential, conserved and less-conserved/variable amino acid positions and to identify stronger responding peptide variants. Each amino acid position in the WT sequence was systematically exchanged by all 20 physiologic amino acids, and the resulting linear and cyclic peptides and peptide variants were printed in triplicate on the peptide substitution scan microarrays. The immunoreactivity of the peptide variants was tested by incubation with Zika acute and convalescent plasma samples. The assays were carried out in a multiplexed format with fluorescently labeled secondary anti-human IgG and anti-human IgM antibodies to simultaneously analyze the IgG and IgM profiles of each sample.

[0271] The inventors identified as strongly conserved amino acid positions K7, E10, T12, G13, N14 in the IgG specific epitopes (wherein the numbering is in reference to SEQ ID NO. 1). In these positions, no amino acid exchange is tolerated. Other amino acid positions were less conserved or even variable. Strongest IgG reactivities were observed for peptide variants of A9P,E; V11D,E; S15D,K and R17D,E (see FIG. **7**). Amino acid position K7 turned out to be essential for binding of anti-Zika virus antibodies in all tested human samples, and was shown to be differential for Zika virus in the corresponding regions of NS2b of other flaviviruses or chikungunya virus. More interestingly, not a single human sample exhibited any specific IgM reactivity against WT peptide WEKDAEVTGNSPRLD (SEQ ID NO. 9) or cyclic WT peptide KDAEVTGNS (SEQ ID NO. 10) and the corresponding peptide variants even in the acute phase (data not shown). The linear WT peptide WEKDAEVTGNSPRLD (SEQ ID NO. 9) and cyclic WT peptide KDAEVTGNS (SEQ ID NO. 10) and selected substituted

peptide variants with strongest IgG reactivities were further evaluated by peptide ELISAs. The following examples and drawings illustrate the present invention without, however, limiting the same thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0272] FIG. **1**: Course of disease and diagnostics (detection of RNA and antibodies).

[0273] A typical time course of virus and antibody kinetics in (flavi-)viral infections after virus infection: After infection, the virus replicates, until the immune system successfully overcomes the infection, generating specific IgM and eventually (after isotype switch) IgG antibodies against the viral proteins.

[0274] FIG. **2**: First screening round (Arrays with whole proteome content):

[0275] (a) The IgG antibody reactivity of Zika patients of different groups was screened against the whole ZIKV proteome (only protein NS2b shown). The most prominent interaction was observed with the protein NS2b. A representative selection of data for relevant peptides (bracket with black asterisk) is shown in (b). Framed peptides represent Seq-IDs NO. 2-7. Fluorescence values are shown in gradual 3-color scale (green: >0; black: >100; red: >1000)

[0276] FIG. **3**: Second screening round (Specific arrays with NS1, NS2a, NS2b content).

[0277] The IgG antibody reactivity showed clear signals in the NS2b protein. A major epitope was identified.

[0278] FIG. **4**: 3D structure of the NS2b-NS3 serine protease complex.

[0279] The identified major epitope is only at the beginning (13 amino acids) part of an ordered 3D crystal structure (GDITWEKDAEVTG) [SEQ ID NO. 2], the missing 8 amino acids of this epitope (highlighted in the circle) are part of a probably unordered linear protrusion of 54 amino acids length (orange line), connected to the amino acid (highlighted with a star).

[0280] FIG. **5**: Comparison of specific Zika NS2b and dengue NS2b reactivity in patients with and without dengue/Zika infection:

[0281] The identified major epitope in the Zika NS2b protein is only prevalent in patients after infection with Zika (convalescent samples, highlighted in circles). In the dengue virus NS2b protein, there is almost no reactivity.

[0282] FIG. **6**: Comparison of specific Zika NS2b IgG reactivity in the second study in Zika and Dengue patients.

[0283] The second more comprehensive study analyzed the IgG and IgM antibody responses to the proteomes of 119 Zika virus strains using a total of 375 disease and control human plasma samples. While we did not observe any specific IgM reactivity against framed peptides representing SEQ ID NOs. 1-10, the major epitope in the Zika NS2b protein shows a clear and differential IgG reactivity in patients after infection with Zika in nearly all convalescent and a high number of acute samples. In contrast, the Zika negative/Dengue positive and Zika negative/Dengue negative samples hardly showed any IgG reactivity. Fluorescence values are shown in gradual 3-color scale (light gray: >0; gray: >100; dark gray: >1000)

[0284] FIG. **7**: Full substitution scan of SEQ ID 9.

[0285] **7**A and B, top: Fluorescence pattern of four arrays stained with Zika acute and convalescent plasma samples. Arrays are framed with polio and hemagglutinin peptides as positive controls.

[0286] **7**A and B, bottom: Amino acid plots calculated from the IgG fluorescence intensities. The IgG reactivity of each amino acid exchange and each peptide variant was referenced to the IgG fluorescence intensity of wild type peptide WEKDAEVTGNSPRLD (SEQ ID NO. 9) set to 100%. The wild type peptide sequence is plotted from the N- to the C-terminus on the x-axis, the relative IgG reactivity of each amino acid substitution as percentage of the wild type peptide at 100% on the y-axis.

