

Another problem underlying the present invention is to provide an assay for distinguishing an acute infection by Flaviviruses, in particular Zika virus, from a vaccination or a previous infection by the same or another Flavivirus, preferably dengue virus.

Another problem underlying the present invention is to provide a vaccine against a flavivirus, preferably Zika virus infection.

Another problem underlying the present invention is to provide a resource-efficient, yet diagnostically reliable test for distinguishing a flavivirus infection from another disease.

Another problem underlying the present invention is to provide a test that may be used during a longer time window between the initial exposure or the onset of symptoms and the day the sample for the diagnosis is obtained.

Another problem underlying the present invention is to provide a test for distinguishing between a primary Flavivirus infection, preferably with a Flavivirus other than the Zika virus, and a secondary Flavivirus infection, preferably Zika infection, which method is diagnostically more reliable than state of the art methods, in particular with regard to avoiding false positive or negative results, and may ideally be applied to samples from patients having an IgM deficiency.

Summary

The problem underlying the present invention is solved by the subject-matter of the attached independent and dependent claims.

In one aspect, the present disclosure refers to a kit comprising a diagnostically useful carrier, wherein the kit further comprises a means for specifically detecting a captured antibody, wherein the diagnostically useful carrier is a carrier comprising a means for specifically capturing an antibody to SEQ ID NO1 in a sample from a subject, and wherein the means for specifically detecting a captured antibody is a secondary antibody binding to the constant region of IgA class antibodies. In another aspect, the present disclosure refers to a kit comprising a diagnostically useful carrier and a polypeptide, wherein the diagnostically useful carrier comprises a means for specifically capturing all IgA class antibodies, which is an antibody binding to all IgA class antibodies, wherein the polypeptide is a recombinant polypeptide comprising SEQ ID NO1 or a variant thereof. In yet another aspect, the present

disclosure refers to a method comprising the step detecting in a sample from a subject, the presence or absence of an antibody to SEQ ID NO1, wherein the antibody is an IgA class antibody. In still another aspect, the present disclosure refers to an *in vitro* use of a polypeptide comprising SEQ ID NO1 or a variant thereof, a diagnostically useful carrier comprising said polypeptide or a kit as disclosed herein or an IgA class antibody to SEQ ID NO1 for the diagnosis of a Zika virus infection. In a further aspect, the present disclosure refers to an *in vitro* use of a polypeptide comprising SEQ ID NO1 or a variant thereof, a diagnostically useful carrier comprising said polypeptide or a kit as disclosed herein or an IgA class antibody to SEQ ID NO1 for distinguishing a primary from a secondary Zika virus infection, wherein the use comprises detecting an IgA or IgM class antibody to SEQ ID NO1, and an IgG class antibody to SEQ ID NO1.

In a first example, the problem underlying the present invention is solved by a polypeptide comprising a sequence selected from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 or a variant thereof, preferably SEQ ID NO1 or a variant thereof, preferably a dimer and/or hexamer, more preferably a dimer, for the diagnosis of a disease.

In a second example, the problem is solved by a diagnostically useful carrier comprising a means for specifically capturing an antibody to SEQ ID NO1 in a sample from a subject.

In a preferred example, the carrier further comprises one or more than one means, which means is for specifically capturing an antibody to an antigen from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9.

In a preferred example, the carrier is selected from the group comprising a bead, preferably a paramagnetic particle, a test strip, a microtiter plate, a blot, preferably from the group comprising western blot, line blot and dot blot, lateral flow test, a glass surface, a slide, a biochip and a membrane, and is preferably a bead a line blot or microtiter plate, more preferably a microtiter plate.

In a third example, the problem is solved by a kit comprising the diagnostically useful carrier according to the present disclosure, optionally as well as a means for specifically detecting a captured antibody.

In a preferred example, the kit comprises the diagnostically useful carrier which further comprises one or more means, which means is for specifically capturing an antibody to one or more further antigens from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9,

wherein the means for specifically capturing an antibody to SEQ ID NO1 and the means for specifically capturing an antibody to one or more further antigens are coated on, preferably covalently linked to separate carriers.

In a preferred example, the kit comprises the diagnostically useful carrier which further comprises one or more means, which means is for specifically capturing an antibody to one or more further antigens from the group SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9,

wherein the means for specifically capturing an antibody to SEQ ID NO1 and the means for specifically capturing an antibody to one or more further antigens are coated on one, preferably covalently linked to one carrier.

In a 4th example, the problem is solved by a method, preferably for diagnosing a disease, comprising the step detecting in a sample from a subject the presence or absence of an antibody to SEQ ID NO1.

In a preferred example, the method further comprises the step detecting in a sample, preferably blood or CSF sample, from a subject the presence or absence of an antibody to one or more further antigens from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9.

In a preferred example, the presence or absence of an antibody to SEQ ID NO1 and the presence or absence of an antibody to one or more further antigens from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 is detected simultaneously.

In a preferred example, the presence or absence of an antibody to SEQ ID NO1 and the presence or absence of an antibody to one or more further antigens is detected in spatially separate binding reactions.

In a preferred example, the presence or absence of an antibody to SEQ ID NO1 and the presence or absence of an antibody to one or more further antigens is detected in a one-pot reaction.

In a preferred example, the method comprises the step contacting the diagnostically useful carrier according to the present disclosure with a sample from the subject.

In a preferred example, the subject suffers from or is suspected of suffering from an infectious disease, preferably a viral infection, more preferably an infection by a flavivirus, preferably from the group comprising Zika, dengue, Yellow fever, TBEV, Usutu, Powassan, West Nile and JEV, preferably Zika.

In a preferred example, the antibody is a mammalian, preferably human antibody, more preferably a human IgA, IgM or IgG class antibody, preferably IgG.

In a 5th example, the problem underlying the present invention is solved by diagnostically useful carrier configured for capturing an IgA class antibody to NS1 from a flavivirus, preferably the Zika virus, in a sample from a subject,

preferably for diagnosing a Flavivirus infection, more preferably for distinguishing a primary from a secondary Flavivirus, preferably Zika virus infection.

In a preferred example, the carrier is in addition configured for capturing and specifically detecting an IgM and/or IgG class antibody, preferably an IgM class antibody, to NS1 from said Flavivirus

and/or is in addition configured for capturing an antibody to an envelope glycoprotein of a Flavivirus.

In another preferred example the diagnostically useful carrier is in complex with an IgA class antibody to NS1 from a Flavivirus.

In another preferred example said complex further comprises the NS1 from a Flavivirus or a variant thereof, and preferably further comprises a detection label which is more preferably associated with an antibody to be detected or the NS1 from a Flavivirus or variant thereof.

In another preferred example, the diagnostically useful carrier comprises a dimer or hexamer of the NS1 of said Flavivirus.

In a 6th example, the problem is solved by a kit comprising the diagnostically useful carrier according to the present disclosure.

In a 7th example, the problem is solved by a method for diagnosing a Flavivirus infection, preferably for distinguishing a primary from a secondary Flavivirus infection, comprising the step

- a) detecting in a first sample from a subject an IgA class antibody to NS1 of said Flavivirus.

In another preferred example, the diagnostically useful carrier according to the present disclosure is used.

In another preferred example, the method further comprises the step

- b) detecting in a second sample from said subject an IgA class antibody to NS1 of said Flavivirus,
wherein the second sample was obtained from said subject at least three days later than the first sample.

In another preferred example, in addition an IgM class antibody to NS1 of said Flavivirus is detected as part of step a) and/or step b), preferably step a).

In another preferred example, an IgG class antibody to NS1 of said Flavivirus is detected in addition in step a) and/or step b), preferably step a).

In another preferred example, in addition in step a) and/or step b), preferably step a), at least one class of antibody to an envelope glycoprotein of said Flavivirus is detected,

wherein preferably the at least one class of antibody to an envelope glycoprotein of said Flavivirus is selected from the group comprising IgG, IgM and IgA, preferably IgA and IgM, or IgA and IgG, or IgM and IgG, more preferably IgA.

In another preferred example, each antibody is detected in spatially separate binding reactions, separated according to antigen and antibody class to be detected.

In another preferred example, the Flavivirus is selected from the group comprising Zika virus, dengue virus, Yellow fever virus, Tick-borne encephalitis virus, Usutu virus, West Nile virus and Japanese encephalitis virus, preferably Zika virus.

In an 8th example, the problem is solved by a use of an IgA class antibody to NS1 from a Flavivirus, preferably the Zika virus, or a diagnostically useful carrier for immobilizing and optionally detecting said antibody, for distinguishing a primary from a secondary Flavivirus infection, preferably Zika virus infection.

In a 9th example, the problem is solved by a use of an IgA class antibody to NS1 from a Flavivirus, preferably the Zika virus, or a diagnostically useful carrier for immobilizing and optionally detecting said antibody, for diagnosing a flaviviral infection, preferably a Zika virus infection, in an IgM-deficient subject.

In a 10th example, the problem is solved by a use of an IgA class antibody to NS1 from a Flavivirus, preferably the Zika virus, or a diagnostically useful carrier for immobilizing and optionally detecting said antibody, for increasing the diagnostic reliability, preferably sensitivity, of a diagnostic assay for diagnosing a flaviviral infection, preferably a Zika infection, more preferably at the early stages of an infection.

The present disclosure sets forth the detection of an antibody to NS1 from Zika virus (SEQ ID NO1) as part of a diagnostic method practiced on a sample from a patient suspected of suffering from an infection, preferably a flaviviral infection, more preferably a Zika virus infection.

The present inventors have surprisingly found that a Zika virus infection may be diagnosed and distinguished from other flaviviral infections by detecting antibodies in a sample from a

patient, with a surprisingly high degree of diagnostic reliability, in particular relative to the NS1 antigens derived from other flaviviruses, with an unexpected low degree of cross reactivity.

The inventors have also surprisingly found that some patients, despite having been exposed to a Flavivirus, do not have titers of IgM that allow for the monitoring of the course of the infection, but have surprisingly dynamic titers of IgA to NS1 of said Flavivirus that may be used. More surprisingly, these IgA antibodies do not show a degree of cross reactivity, as would have to be expected, that would make the distinction between an acute Zika virus infection and a previous Flavivirus infection, preferably with a Flavivirus other than the Zika virus, insufficiently reliable.

The inventors have also surprisingly found that Zika virus NS1 antigen exists in oligomeric forms and complexes that have surprising properties relevant for application in diagnostic assays, among them monomers, dimers and hexamers, and complexation with mammalian apolipoproteins, which, when in complex with Zika virus NS1, enhance the diagnostic reliability and stability of the antigen.

The disclosure relates to a diagnostically useful carrier, which is preferably a solid carrier made from an artificial material such as glass or plastic for contacting a means, which means is associated with said carrier, which means is for specifically capturing an antibody with a bodily fluid sample from a subject, preferably a mammalian subject, more preferably a human subject. In a preferred example, the term “captured” or “specifically captured”, as used herein, means that the binding between the means for specifically capturing and the antibody to be captured is stronger than a binding reaction characterized by a dissociation constant of 1×10^{-5} M, more preferably 1×10^{-7} M, more preferably 1×10^{-8} M, more preferably 1×10^{-9} M, more preferably 1×10^{-10} M, more preferably 1×10^{-11} M, more preferably 1×10^{-12} M, as determined by surface plasmon resonance using Biacore equipment at 25 °C in PBS buffer at pH 7. The diagnostically useful carrier may comprise one or more controls, preferably selected from a control confirming that sample has been added and/or a control confirming that a secondary antibody has been added.

In a preferred example, the antibody captured specifically may be an antibody from a certain antibody class, preferably selected from IgG, IgM and IgA, more preferably IgA. In another preferred example, the antibody captured specifically may be an antibody to a Flavivirus antigen, preferably selected from the group comprising NS1 and Envelope glycoprotein,

preferably NS1. In a more preferred example, the antibody captured specifically is an IgM or IgA, preferably IgA class antibody to SEQ ID NO1, preferably to an epitope from SEQ ID NO1 sufficiently long to be recognized by an antibody, which epitope comprises, with reference to SEQ ID NO1, one or more amino acids from the group comprising Arg62, Ile66, Arg 69, Glu72, Glycine73, the latter of which may be substituted with serine or alanine, preferably the peptide comprising the sequence Arg 62 to Glycine 73; one or more amino acids from the group comprising Gln102, Pro105, the latter of which could be substituted with a neutral amino acid and a short side chain such as Ser or Ala, and Glu110; preferably the peptide comprising the sequence Gln102 to Glu110; the peptide comprising residues Ser121 to Thr129, the peptide comprising residues Asp138 to Lys141, the peptide comprising residues Asp174 to Glu178 and the peptide comprising Ser322 to Lys326. In a preferred example, the solid carrier is a diagnostic device, more preferably selected from the group comprising a bead, preferably a paramagnetic particle, a test strip, a microtiter plate, blot, a glass surface, a biochip and a membrane, more preferably from the group comprising a bead, a blot, a test strip and a microtiterplate.