[0287] FIG. **8**: IgG reactivity in different patient sera towards the WT peptide and cyclic Zika NS2b peptide variants.

[0288] The cyclic peptides and WT peptide were tested by a chemiluminescence ELISA using sera of Zika acute and Zika convalescent patients. A healthy control (HC) sera was included. The ELISA assays were performed under stringent conditions, that is at high serum dilutions (1:1000) to better evaluate the diagnostic potential of the peptide marker. The ELISA results are given in chemiluminescence units (CLUs). Samples were considered as positive, if the CLU of a respective sera is >=2 times over HC value. Each Zika serum exhibited a positive response towards at least one of the cyclic peptide variants.

[0289] A) IgG reactivity towards test peptides in Zika acute patient sera; the numbers at the y-axis indicate the chemiluminescence intensity, serum identifier are plotted at the x-axis.

[0290] B) IgG reactivity towards test peptides in Zika convalescent patient sera; the numbers at the y-axis indicate the chemiluminescence intensity, serum identifier are plotted at the x-axis.

EXAMPLES

[0291] 1. NS2b ZIKV Peptide Microarray

[0292] The whole proteome sequences of all at that time point publicly available 15 zika virus strains were retrieved from the NCBI database and translated into 15-mer peptides with a peptide-peptide overlap of 12 amino acids. Peptide arrays were produced by the company PEPperPRINT GmbH (Heidelberg, Germany) in a laser printing process on glass slides, coated with a PEGMA/PMMA graft copolymer, which were functionalized with a βAla-βAla-linker. In brief, a layer of amino acid particles, containing Fmoc-amino acid pentafluorophenyl esters, was printed layer after layer onto the functionalized glass slides, with intermittent melting (i.e. coupling) steps at 90° C. and chemical washing and capping steps (Stadler et al., 2008). Peptides were generated in duplicates on the arrays, which were screened for IgG responses in human sera.

[0293] 2. Proteome-Wide Zika Virus Peptide Microarrays

[0294] The proteome sequences of 119 Zika virus strains from Nicaragua, Colombia, Guatemala, Honduras, Mexico, Panama and the USA were obtained from sequencing data. A homology analysis and sequence alignment were applied to remove redundant sequences, and unique Zika virus sequences translated into microarrays with 4356 different 15 amino acid peptides, which were printed in duplicate. Peptide microarrays were produced by PEPperPRINT GmbH (Heidelberg, Germany) in a laser printing process on glass slides, coated with a PEGMA/PMMA graft copolymer, which were functionalized with a βAla-βAla-linker. In brief, a layer of amino acid particles, containing Fmoc-amino acid pentafluorophenyl esters, was printed layer after layer onto

the functionalized glass slides, with intermittent melting (i.e. coupling) steps at 90° C. and chemical washing and capping steps (Stadler et al., 2008).

[0295] 3. Immunostaining of Peptide Microarrays

[0296] Peptide microarrays were placed in incubation trays (PEPperPRINT GmbH, Heidelberg, Geimany) and blocked for 30 min at room temperature at 120 RPM orbital shaking with western blot blocking buffer MB-070 (Rockland, USA).

[0297] In the first study, sera were diluted 1:1000 in PBS buffer with 0.05% Tween 20 pH 7.4 (PBST) and 10% blocking buffer, incubating the sera for 16 h at 4° C. and 50 RPM orbital shaking. Peptide microarrays were washed three times shortly with PBST, followed by an incubation with a 1:2500 dilution of the secondary antibody (goat anti-human IgG Fc specific DyLight 680, Rockland, USA), together with the control antibody, diluted 1:500 (anti-c-Myc antibody, PEPperPRINT, Germany), for 30 min at room temperature and 120 RPM orbital shaking. The peptide microarrays were washed 3×10 s with PBST and rinsed with deionized water. After drying in a stream of air, fluorescent images were acquired using an Odyssey Imaging System (LI-COR, USA) at 700 nm with a resolution of 21 μm and a scanning sensitivity of 7. Image analysis and quantification was performed with the PepSlide Analyzer software (Sicasys Software GmbH, Heidelberg, Germany).

[0298] In the second study, human plasma samples were diluted 1:250 in PBS buffer with 0.05% Tween 20 pH 7.4 (PBST) and 10% blocking buffer, incubating the sera for 16 h at 4° C. and 140 RPM orbital shaking. Peptide microarrays were washed three times shortly with PBST, followed by an incubation with a 1:5000 dilution of the secondary antibodies (goat anti-human IgG Fc specific DyLight680 and goat anti-human IgM μ chain specific DyLight800, Rockland, USA) for 30 min at room temperature and 140 RPM orbital shaking.

[0299] Samples used for the peptide array experiments of the first study were collected from individuals with acute febrile illnesses enrolled in a prospective cohort study from May 2015 to April 2016. The cohort was established in an urgent health care clinic in the Recife Metropolitan Region as part of the International Research Consortium on Dengue Risk Assessment, Management and Surveillance-IDAMS (www.idams.eu; Jaenisch et al., 2013; Jaenisch et al., 2016). The age of patients from whom sera were used for the assays varied from 9 to 57 years old, where 7 were females and 5 were males. Sample collection was performed in the first day of recruitment (Day 1—acute sample), which following the IDAMS protocol corresponds to the period within the first 72h of the febrile period, and on the convalescent phase (Day 10-30 after recruitment—convalescent sample). For molecular viral diagnosis, quantitative real-time PCR (qRT-PCR) for Dengue (DENV) and Zika (ZIKV) viruses was performed. Protocols were slightly modified from previously reported assays (Warrilow et al., 2002; Lances et al., 2012; Lanciotti et al., 2007). Positive controls were viruses extracted from cell culture, and the negative control was water. As for serology, samples were assayed for anti-DENV IgM and IgG and anti-ZIKV IgM, through ELISA. The Panbio® Dengue Capture ELISA was used for the anti-DENV IgM and IgG assays, following the manufacturer's protocol. The anti-ZIKV IgM ELISA protocol was that of the Centers for Diseases Control and Prevention (CDC) (MMWR 2016; Granger et al., 2017).

[0300] According to the assays results, each sample was classified as:

[0301] 1) ZIKV Positive/DENV Naïve, if they were positive for ZIKV qRT-PCR and negative for DENV qRT-PCR in the acute phase and/or positive for anti-ZIKV IgM with titers >2 times those for anti-DENV IgM in the convalescent phase, and negative for anti-DENV IgG in the acute phase;

[0302] 2) ZIKA Positive/DENV Exposed, if they were positive for ZIKV qRT-PCR and negative for DENV qRT-PCR in the acute phase and/or positive for anti-ZIKV IgM with titers >2 times those for anti-DENV IgM in the convalescent phase, and positive for anti-DENV IgG in the acute phase;

[0303] 3) Naïve, if they were negative for DENV and ZIKV qRT-PCRs in the acute phase, and negative for anti-DENV and anti-ZIKV IgM and IgG in both acute and convalescent phases.

[0304] In the second study, human plasma samples were pre-collected and purchased from the Nicaraguan Biorepository at the Sustainable Sciences Institute and tested by PCR for Dengue (DENV) and Zika (ZIKV) viremia by polymerase chain reaction. The day of enrollment after onset of symptoms was in the range of 4-6 days for acute plasma samples. Collection day of convalescent sample after onset of symptoms was in the range of 14-21 days. The 375 samples were classified as:

[0305] 1) ZIKV Positive/DENV Naïve, if they were positive for ZIKV PCR and negative for DENV PCR in the acute or convalescent phase;

[0306] 2) ZIKA Positive/DENV Positive, if they were positive for ZIKV PCR and positive for DENV PCR in the acute or convalescent phase;

[0307] 3) ZIKA Negative/DENV Positive, if they were positive for DENV PCR in the convalescent phase and collected before the Zika virus outbreak in 2015.

[0308] 2) ZIKA Negative/DENV Negative, if they were negative for ZIKV PCR and negative for DENV PCR.

[0309] 4. Full Substitution Scan

[0310] A substitution analysis was performed with the WT peptide WEKDAEVTGNSPRLD (SEQ ID No. 9) and cyclic peptide KDAEVTGNS (SEQ ID NO. 10) to determine essential, conserved and less-conserved/variable amino acid positions and to identify stronger reacting peptide variants.

[0311] Each amino acid position in the WT sequence was systematically exchanged by all 20 physiologic amino acids, and the resulting peptides and peptide variants were printed in triplicate on peptide microarrays. The immunoreactivity of the peptide variants was tested by incubation with Zika acute and convalescent plasma samples. The assay was carried out in a multiplexed format with fluorescently labeled secondary anti-human IgG and anti-human IgM antibodies to simultaneously analyze the IgG and IgM profiles of each sample.

[0312] Results are shown in FIGS. **7**A and **7**B. The IgG specific epitopes are strongly conserved in positions K7, E10, T12, G13, N14 (numbering in reference to SEQ ID NO. 1). In these positions, no amino acid exchange is tolerated. Other amino acid positions are less conserved or even variable. Strongest IgG reactivities were observed for peptide variants of A9P,E; V11D,E; S15D,K and R17D,E. Interestingly, amino acid position K7 turned out to be essential for binding of anti-Zika virus antibodies in all

tested human samples, and was shown to be differential for Zika virus in the corresponding regions of NS2b of other flaviviruses.

[0313] 5. Cyclic Peptides

[0314] For ELISA tests, four cyclic peptides with a Biotin tag preceding the C-terminal cysteine residue were synthesized. The non-cyclic WT peptide ("WT peptide") was synthesized with a C-terminal Biotin tag at the C-terminus, the amino acid sequence was identical to SEQ ID NO. 9.