The diagnostically useful carrier may be a microtiter plate comprising a range of wells configured for an immunoassays such as an ELISA assay. In a preferred example, the term "microtiter plate" is a diagnostic device, preferably made from glass or plastic, more preferably plastic, comprising one or more, preferably more than one, more preferably at least 8 wells, in which reactions in liquid buffer may be run separately without cross-contamination. At least one of the well is coated with a polypeptide, preferably an antigenic polypeptide that may be used to specifically capture a diagnostically useful antibody. If more than one means for specifically detecting an antigen is used, then preferably each means is in a well separate from other means. The microtiter plate may be used for running several samples in parallel, preferably in an automated fashion. The wells are preferably compatible with at least one routine detection techniques such colourimetry, immunofluorescence, detection of enzymatic activity, chemiluminescence, radioactivity or the like. Suitable microtiter plates are commercially available. If the diagnostically useful carrier is a microtiter plate, it is preferred that at least 50%, 60%, 70%, 80% or 90%, preferably 50% of any Flavivirus NS1, preferably Zika virus NS1, is a hexamer or dimer, preferably dimer.

The diagnostically useful carrier may be a bead configured for an immunoassay comprising a polypeptide comprising SEQ ID NO1 or a variant thereof. In a more preferred example, the bead is a paramagnetic microparticle which may be removed from a solution and concentrated, preferably at the surface of a vessel, by applying a magnetic field. The bead comprises an antigen linked to the bead by a covalent or non-covalent bond. If more than

one bead is used, a preparation of beads may be made by covalently attaching to the beads a universal ligand which binds strongly to a tag which may be fused to one or more polypeptides according to the present disclosure by way of genetic engineering. The preparation of beads may then be divided into more than one batches, and each batch is contacted with a different polypeptide fused to a tag to the effect that a range of carriers, each with a different immobilized polypeptide, is produced. A mixture of beads, for example one of which carrying a polypeptide comprising SEQ ID NO1 or a variant thereof, and at least one more carrying a polypeptide comprising a sequence selected from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 may be made. If the diagnostically useful carrier is a bead, it is preferred that at least 50%, 60%, 70%, 80% or 90%, preferably 50% of any Flavivirus NS1, preferably Zika virus NS1, is a monomer, hexamer or dimer, preferably monomer.

The diagnostically useful carrier may be a line blot (Raoult, D., and Dasch, G. A. (1989), The line blot: an immunoassay for monoclonal and other antibodies. Its application to the serotyping of gram-negative bacteria. J. Immunol. Methods, 125 (1-2), 57-65; WO2013041540). In a preferred example, the term "line blot", as used herein, refers to a test strip, more preferably membrane-based, that has been coated with one or more means for capturing an antibody, preferably a polypeptide each. If two or more means are used, they are preferably spatially separated on the carrier. Preferably, the width of the bands is at least 30, more preferably 40, 50, 60, 70 or 80 % of the width of the test strip. The test strip may comprise one or more control bands for confirming that it has been contacted with sample sufficiently long and under adequate conditions, in particular in the presence of human serum, antibody conjugate, or both. A multitude of line blots are commercially available, for example from EUROIMMUN AG, Lübeck, Germany. If the diagnostically useful carrier is a line blot, it is preferred that at least 50%, 60%, 70%, 80% or 90%, preferably 50% of any Flavivirus NS1, preferably Zika virus NS1, is a hexamer or dimer, preferably a hexamer.

The diagnostically useful carrier may be a glass slide coated with a cell, preferably a eukaryotic cell, more preferably an insect or mammalian cell, preferably a human cell expressing a Flavivirus NS1, preferably a polypeptide comprising SEQ ID NO1. The cell may be a live cell, but is preferably a fixed cell. Immunofluorescence may then be used to detect the antibody. Preferably the cell is a recombinant cell overexpressing the Flavivirus NS1. The

Flavivirus NS1 may be localized at a cell membrane, preferably at the surface of the cell. If the diagnostically useful carrier is a glass slide, it is preferred that at least 50%, 60%, 70%, 80% or 90%, preferably 50% of any Flavivirus NS1, preferably Zika virus NS1, is a hexamer or dimer, preferably a dimer.

According to the present disclosure, the diagnostically useful carrier is configured for capturing an antibody to a Flavivirus antigen. The carrier comprises one or more means for specifically capturing an antibody, preferably one or more, more preferably two or more, more preferably three or more, more preferably four or more such means, each of them capable of specifically capturing a different antibody. Alternatively, one or more carriers, preferably two or more, three or more or four or more, each comprising a means for specifically capturing an antibody, may be used. The means for specifically capturing an antibody may be an antibody binding to all antibodies of a certain Ig class, preferably selected from the group comprising IgG, IgM and IgA, more preferably IgA class antibodies. Said means is preferably immobilized on said carrier. In a preferred example, the means for specifically capturing an antibody is a polypeptide, preferably a dimer and/or hexamer, more preferably a dimer, comprising or consisting of an antigen from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 or a variant thereof, preferably SEQ ID NO1, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8 and SEQ ID NO9 or a variant thereof, and at least one, most preferably one of SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5 or a variant thereof. In another preferred example, a polypeptide comprising SEQ ID NO1, a polypeptide comprising SEQ ID NO6, a polypeptide comprising SEQ ID NO7, a polypeptide comprising SEQ ID NO8, and at least one polypeptide, preferably all polypeptides, from the group comprising a polypeptide comprising SEQ ID NO 2, a polypeptide comprising SEQ ID NO 3, a polypeptide comprising SEQ ID NO 4 and a polypeptide comprising SEQ ID NO 5 or a variant thereof is used. In another preferred example, a polypeptide comprising SEQ ID NO1, a polypeptide comprising SEQ ID NO2, a polypeptide comprising SEQ ID NO3, a polypeptide comprising SEQ ID NO4, a polypeptide comprising SEQ ID NO5 and a polypeptide comprising SEQ ID NO6 or a variant thereof is used. Preferably at least 0.001, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 5, 10 or 100 µg of polypeptide are used for each carrier as a means for specifically capturing an antibody.

In a preferred example, the diagnostically useful carrier comprises one or more means for specifically capturing an antibody to a Flavivirus envelope glycoprotein, preferably a

antigenic polypeptide comprising a sequence selected from the group comprising SEQ ID NO11, SEQ ID NO12, SEQ ID NO13, SEQ ID NO14, SEQ ID NO15, SEQ ID NO16, SEQ ID NO17, SEQ ID NO18 and SEQ ID NO27 and a variant thereof.

The diagnostically useful carrier may comprise one or more means, each for capturing an antibody to a Flavivirus antigen from the group comprising NS1 of a Flavivirus and Envelope glycoprotein of a Flavivirus, preferably comprising two means for capturing two antibodies, one to NS1 and one to envelope glycoprotein antigens from the same Flavivirus, more preferably one to NS1 from Zika virus and one to the envelope glycoprotein from Zika virus (SEQ ID NO1 and SEQ ID NO11, respectively).

Said antigen, together with the insoluble carrier to which it is attached, may be separated from a sample from a subject in a straightforward manner, for example by filtration, centrifugation, magnetism or decanting. Said antigen may be immobilized in a reversible or irreversible manner. For example, the immobilization is reversible if the molecule interacts with the carrier via ionic interactions which may be masked by addition of a high concentration of salt or if the molecule is bound via a cleavable covalent bond or a non-covalent bond. By contrast, the immobilization is irreversible if the molecule is tethered to the carrier via a covalent bond that cannot be cleaved in aqueous solution. The polypeptide may be indirectly immobilized, for example by immobilizing an antibody or other entity having affinity to the polypeptide, followed by addition of the polypeptide and formation of a polypeptide-antibody complex. A non-covalent bond may be made by chemically attaching a ligand to the carrier, preferably via a covalent bond, and fusing to the polypeptide according to the present disclosure a polypeptide having affinity to the ligand. In a preferred example, the ligand is selected from the group comprising biotin, in which case the polypeptide having affinity may be streptavidin or a variant thereof binding to biotin, glutathione (polypeptide having affinity: glutathione-S-transferase), Nickel (polypeptide having affinity: His tag), Flag tag (polypeptide having affinity: anti-flag antibody), carbohydrate such as maltose or cellulose (polypeptide having affinity: maltose or cellulose binding protein), and is preferably biotin.

The polypeptide according to the present disclosure comprising SEQ ID NO1 or a variant thereof or, in addition a polypeptide comprising a sequence selected from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 and a variant thereof, may be immobilized via the diagnostically relevant antibody to be detected, which is immobilized on

the carrier via another antibody directly attached to the carrier. The other antibody may be an Ig class-specific antibody, preferably from the group comprising IgM, IgG and IgA-class specific antibody, more preferably an IgA class specific antibody. The binding site of such a class-specific antibody, which is commercially available, may be the constant region of a human antibody.

The teachings of the present disclosure may not only be carried out using polypeptides, for example SEQ ID NO1, optionally in combination with one or more further antigens such as SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9, having the exact sequences referred to in this application explicitly, for example by function, name, sequence or accession number, or implicitly, but also using variants of such polypeptides.

In a preferred example, the term "variant", as used herein, may refer to at least one fragment of the full length sequence referred to, more specifically one or more amino acid or nucleic acid sequence which is, relative to the full-length sequence, truncated at one or both termini by one or more amino acids. Such a fragment comprises or encodes for a peptide having at least 10, 15, 25, 50, 75, 100, 150, 200, 250 or 300 successive amino acids of the original sequence or a variant thereof. The total length of the variant may be at 25, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids.

In another preferred example, the term "variant" relates not only to at least one fragment, but also a polypeptide or a fragment thereof comprising amino acid sequences, preferably a fragment comprising at least 25, more preferably 50, more preferably 200, more preferably 300 successive amino acids, that are at least 40, 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98 99, 99.4, 99.5, 99.6, 99.7, 99.8 or 99.9 % identical to the reference amino acid sequence referred to or the fragment thereof, wherein amino acids other than those essential for the biological activity, for example the ability to bind specifically to an antibody of interest, or the fold or structure of the polypeptide are deleted or substituted and/or one or more such essential amino acids are replaced in a conservative manner and/or amino acids are added or deleted such that the biological activity of the polypeptide is at least partially preserved. Known methods comprise various methods that may be used to align two given nucleic acid or amino acid sequences and to calculate the degree of identity, see for example Arthur Lesk (2008), Introduction to bioinformatics, Oxford University Press, 2008, 3rd edition. In a preferred example, the ClustalW software (Larkin, M. A., Blackshields, G., Brown, N. P.,

Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. (2007): Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948) is used applying default settings.

In a preferred example, variants may, in addition, comprise chemical modifications, for example isotopic labels or covalent modifications such as glycosylation, phosphorylation, acetylation, decarboxylation, citrullination, hydroxylation and the like. The person skilled in the art is familiar with methods for the modification of polypeptides. Moreover, variants may also be generated by way of fusion with other known polypeptides or variants thereof.

The variant of the polypeptide has biological activity. In a preferred example, such biological activity is the ability to bind to, preferably capture specifically the respective antibody if the variant is a variant of a sequence from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO9, SEQ ID NO11, SEQ ID NO12, SEQ ID NO13, SEQ ID NO14, SEQ ID NO15, SEQ ID NO16, SEQ ID NO 17, SEQ ID NO18, SEQ ID NO19 and SEQ ID NO27, preferably SEQ ID NO1. For example, a variant of SEQ ID NO1 has the ability to bind specifically to an antibody to SEQ ID NO1 in a sample obtained from a subject suspected of suffering from a viral infection. Such variants have at least one epitope recognized by the antibody to be captured, for example one epitope in SEQ ID NO1 if an antibody to SEQ ID NO1 is captured. The person skilled in the art is capable of designing variants by starting from the original SEQ ID NO1 sequence, introducing modifications such as point mutations, truncations and the like and subsequently confirming that the variant still has biological activity by testing whether said variant binds to an antibody to SEQ ID NO1 in a sample obtained from a subject suffering from the disease to be diagnosed, preferably an infection, more preferably a viral infection, more preferably an infection with a Flavivirus, most preferably an infection with a Zika virus. The 3D protein structure of the Zika Virus NS1 and related Flaviviruses have been published and may be used for guidance in the design of variants and choice of the sequences that may be varied without compromising the biological activity and to distinguish them from important epitopes (for example Xu *et al.*, Contribution of intertwined loop to membrane association revealed by Zika virus full-length NS1 structure (EMBO J., published on August 30, 2016, open access; Akey *et al.*, Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system, *Science* 21;343(6173):881-5. doi: 10.1126/science; WO2015/095735). For example, with reference to SEQ ID NO1, regions that are unique to Zika NS1 and should not be substituted, in particular not in a non-conservative manner, include residues 62 to73, preferably those comprising Arg62, Ile66,

Arg 69, Glu72, Glycine73 (which could be substituted with a neutral amino acid); 102 to 110, preferably Gln102, Pro105 (which could be substituted with a neutral amino acid and a short side chain such as Ser or Ala) and Glu110; residues 121 to 129, residues 138 to 141, 174 to 178 and 322 to 326. The biological activity of mammalian, preferably bovine apolipoprotein provided and used according to the present disclosure is the ability to bind to and form a complex with a polypeptide comprising Flavivirus NS1, preferably SEQ ID NO1. Variants may be identified by identifying naturally occurring fragments of such apolipoproteins derived from the full-length protein or a precursor thereof, for example by purifying them using NS1 as an affinity ligand followed by N-terminal Edman sequencing and/or tryptic digest in combination with mass spectrometry, and using them to practice the disclosure. Conservative amino acid substitutions may be used for all variants.