[0315] The cyclic peptides were cyclized via a thioether bond. Therefore, a C-terminal cysteine residue was added to the amino acid sequence of the peptide and the N-terminus carried a bromoacetyl group. The cyclization was via the thiol group, i.e. the cysteine side chain, and the bromacetylated N-terminus.

Peptide		SEQ ID NO.
WT	WEKDAEVTGNSPRLD	9
Peptide 1c (cyclized WT)	CH_2CONH—WEKDAEVTGNSPRLD—Cys (S)	9
Peptide 2c (cyclic, di-substituted)	CH_2CONH—WEKDPEDTGNSPRLD—Cys (S)	11
Peptide 3c (cyclic, mono-substituted)	CH_2CONH—WEKDAEDTGNSPRLD—Cys (S)	12
Peptide 4c (cyclic, tri-substituted)	CH_2CONH—WEKDPEDTGNKPRLD—Cys (S)	13

[0316] 6. ELISA Tests

[0317] 6.1 Protocol

[0318] Peptide ELISA-Tests were performed on Streptavidin functionalized 96 well microtiterplates (Lumitrac, Greiner BioOne, Germany). The plates were coated with the respective biotinylated peptides with a biotin tag positioned at the C-terminus, preceding the C-terminal cysteine. The plates were incubated overnight at 4° C. with test sera at a dilution of 1:1000 (v/v) in PBST/10% Rockland (PBST=Phosphate buffered saline pH 7.2, 0.05% Tween 20). Thereafter, plates were washed 3 times with PBST and incubated for 1 h at 20° C. with the detection antibody (goat anti-human Fc peroxidase conjugate) at a dilution of 1: 10000 in PBST/10% Rockland. The plates were washed again 3 times with PBST, followed by addition of the peroxidase substrate solution (BM Chemiluminescence ELISA substrate, Sigma Aldrich). The chemiluminescence was measured after 10 minutes at 425 nm using an automated plate reader (CLARIOstar®. BMG LABTECH GmbH, Germany). The chemiluminescence intensity is expressed in chemiluminescence units (CLU), the intensity expressing the immunoreactivity of tested sera.

[0319] 6.2 Samples/Tested Sera

[0320] Samples used for the ELISA tests were pre-collected and purchased from the Nicaraguan Biorepository at the Sustainable Sciences Institute and tested for Dengue (DENV) and Zika (ZIKV) viremia by polymerase chain reaction.

[0321] 6.3 ELISA Results:

[0322] The ELISA results are given in CLUs and shown in FIGS. **8**A and **8**B. Samples were considered as positive, if the CLU of a respective sera is >=2 times over HC value. All tested cyclic peptides and the WT peptide detected Zika specific antibody responses in acute patient sera as well as in convalescent patient sera. A positive IgG response was observed for each Zika serum to at least one of the tested peptide variants.

[0323] The features disclosed in the foregoing description, in the claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

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<223> OTHER INFORMATION: Peptide with SEQ ID NO.9 substituted in position 9

<400> SEQUENCE: 12

Trp Glu Lys Asp Ala Glu Asp Thr Gly Asn Ser Pro Arg Leu Asp
1 5 10 15

<210> SEQ ID NO 13
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide with SEQ ID NO.9 substituted in position 9, 11 and 15

<400> SEQUENCE: 13

Trp Glu Lys Asp Pro Glu Asp Thr Gly Asn Lys Pro Arg Leu Asp
1 5 10 15

<210> SEQ ID NO 14
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide with SEQ ID NO. 10 substituted in position 9 and 11

<400> SEQUENCE: 14

Lys Asp Pro Glu Asp Thr Gly Asn Ser

-continued

1 5

<210> SEQ ID NO 15
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide with SEQ ID NO. 10 substituted in position 9

<400> SEQUENCE: 15

Lys Asp Ala Glu Asp Thr Gly Asn Ser
1 5

<210> SEQ ID NO 16
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide with SEQ ID NO. 10 substituted in position 9, 11 and 15

<400> SEQUENCE: 16

Lys Asp Pro Glu Asp Thr Gly Asn Lys
1 5

<210> SEQ ID NO 17
<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Flavivirus Zika

<400> SEQUENCE: 17

Ser Trp Pro Pro Ser Glu Val Leu Thr Ala Val Gly Leu Ile Cys Ala
1 5 10 15

Leu Ala Gly Gly Phe Ala Lys Ala Asp Ile Glu Met Ala Gly Pro Met
20 25 30

Ala Ala Val Gly Leu Leu Ile Val Ser Tyr Val Val Ser Gly Lys Ser
35 40 45

Val Asp Met Tyr Ile Glu Arg Ala Gly Asp Ile Thr Trp Glu Lys Asp
50 55 60

Ala Glu Val Thr Gly Asn Ser Pro Arg Leu Asp Val Ala Leu Asp Glu
65 70 75 80

Ser Gly Asp Phe Ser Leu Val Glu Glu Asp Gly Pro Pro Met Arg Glu
85 90 95

Ile Ile Leu Lys Val Val Leu Met Ala Ile Cys Gly Met Asn Pro Ile
100 105 110

Ala Ile Pro Phe Ala Ala Gly Ala Trp Tyr Val Tyr Val Lys Thr Gly
115 120 125

Lys Arg
130

<210> SEQ ID NO 18
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: West Nile virus

<400> SEQUENCE: 18

Ala Asp Ile Thr Trp Glu Ser Asp Ala Glu Ile Thr Gly Ser Ser Glu
1 5 10 15

-continued

Arg Val Asp Val Arg
20

<210> SEQ ID NO 19
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: West Nile virus

<400> SEQUENCE: 19

Gly Trp Pro Ala Thr Glu Val Met Thr Ala Val Gly Leu Met Phe Ala
1 5 10 15

Ile Val Gly Gly Leu Ala Glu Leu Asp Ile Asp Ser Met Ala Ile Pro
20 25 30

Met Thr Ile Ala Gly Leu Met Phe Ala Ala Phe Val Ile Ser Gly Lys
35 40 45

Ser Thr Asp Met Trp Ile Glu Arg Thr Ala Asp Ile Thr Trp Glu Ser
50 55 60

Asp Ala Glu Ile Thr Gly Ser Ser Glu Arg Val Asp Val Arg Leu Asp
65 70 75 80

Asp Asp Gly Asn Phe Gln Leu Met Asn Asp Pro Gly Ala Pro Trp Lys
85 90 95

Ile Trp Met Leu Arg Met Ala Cys Leu Ala Ile Ser Ala Tyr Thr Pro
100 105 110

Trp Ala Ile Leu Pro Ser Val Ile Gly Phe Trp Ile Thr Leu Gln Tyr
115 120 125

Thr Lys Arg
130

<210> SEQ ID NO 20
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 1

<400> SEQUENCE: 20

Ala Glu Val Ser Trp Glu Glu Glu Ala Glu His Ser Gly Ala Ser His
1 5 10 15

Asn Ile Leu Val Glu
20

<210> SEQ ID NO 21
<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 1

<400> SEQUENCE: 21

Ser Trp Pro Leu Asn Glu Gly Ile Met Ala Val Gly Ile Val Ser Ile
1 5 10 15

Leu Leu Ser Ser Leu Leu Lys Asn Asp Val Pro Leu Ala Gly Pro Leu
20 25 30

Ile Ala Gly Gly Met Leu Ile Ala Cys Tyr Val Ile Ser Gly Ser Ser
35 40 45

Ala Asp Leu Ser Leu Glu Lys Ala Ala Glu Val Ser Trp Glu Glu Glu
50 55 60

Ala Glu His Ser Gly Ala Ser His Asn Ile Leu Val Glu Val Gln Asp
65 70 75 80

Asp Gly Thr Met Lys Ile Lys Asp Glu Glu Arg Asp Asp Thr Leu Thr

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                85                  90                  95

Ile Leu Leu Lys Ala Thr Leu Leu Ala Ile Ser Gly Val Tyr Pro Met
            100                 105                 110

Ser Ile Pro Ala Thr Leu Phe Val Trp Tyr Phe Trp Gln Lys Lys Lys
        115                 120                 125

Gln Arg
    130


<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 2

<400> SEQUENCE: 22

Ala Asp Val Lys Trp Glu Asp Gln Ala Glu Ile Ser Gly Ser Ser Pro
1               5                   10                  15

Ile Leu Ser Ile Thr
            20


<210> SEQ ID NO 23
<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 2

<400> SEQUENCE: 23

Ser Trp Pro Leu Asn Glu Ala Ile Met Ala Val Gly Met Val Ser Ile
1               5                   10                  15

Leu Ala Ser Ser Leu Leu Lys Asn Asp Ile Pro Met Thr Gly Pro Leu
            20                  25                  30

Val Ala Gly Gly Pro Leu Thr Val Cys Tyr Val Leu Thr Gly Arg Ser
        35                  40                  45

Ala Asp Leu Glu Leu Glu Arg Ala Ala Asp Val Lys Trp Glu Asp Gln
    50                  55                  60

Ala Glu Ile Ser Gly Ser Ser Pro Ile Leu Ser Ile Thr Ile Ser Glu
65                  70                  75                  80

Asp Gly Ser Met Ser Ile Lys Asn Glu Glu Glu Glu Gln Thr Leu Thr
                85                  90                  95

Ile Leu Ile Arg Thr Gly Leu Leu Val Ile Ser Gly Leu Phe Pro Val
            100                 105                 110

Ser Ile Pro Ile Thr Ala Ala Ala Trp Tyr Leu Trp Glu Val Lys Lys
        115                 120                 125

Gln Arg
    130


<210> SEQ ID NO 24
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 3

<400> SEQUENCE: 24

Pro Asp Val Thr Trp Glu Glu Glu Ala Glu Gln Thr Gly Val Ser His
1               5                   10                  15

Asn Leu Met Ile Thr
            20


<210> SEQ ID NO 25
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-continued