If a polypeptide is used as the means for specifically capturing an antibody, said polypeptide, preferably comprising one or more sequences selected from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO9, SEQ ID NO10, SEQ ID NO11, SEQ ID NO12, SEQ ID NO13, SEQ ID NO14, SEQ ID NO15, SEQ ID NO16, SEQ ID NO17, SEQ ID NO18, SEQ ID NO19 and SEQ ID NO27, preferably SEQ ID NO1, when used to carry out the teachings of the present disclosure, may be provided in any form and at any degree of purification, from tissues or cells comprising said polypeptide in an endogenous form, more preferably cells overexpressing the polypeptide, crude or enriched lysates of such cells, to purified and/or isolated polypeptide which may be essentially pure. In a preferred example, the term "overexpressing", as used herein, means that the cell, preferably a eukaryotic, more preferably a mammalian or insect, more preferably a mammalian, more preferably a human cell, most preferably a HEK293 or HEK293T cell, has been genetically engineered such that it expresses more of the protein of interest than a non-engineered wild type cell would. In a preferred example, the polypeptide is a native polypeptide, wherein the term "native polypeptide", as used herein, refers to a folded polypeptide comprising at least 15, 30, 50, 100, 150, 200, 300 or 350 amino acids, preferably more than 30 amino acids, more preferably to a folded polypeptide purified from tissues or cells, more preferably from mammalian cells or tissues, optionally from non-recombinant tissues or cell. In another preferred example, the polypeptide is a linear peptide having at least 7, more preferably at least 10 amino acid residues. If a native polypeptide is used, it is preferably enriched compared to its natural state. A recombinant polypeptide may comprise a C-terminal or N-terminal tag for affinity purification, immobilization or detection such as a His tag, as exemplified by SEQ ID NO10, or a streptavidin tag, preferably a streptavidin, which tag may preferably be removed by

cleavage using a protease recognizing a protease cleavage site in a polypeptide linker between the tag and the N terminus or C-terminus, respectively, as part of the purification or method. The cleaved polypeptide may subsequently be attached to a diagnostically useful carrier to yield the diagnostically useful carrier according to the present disclosure. In another preferred example, the means for specifically capturing an antibody is a Zika virus-infected eukaryotic, preferably human cell. Such a cell may be evaluated by fluorescence microscopy. The cells may be transiently or stably transfected, preferably transiently transfected.

According to the present disclosure, a nucleic acid encoding the polypeptide according to the present disclosure such as a polypeptide comprising SEQ ID NO1 or a variant thereof, optionally with an inducible promotor, which polypeptide is preferably for use for the diagnosis of a disease or the manufacture of a kit or reagent for such use, is provided. Said nucleic acid may be a vector, preferably for expressing said nucleic acid. A eukaryotic or prokaryotic, preferably eukaryotic cell comprising this vector and preferably expressing the polypeptide encoding by the vector, is also provided. The nucleic acid, the vector and the cell may be used for the manufacture of a kit for use according to the present disclosure such as use of an antibody to NS1, preferably IgA class antibody to NS1 from a Flavivirus, preferably the Zika virus, or a diagnostically useful carrier for immobilizing and optionally detecting said antibody, for distinguishing a primary from a secondary, preferably the Zika virus infection use of an IgA class antibody to NS1 from a Flavivirus, preferably the Zika virus, or a diagnostically useful carrier for immobilizing and optionally detecting said antibody, for diagnosing a flaviviral infection, preferably a Zika virus infection, in an IgM-deficient subject, such as use of an IgA class antibody to NS1 from a Flavivirus, preferably the Zika virus, or a diagnostically useful carrier for immobilizing and optionally detecting said antibody, for increasing the diagnostic reliability, preferably sensitivity, of a diagnostic assay for diagnosing a flaviviral infection, preferably a Zika infection, more preferably at the early stages of an infection or such as for distinguishing a primary from a secondary, preferably the Zika virus infection or such as for increasing the diagnostic reliability, preferably sensitivity, of a diagnostic assay for diagnosing a flaviviral infection, preferably a Zika infection, more preferably at the early stages of an infection. The nucleic acid may be expressed, the polypeptide encoded purified and used, preferably coated on a diagnostically useful carrier, in order to make the diagnostically useful carrier according to the present disclosure. In a preferred example, the term “early stage” refers to the time period before the first 60, preferably first 40 days after symptom onset, wherein more preferably no increase in IgG class antibodies can be observed.

A polypeptide provided or used in a method or as part of a carrier or used in any other way according to the present disclosure may be glycosylated or non-glycosylated, preferably glycosylated. A glycosylated polypeptide comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO8, SEQ ID NO19 or SEQ ID NO9 or a variant thereof may be obtained by purifying the polypeptide from a eukaryotic cell, preferably a HEK293 or HEK293T cell. A homogeneously glycosylated polypeptide may be obtained by purifying the polypeptide from the cytosolic fraction of a eukaryotic cell, a heterogeneously glycosylated polypeptide may be obtained by purifying the polypeptide from the cell culture supernatant medium following culture of a eukaryotic cells expressing the polypeptide. A non-glycosylated polypeptide may be obtained by enzymatic deglycosylation of a polypeptide purified from a eukaryotic cell or by purification of a polypeptide expressed in a prokaryotic cell.

In a preferred example, a polypeptide comprising a sequence from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 or a variant thereof, preferably SEQ ID NO1 or a variant thereof, may be provided or used, as part of a diagnostically useful carrier, method or use according to the present disclosure in various oligomeric forms that comprise one or more than one monomer, for example 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 monomers, and may be used, for example, as a means for capturing an antibody to Flavivirus N1, preferably comprising SEQ ID NO1 or a variant thereof, optionally an IgA class antibody to a Flavivirus NS1, in the form of a monomer, dimer or a hexamer, preferably a dimer. In a preferred example said monomer, dimer or hexamer, preferably dimer, has been purified from a eukaryotic cell, preferably recombinant eukaryotic cell, such as a HEK293T or HEK293 cell, preferably from the cytoplasmic fraction, which contains the dimeric form, or the cell medium in which the cell was grown, which contains the hexameric form secreted into the medium, preferably from the cytosolic fraction. In another preferred example, a mixture of oligomeric forms, preferably purified from a eukaryotic cell, is used, wherein the molar ratio of dimer to hexamer is at least 0.1:1, preferably, 0.5:1, 1:1, 1.5:1, 2:1, 5:1 or 10:1. In another preferred example, a mixture of oligomeric forms, preferably purified from a eukaryotic cell, is used, wherein the molar ratio of hexamer to dimer is at least 0.1:1, preferably, 0.5:1, 1:1, 1.5:1, 2:1, 5:1 or 10:1. In a preferred example, the hexamer is used as a means to capture

an IgG class. In another preferred example, the dimer is used to capture an IgM class antibody.

Alternatively, a prokaryotic cell or chemical synthesis may be used to express or obtain and to purify the polypeptide oligomer, preferably dimer or hexamer, optionally by chemical crosslinking and isolating the oligomer, preferably dimer or hexamer. The person skilled in the art is familiar with techniques for isolating or enriching certain oligomeric forms, for example using size-exclusion chromatography. The interface of the monomers in an oligomeric form, which causes the monomers to associate to the oligomer, is preferably made of SEQ ID NO1 or a variant thereof that is part of sequence of the monomers in the oligomeric form.