<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 3

<400> SEQUENCE: 25

Ser Trp Pro Leu Asn Glu Gly Val Met Ala Val Gly Leu Val Ser Ile
1 5 10 15

Leu Ala Ser Ser Leu Leu Arg Asn Asp Val Pro Met Ala Gly Pro Leu
20 25 30

Val Ala Gly Gly Leu Leu Ile Ala Cys Tyr Val Ile Thr Gly Thr Ser
35 40 45

Ala Asp Leu Thr Val Glu Lys Ala Pro Asp Val Thr Trp Glu Glu Glu
50 55 60

Ala Glu Gln Thr Gly Val Ser His Asn Leu Met Ile Thr Val Asp Asp
65 70 75 80

Asp Gly Thr Met Arg Ile Lys Asp Asp Glu Thr Glu Asn Ile Leu Thr
85 90 95

Val Leu Leu Lys Thr Ala Leu Leu Ile Val Ser Gly Ile Phe Pro Tyr
100 105 110

Ser Ile Pro Ala Thr Leu Leu Val Trp His Thr Trp Gln Lys Gln Thr
115 120 125

Gln Arg
130

<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Yellow fever virus

<400> SEQUENCE: 26

Gly Glu Val Ser Trp Glu Glu Glu Ala Glu Ile Ser Gly Ser Ser Ala
1 5 10 15

Arg Tyr Asp Val Ala
20

<210> SEQ ID NO 27
<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Yellow fever virus

<400> SEQUENCE: 27

Ser Ile Pro Val Asn Glu Ala Leu Ala Ala Ala Gly Leu Val Gly Val
1 5 10 15

Leu Ala Gly Leu Ala Phe Gln Glu Met Glu Asn Phe Leu Gly Pro Ile
20 25 30

Ala Val Gly Gly Leu Leu Met Met Leu Val Ser Val Ala Gly Arg Val
35 40 45

Asp Gly Leu Glu Leu Lys Lys Leu Gly Glu Val Ser Trp Glu Glu Glu
50 55 60

Ala Glu Ile Ser Gly Ser Ser Ala Arg Tyr Asp Val Ala Leu Ser Glu
65 70 75 80

Gln Gly Glu Phe Lys Leu Leu Ser Glu Glu Lys Val Pro Trp Asp Gln
85 90 95

Val Val Met Thr Ser Leu Ala Leu Val Gly Ala Ala Leu His Pro Phe
100 105 110

-continued

Ala Leu Leu Leu Val Leu Ala Gly Trp Leu Phe His Val Arg Gly Ala
115 120 125

Arg Arg
130

<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 4

<400> SEQUENCE: 28

Ala Asn Val Gln Trp Asp Glu Met Ala Asp Ile Thr Gly Ser Ser Pro
1 5 10 15

Ile Ile Glu Val Lys
20

<210> SEQ ID NO 29
<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Dengue virus type 4

<400> SEQUENCE: 29

Ser Trp Pro Leu Asn Glu Gly Ile Met Ala Val Gly Leu Val Ser Leu
1 5 10 15

Leu Gly Ser Ala Leu Leu Lys Asn Asp Val Pro Leu Ala Gly Pro Met
20 25 30

Val Ala Gly Gly Leu Leu Leu Ala Ala Tyr Val Met Ser Gly Ser Ser
35 40 45

Ala Asp Leu Ser Leu Glu Lys Ala Ala Asn Val Gln Trp Asp Glu Met
50 55 60

Ala Asp Ile Thr Gly Ser Ser Pro Ile Ile Glu Val Lys Gln Asp Glu
65 70 75 80

Asp Gly Ser Phe Ser Ile Arg Asp Val Glu Glu Thr Asn Met Ile Thr
85 90 95

Leu Leu Val Lys Leu Ala Leu Ile Thr Val Ser Gly Leu Tyr Pro Leu
100 105 110

Ala Ile Pro Val Thr Met Thr Leu Trp Tyr Met Trp Gln Val Lys Thr
115 120 125

Gln Arg
130

<210> SEQ ID NO 30
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Tick-borne encephalitis virus

<400> SEQUENCE: 30

Gly Cys Val Glu Trp Tyr Pro Glu Leu Val Asn Glu Gly Gly Glu Val
1 5 10 15

Ser Leu Arg Val Arg
20

<210> SEQ ID NO 31
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Tick-borne encephalitis virus

<400> SEQUENCE: 31

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Ser Phe Ser Glu Pro Leu Thr Val Val Gly Val Met Leu Thr Leu Ala
1               5                   10                  15

Ser Gly Met Met Arg His Thr Ser Gln Glu Ala Leu Cys Ala Leu Ala
            20                  25                  30

Val Ala Ser Phe Leu Leu Leu Met Leu Val Leu Gly Thr Arg Lys Met
        35                  40                  45

Gln Leu Val Ala Glu Trp Ser Gly Cys Val Glu Trp Tyr Pro Glu Leu
    50                  55                  60