An oligomer comprising more than one monomer may be stabilized by a non-covalent or covalent bond, preferably covalent bond between the two or more monomers that form such oligomer. In a preferred example, the oligomer is stabilized by one or more covalent bond via one or more Cystein side chains between the monomers. The covalent bond may be a disulfide bond or comprise a linker comprising two functional groups that are reactive with thiol groups, which linker links two side chain residues following reaction of the two functional groups with two thiol groups. In a more preferred example, this covalent bond is between two cysteine residues side chain that would not normally form a disulfide bond in the native, as mentioned in (Xu *et al.*, (Contribution of intertwined loop to membrane association revealed by Zika virus full-length NS1 structure (EMBO J., published on August 30, 2016, open access), natural state of the protein. In another preferred example, the oligomer is stabilized by non-covalent bonds between monomers, and at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 11 or 12 cystein residues are oxidized, forming intermonomeric disulfide bonds.

In a preferred example, the Flavivirus NS1, preferably SEQ ID NO1 or a variant thereof, is in complex with a lipid, which lipid is preferably derived from a cell membrane, more preferably from a Eukaryotic cell membrane such as a HEK293 cell membrane.

Oligomeric forms such as dimers or hexamers may be used, when practicing the present disclosure, in the form of homogenous or heterogeneous oligomers, wherein homogeneous oligomers comprise two or more different monomers, optionally derived from different flavivirus sequences from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 or a variant thereof, preferably of SEQ ID NO1 or a variant thereof. For

example, a heterogeneous dimer may comprise a monomer comprising SEQ ID NO1 and a monomer comprising SEQ ID NO2. By contrast, heterogeneous oligomers comprise two monomers which are both derived from the same sequence from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 or a variant thereof, preferably of SEQ ID NO1 or a variant thereof, and are optionally identical.

In a preferred example, the antibody to be detected may be an antibody to a monomer, dimer and/or hexamer, preferably to a dimer, of a polypeptide from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8 and SEQ ID NO9 or a variant thereof, preferably SEQ ID1 or a variant thereof. Said antibody or an antibody binding to a polypeptide from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 or a variant thereof, preferably SEQ ID1 or a variant thereof regardless of the oligomeric state, may be provided as an isolated and/or recombinant antibody or antibody fragment.

In another preferred example, the polypeptide comprising a sequence from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 and a variant thereof, preferably SEQ ID1 or a variant thereof, preferably a dimer and/or hexamer, preferably the hexamer, is used in a mixture with a mammalian, preferably non-human polypeptide such as a bovine polypeptide such as mammalian Apolipoprotein A-I, more preferably human (NCBI Reference Sequence: NP_000030.1) or bovine Apolipoprotein A-I (GenBank: AAI02942.1; all data base codes cited in this document refer to the entry in the respective data base at the date of priority), or human (NCBI: NP_000375.2) or bovine Apolipoprotein B-100 isoform X1 (NCBI: XP_015329038.1 or a variant thereof) most preferably bovine Apolipoprotein A-I or a variant thereof. The mixture may comprise a complex of the polypeptide comprising a sequence from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 and a variant thereof, preferably SEQ ID NO1 or a variant thereof, preferably a dimer and/or hexamer, preferably the hexamer, and, as a second component of the complex, a mammalian Apolipoprotein A-I, more preferably human (NCBI Reference Sequence: NP_000030.1) or bovine Apolipoprotein A-I (GenBank: AAI02942.1), most preferably bovine Apolipoprotein A-I or a variant thereof, which complex may be used for practicing the invention, for example for the diagnosis of a

disease, more specifically as a means for capturing an antibody. In the mixture, preferably the complex, the molar ratio between polypeptide monomers and the mammalian Apolipoprotein A-1 may be at least 1:1, 2:1, 5:1, 6:1, 10:1, 50:1 or 100:1.

According to the present disclosure, the polypeptide may be a recombinant protein, wherein the term “recombinant”, as used herein, refers to a polypeptide produced using genetic engineering approaches at any stage of the production process, for example by fusing a nucleic acid encoding the polypeptide to a strong promoter for overexpression in cells or tissues or by engineering the sequence of the polypeptide itself. The person skilled in the art is familiar with methods for engineering nucleic acids and polypeptides encoded (for example, described in Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989), Molecular Cloning, CSH or in Brown T. A. (1986), Gene Cloning – an introduction, Chapman & Hall) and for producing and purifying native or recombinant polypeptides (for example Handbooks „Strategies for Protein Purification“, „Antibody Purification“, published by GE Healthcare Life Sciences, and in Burgess, R. R., Deutscher, M. P. (2009): Guide to Protein Purification). In another preferred example, the polypeptide according to the present disclosure and used for the various examples of the present disclosure is an isolated polypeptide, wherein the term “isolated”, as used herein, means that the polypeptide has been enriched compared to its state upon production using a biotechnological or synthetic approach and is preferably pure, *i.e.* at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 or 99 percent of the polypeptide in the respective sample consists of said polypeptide as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining and visual inspection.

The subject according to the present disclosure is an organism producing antibodies, preferably IgA, IgM and/or IgG class antibodies, more preferably from a mammal, most preferably a human. According to the present disclosure, IgM and IgG class antibodies to SEQ ID NO1 may be detected in separate assay reactions, for example to determine the time when the subject was infected for the first time.

Within the scope of the present disclosure is a diagnostically useful carrier comprising a means for specifically capturing an antibody to an antigen such as SEQ ID NO1. In a preferred example, the term “specifically capturing an antibody”, as used herein, refers to the ability to bind specifically to the antibody of interest, preferably an IgA, IgM or IgG class antibody, to the effect that it is bound and removed from the sample, whereas other antibodies, preferably from the same class and/or to another antigen, are essentially not

bound and remain in the sample. The antibody is preferably an antibody that binds to the antigen of interest only such as the one represented by SEQ ID NO1, but not to other homologous antigens from other viruses such as those represented by SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8 and SEQ ID NO9.

The diagnostically useful carrier according to the disclosure serves as a scaffold for the one or more means for specifically capturing an antibody, preferably a diagnostically relevant antibody to a Flavivirus antigen such as the one represented by SEQ ID NO1. Said carrier is suitable for carrying out a diagnostic method. By using a carrier rather than free, soluble means for specifically capturing an antibody, it is more straightforward to isolate and separate from the sample a complex comprising the means and the antibody and to wash said complex, for example for the purpose of removing any molecules binding non-specifically to the means, complex or carrier. In a preferred example, the diagnostically useful carrier is a diagnostic device, preferably selected from the group comprising a bead, preferably a paramagnetic particle, a test strip, a microtiter plate, a blot and a membrane, and is preferably a line blot or microtiter plate, more preferably a microtiter plate.

In a preferred example, the diagnostically useful device is a microtiter plate comprising a well coated with a means for specifically capturing an antibody to SEQ ID NO1, which means is preferably a polypeptide comprising SEQ ID NO1 or a variant thereof. In addition, said well comprises a means for detecting an antibody to at least one of SEQ ID NO2, SEQ ID NO3, SEQ ID NO4 and SEQ ID NO5, preferably all of them, preferably a polypeptide comprising at least one of SEQ ID NO2, SEQ ID NO3, SEQ ID NO4 and SEQ ID NO5 or a variant thereof. In addition, said well comprises means for specifically capturing an antibody to each of SEQ ID NO6, SEQ ID NO 7, SEQ ID NO8 and SEQ ID NO9, preferably a polypeptide comprising SEQ ID NO6 or a variant thereof, SEQ ID NO 7 or a variant thereof, SEQ ID NO 8 or a variant thereof and SEQ ID NO9 or a variant thereof. In addition, a separate well may include one or more antigens for detecting a chikungunya virus infection.

In a preferred example, the term “specifically detecting a captured antibody”, as used herein, means that the antibody binding specifically to the means for specifically capturing the antibody, preferably a polypeptide comprising SEQ ID NO1 or a variant thereof, following capture, is detected rather than any other antibody present in the sample. In a preferred

example, the term “binding specifically”, as used herein, means that the binding is stronger than a binding reaction characterized by a dissociation constant of 1×10^{-5} M, more preferably 1×10^{-7} M, more preferably 1×10^{-8} M, more preferably 1×10^{-9} M, more preferably 1×10^{-10} M, more preferably 1×10^{-11} M, more preferably 1×10^{-12} M, as determined by surface plasmon resonance using Biacore equipment at 25 °C in PBS buffer at pH 7.

In a preferred example, the means for specifically capturing an antibody to SEQ ID NO 1 and the means for specifically capturing an antibody to one or more further antigens, preferably selected from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9, are on separate carriers. This means that the means are not attached to a single carrier, but one or more carriers that are separate and/or separable without damaging them. For example, the means for specifically capturing an antibody to SEQ ID NO 1 may be attached to a first test strip, and the means for specifically capturing an antibody to SEQ ID NO 2 is attached to another test strip which is separate from the first test strip.

In a preferred example, the means for specifically capturing an antibody to SEQ ID NO 1 and the means for specifically capturing an antibody to one or more further antigens, preferably selected from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9, are on one, preferably covalently linked to one carrier. This means that the means are attached to one carrier which may not be disassembled, without damaging the carrier, such that the means are on separate carriers. For example, the means may be all coated on one test strip, particular in the form of a line blot.

According to the present disclosure, a means for specifically detecting a captured antibody is provided, optionally as part of a kit.

The inventive teachings provide a kit, preferably for diagnosing an infection, more preferably for diagnosing a flavivirus infection, most preferably a Zika virus infection. Such a kit is a container that comprises specific reagents required to practice the inventive method, in particular the diagnostically useful carrier according to the present disclosure, optionally in addition to one or more solutions required to practice the inventive method, preferably selected from or all from the group comprising sample dilution buffer, washing buffer and buffer comprising a means for detecting any specifically captured antibody, such as a

secondary antibody and optionally a means for detecting the specifically captured antibody, which may optionally be attached to the secondary antibody, for example a fluorescent, enzymatically active, radioactive, chemiluminescent, preferably electrochemiluminescent label or a spin label. The kit may comprise a chemical solution for carrying out a detection reaction such as 3,3',5,5'-tetramethylbenzidine, *p*-Nitrophenyl Phosphate, 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid or *o*-phenylenediamine dihydrochloride for a colorimetric reaction tripropylamin for an electrochemiluminescence reaction. Furthermore, it may comprise instructions detailing how to use the kit and the inventive diagnostically useful carrier for contacting the inventive polypeptide with a bodily fluid sample from a subject, preferably a human subject, for example a line blot, wherein the inventive means for specifically capturing SEQ ID NO1, preferably a polypeptide comprising SEQ ID NO1 or a variant thereof, is immobilized on the line blot. Furthermore, the kit may comprise a positive control, for example a recombinant antibody known to bind to SEQ ID NO1, and a negative control, for example a protein having no detectable affinity to SEQ ID NO1. Finally, the kit may comprise a standard solution comprising a SEQ ID NO1-binding antibody for preparing a calibration curve. In a preferred example, the kit comprises a device, preferably a blot-based device such as a line blot coated with a means for specifically capturing an antibody to SEQ ID NO1 and, optionally, an antibody to one or more further antigens such as SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and/or SEQ ID NO9. The kit may comprise one or more further control selected from a control confirming that sample has been added and/or a control confirming that a secondary antibody has been added.

According to the disclosure, a means for detecting the one or more captured antibodies may be used. The person skilled in the art is aware of many methods that may be used, which are also described in the state of the art, for example in Zane, H. D. (2001), Immunology – Theoretical & Practical Concepts in Laboratory Medicine, W. B. Saunders Company, in particular in Chapter 14. In a preferred example, a secondary antibody binding to the constant region of the one or more captured antibodies, which is the corresponding primary antibody, is used, which secondary antibody may be associated with a label that is straightforward to detect. Alternatively, an antigenic polypeptide, preferably from the group of polypeptides comprising a sequence from the group comprising SEQ ID NO1, SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and SEQ ID NO9 and a variant thereof, preferably SEQ ID NO1, or a variant thereof may be used to detect the diagnostically relevant antibody, preferably following its immobilization, wherein said polypeptide preferably comprises a label that is straightforward

to detect. Such antigenic polypeptide may bind to any immobilized diagnostically useful antibody to allow the specific detection. The label that is straightforward to detect may be selected from the group comprising label that is straightforward to detect, for example a fluorescent, chemiluminescent such as electrochemiluminescent, radioactive label, spin label or enzymatically active label, the latter of which may catalyze a chemiluminescent reaction, or it may bring about the generation of a molecule detectable or a signal such as a photon using colorimetry, fluorescence detection such as fluorescence microscopy, photomultiplication or spectroscopy or another analytical method.

In a preferred example, the term „diagnosis“, as used herein, refers to any kind of procedure aiming to obtain information instrumental in the assessment whether a patient suffers or is likely or more likely than the average or a comparative subject, the latter preferably having similar symptoms, to suffer from a certain disease or disorder in the past, at the time of the diagnosis or in the future, to find out how the disease is progressing or is likely to progress in the future or to evaluate the responsiveness of a patient with regard to a certain treatment, for example the administration of suitable drugs such as drugs for the desensitization of allergic patients. In other words, the term “diagnosis” comprises not only diagnosing, but also prognosticating and/or monitoring the course of a disease or disorder.