Val Asn Glu Gly Gly Glu Val Ser Leu Arg Val Arg Gln Asp Ala Met
65                  70                  75                  80

Gly Asn Phe His Leu Thr Glu Leu Glu Lys Glu Glu Arg Met Met Ala
                85                  90                  95

Phe Trp Leu Ile Ala Gly Leu Ala Ala Ser Ala Ile His Trp Ser Gly
            100                 105                 110

Ile Leu Gly Val Met Gly Leu Trp Thr Leu Thr Glu Met Leu Arg Ser
        115                 120                 125

Ser Arg Arg
    130


<210> SEQ ID NO 32
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Japanese encephalitis virus

<400> SEQUENCE: 32

Ala Thr Asp Met Trp Leu Glu Arg Ala Ala Asp Ile Ser Trp Glu Met
1               5                   10                  15

Asp Ala Ala Ile Thr
            20


<210> SEQ ID NO 33
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Japanese encephalitis virus

<400> SEQUENCE: 33

Gly Trp Pro Ala Thr Glu Phe Leu Ser Ala Val Gly Leu Met Phe Ala
1               5                   10                  15

Ile Val Gly Gly Leu Ala Glu Leu Asp Ile Glu Ser Met Ser Ile Pro
            20                  25                  30

Phe Met Leu Ala Gly Leu Met Ala Val Ser Tyr Val Val Ser Gly Lys
        35                  40                  45

Ala Thr Asp Met Trp Leu Glu Arg Ala Ala Asp Ile Ser Trp Glu Met
    50                  55                  60

Asp Ala Ala Ile Thr Gly Ser Ser Arg Arg Leu Asp Val Lys Leu Asp
65                  70                  75                  80

Asp Asp Gly Asp Phe His Leu Ile Asp Asp Pro Gly Val Pro Trp Lys
                85                  90                  95

Val Trp Val Leu Arg Met Ser Cys Ile Gly Leu Ala Ala Leu Thr Pro
            100                 105                 110

Trp Ala Ile Val Pro Ala Ala Phe Gly Tyr Trp Leu Thr Leu Lys Thr
        115                 120                 125

Thr Lys Arg
    130
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-continued

<210> SEQ ID NO 34
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 9, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 34

Trp Glu Xaa Asp Ala Glu Val Thr Gly Asn Ser Pro Arg Leu Asp
1 5 10 15

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 10, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 35

Xaa Asp Ala Glu Val Thr Gly Asn Ser
1 5

<210> SEQ ID NO 36
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 11, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 36

Trp Glu Xaa Asp Pro Glu Asp Thr Gly Asn Ser Pro Arg Leu Asp
1 5 10 15

<210> SEQ ID NO 37
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 12, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 37

Trp Glu Xaa Asp Ala Glu Asp Thr Gly Asn Ser Pro Arg Leu Asp
1 5 10 15

<210> SEQ ID NO 38
<211> LENGTH: 15

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 13, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 38

Trp Glu Xaa Asp Pro Glu Asp Thr Gly Asn Lys Pro Arg Leu Asp
1 5 10 15

<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 14, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 39

Xaa Asp Pro Glu Asp Thr Gly Asn Ser
1 5

<210> SEQ ID NO 40
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 15, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 40

Xaa Asp Ala Glu Asp Thr Gly Asn Ser
1 5

<210> SEQ ID NO 41
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Peptide, derived from SEQ ID NO. 16, with K7 substitution, wherein X is any amino acid except K (lysine)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 41

Xaa Asp Pro Glu Asp Thr Gly Asn Lys
1 5

1-26. (canceled)

27. A protein NS2b or at least one fragment thereof as a biomarker or diagnostic marker for use in a method for the diagnosis and/or prognosis of Zika virus infections.

28. The NS2b protein or at least one fragment thereof according to claim **27**, wherein the fragment comprises an amino acid sequence of the ZIKV polyproteome of positions 1429-1449 of Zika Uganda Strain MR766_NIID (SEQ ID NO: 1).

29. The NS2b fragment according to claim **27**, wherein the fragment comprises or is a peptide having

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1.

30. The NS2b fragment according to claim **29**, wherein the amino acid substitution(s) are

A9P, A9E, A9M, A9S, A9T, A9K;

V11D, V11E, V11T, V11A, V11N, V11S, V11M, V11L or I11D, I11E, I11T, I11A, I11N, I11 S, I11M, I11L;

S15D, S15K, S15M, S15A, S15R, S15N; and/or

R17D, R17E, R17T.

31. The NS2b fragment according to claim **27**, wherein the fragment comprises or is a cyclic peptide having

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

wherein the cyclization is via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

or

head-to-tail cyclization.

32. A peptide or a cyclic peptide that is either:

A) a peptide comprising an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1 or

B) a cyclic peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

wherein the cyclization is via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

or

head-to-tail cyclization.

33. The peptide of claim **32** having a length of 5 to 130 amino acids.

34. The peptide or the cyclic peptide of claim **32**, comprising at least one further component(s), which is/arc selected from

label(s),

tag(s),

linker or anchoring group(s)

or combinations thereof,

wherein said at least one further component(s) is/are covalently coupled to said peptide via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

35. A composition or an array comprising:

(a) at least one peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs: 1 to 10, or

(b) at least one peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

and/or

(c) at least one cyclic peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

wherein the cyclization is via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

or

head-to-tail cyclization,

(d) a protein or at least one fragment thereof of claim **1**.

36. A multimer compound comprising at least two of component(s) (a) to (d):

(a) a peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs: 1 to 10, or

(b) a peptide comprising an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

(c) a cyclic peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

wherein the cyclization is via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,

or

head-to-tail cyclization,

and/or

(d) a protein NS2b or at least one fragment thereof of claim **1**,

wherein at least two of component(s) (a) to (d) are covalently coupled to each other or are connected via a linear or cyclic scaffold.

37. A peptide-based compound comprising:
(i) at least one peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs: 1 to 10, or
an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,
and/or at least one cyclic peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1
wherein the cyclization is via
thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,
or
head-to-tail cyclization
and
(ii) at least one further component coupled to the peptide and/or cyclic peptide (i).

38. The peptide-based compound of claim **37**, wherein the peptide and/or cyclic peptide has a length of at least 5 amino acids.

39. The peptide-based compound of claim **37**, wherein the at least one further component is selected from
label(s),
tag(s),
linker or anchoring group(s),
or combinations thereof,
wherein the at least one further component(s) is/are preferably covalently coupled to said peptide and/or cyclic peptide via a linker, an amino acid side chain, and/or to the N- and/or C-terminus.

40. A method for the diagnosis and/or prognosis of Zika virus infections, comprising the steps of
(a) providing a sample of a patient to be tested,
(b) providing
(1) protein NS2b or at least one fragment thereof;
(2) a peptide-based compound comprising
(i) at least one peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs: 1 to 10, or
an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,
and/or at least one cyclic peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1
wherein the cyclization is via
thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,
or
head-to-tail cyclization
and
(ii) at least one further component coupled to the peptide and/or cyclic peptide (i);
(3) a peptide comprising an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1;
(4) at least one cyclic peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,
wherein the cyclization is via
thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,
or
head-to-tail cyclization
(5) a composition or an array comprising
(a) at least one peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs: 1 to 10, or
(b) at least one peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,
and/or
(c) at least one cyclic peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,
wherein the cyclization is via
thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus,
or
head-to-tail cyclization,
(d) a protein NS2b or at least one fragment thereof of claim **1**;
and/or
(6) a multimer compound comprising at least two of component(s) (a) to (d):
(a) a peptide comprising
an amino acid sequence selected from SEQ ID NOs: 1 to 10, or
an amino acid sequence having at least about 50% sequence identity to an amino acid sequence of SEQ ID NOs: 1 to 10, or
(b) a peptide comprising an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or

four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

(c) a cyclic peptide comprising

an amino acid sequence selected from SEQ ID NOs: 1 to 10, or

an amino acid sequence selected from SEQ ID NOs: 1 to 10 having one, two, three or four amino acid substitution(s) in position 9, 11, 15 and/or 17 in reference to the amino acid sequence of SEQ ID NO: 1,

wherein the cyclization is via

thioether formation, wherein the peptide comprises an additional cysteine residue at or near the C-terminus, or

head-to-tail cyclization,

and/or

(d) a protein NS2b or at least one fragment thereof,

wherein at least two of component(s) (a) to (d) are coupled to each other or are connected via a linear or cyclic scaffold;

(c) performing an immunoassay, comprising detecting an anti-Zika antibody response in said patient sample.

41. The method of claim **40**, comprising the use of a negative control, wherein said negative control is a NS2b peptide, cyclic peptide or a peptide-based compound comprising an amino acid substitution in position K7 in reference to the amino acid sequence of SEQ ID NO: 1.

42. The method according to claim **40**, wherein the NS2b fragment comprises an amino acid sequence of the ZIKV polyproteome of positions 1429-1449 of Zika Uganda Strain MR766 NIID (SEQ ID NO: 1)

43. The method according to claim **40**, wherein the NS2b fragment has the following substitutions:

A9P, A9E, A9M, A9S, A9T, A9K;

V11D, V11E, V11T, V11A, V11N, V11S, V11M, V111, or I11D, I11E, MT, I11A, I11N, I11S, I11M, I11L;

S15D, S15K, S15M, S15A, S15R, S15N; and/or

R17D, R17E, R17T.

44. The method according to claim **40**, wherein one fragment is used that comprises more than one of said peptides, or more than one fragment each comprising or consisting of one peptide are used.

45. The NS2b fragment according to claim **29**, wherein the amino acid substitution(s) are A9P, A9E, V11D, MD, V11E, I11E, S15D, S15K, and/or R17D, R17E.

46. The NS2b fragment according to claim **29**, comprising an amino acid sequence selected from SEQ ID NOs: 11 to 16.

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