Therefore, the term “diagnosis” does preferably not imply that the diagnostic methods or agents according to the present disclosure will be definitive and sufficient to finalize the diagnosis on the basis of a single test, let alone parameter, but may refer to a contribution to what is referred to as a “differential diagnosis”, *i.e.* a systematic diagnostic procedure considering the likelihood of a range of possible conditions on the basis of a range of diagnostic parameters. The term “diagnosis” may also refer to a method or agent used to choose the most promising treatment regime for a patient. In other words, the method or agent may relate to selecting a treatment regimen for a subject.

The present disclosure relates to a method comprising the step detecting in a sample from a subject the presence or absence of an antibody to an antigenic polypeptide such as a polypeptide comprising a SEQ ID NO1 or a variant thereof. This method preferably comprises immobilizing said antibody followed by specific detection of said antibody, for example by way of the steps a) providing a sample from a subject, b) contacting the sample with the diagnostically useful carrier according to the present disclosure under conditions compatible with the formation of a complex comprising the diagnostically useful carrier and

the antibody, more specifically the means for specifically capturing the antibody and the antibody, c) isolating any said complex, for example by removing the sample, d) optionally washing said complex, and e) optionally detecting said complex. The method is preferably an *in vitro* method. The detection of the complex for the prognosis, diagnosis, methods or test kit according to the present disclosure comprises the use of a method selected from the group comprising immunodiffusion techniques, immunoelectrophoretic techniques, light scattering immunoassays, agglutination techniques, labeled immunoassays such as those from the group comprising radiolabeled immunoassay, enzyme immunoassays such as colourimetric assays, chemiluminescence, preferably electrochemiluminescence, immunoassays and immunofluorescence techniques. The person skilled in the art is familiar with these methods, which are also described in the state of the art, for example in Zane, H. D. (2001): Immunology – Theoretical & Practical Concepts in Laboratory Medicine, W. B. Saunders Company, in particular in Chapter 14. The method may further involve testing the avidity of antibodies to SEQ ID NO1 in the sample, preferably of antibodies to SEQ ID NO1.

A product obtained when practicing the inventive method is a diagnostically useful carrier comprising a means for specifically detecting an antibody to SEQ ID NO1 in complex with the antibody to SEQ ID NO1 and optionally a means for specifically detecting the antibody to SEQ ID NO1 such as a secondary antibody. If the antibody to SEQ ID NO1 is an IgM class antibody, the secondary antibody is a labeled antibody binding to a constant region of IgM class antibodies. If the antibody to SEQ ID NO1 is an IgG class antibody, the secondary antibody is a labeled antibody binding to a constant region of IgG class antibodies. If the antibody to SEQ ID NO1 is an IgA class antibody, the secondary antibody is a labeled antibody binding to a constant region of IgA class antibodies. The diagnostically useful carrier may be a microtiter plate with one or more than one wells, one well comprising a means for specifically capturing an antibody to SEQ ID NO1, and at least one or more, two or more, three or more, for or more wells each comprising a means for specifically capturing an antibody to a sequence from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and/or SEQ ID NO9.

In many cases, detecting the absence or presence of an antibody, optionally meaning determining whether the concentration of the antibody is beyond a certain threshold, often suggested by the detection limit, in the sample, is sufficient for the diagnosis. If the antibody can be detected, this could be information used for clinician's diagnosis and indicates an increased likelihood that the patient suffers from a disease. In a preferred example, the relative concentration of the antibody in serum, compared to the level that may be found in

an average healthy subject, may be determined. In a preferred example, the term “detecting the presence or absence”, as used herein, means that it is sufficient to check whether a signal sufficiently beyond any background level may be detected using a suitable complex detection method that indicates that the antibody of interest is present or more antibody of interest is present than would be in a healthy subject. In a more preferred example, this may involve determining whether the concentration is at least 0.1, preferably 0.2, 0.5, 1, 2, 5, 10, 20, 25, 50, 100, 200, 500, 1000, 10000 or 100000 times higher than the concentration of the antibody of interest found in the average healthy subject.

The disease to be diagnosed is an infection, preferably a viral, more preferably Flavivirus, most preferably Zika virus infection. Preferably, a Zika virus infection may be distinguished from another Flavivirus infection, more preferably from an infection with a Flavivirus selected from the group comprising dengue virus, Yellow fever virus, Tick-borne encephalitis virus, Usutu virus, West Nile virus and Japanese encephalitis virus or all of them, preferably from the dengue virus.

The invention may be used to provide a prognosis whether a pregnant woman's newborn child is likely to suffer from a deformity if the sample from the pregnant woman is tested. Preferably, the pregnant woman may have symptoms suggesting that she may suffer from a flaviviral infection or may very actually suffer from an infection.

The invention may be used to diagnose whether deformities in a child, such as microcephaly, are a result of a previous Zika virus infection or not.

The invention may be used to diagnose whether a subject is suffering or likely to suffer, following onset of the infection, from an autoimmune disease such as the Guillan Barré syndrome. More specifically, if antibodies to SEQ ID NO1 are detected in a sample from a subject, said subject is more likely to suffer from an autoimmune disease than a subject having no antibodies to SEQ ID NO1.

The invention may be used to test samples comprising autoimmune antibodies such as ANA autoantibodies which may obscure results obtained using conventional assays and so may be used in combination with a method comprising the step detecting in a sample from a subject ANA autoantibodies. Commercially available kits may be used for detecting ANAs for example EUROPLUS ANA Mosaic 20A, ANA screen 11, ANA Profile 3 or Anti-ENA

ProfilePlus. This may allow distinguishing an autoimmune disease from a viral infection or an antibody relating to an autoimmune disease and those relating to a viral infection.

The invention may be used to distinguish Flavivirus infections from other viral infections, preferably alphavirus infections, more preferably a chikungunya virus infection.

The invention may be used to screen blood given by blood donors for previous infections.

In a preferred example, the absence or presence of one or more antibodies, such as an antibody to SEQ ID NO1, is detected simultaneously, *i.e.* at the same time. This is convenient in terms of efficient diagnostic procedures, as a maximum of diagnostic information is obtained in a given period of time. Of course, a prerequisite is that sufficient capacity is available for running all reactions.

In a preferred example, the absence or presence of at least two antibodies, such as an antibody to SEQ ID NO1 and one and more antibodies to an antigen from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4, SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO9, SEQ ID NO10, SEQ ID NO11, SEQ ID NO12, SEQ ID NO13, SEQ ID NO14, SEQ ID NO15, SEQ ID NO16, SEQ ID NO17, SEQ ID NO18 and SEQ ID NO27, is detected in spatially separate reactions. This means that these reactions run in different reaction mixtures in separate vessels, for example separate wells of a microtiter plate or separate compartments each comprising a different bead or the same compartment used subsequently with more than one bead.

If more than one antibody is to be detected, the method may, in another preferred example, be carried out in a one-pot reaction. Preferably, the term “one-pot reaction”, as used herein, means that two or more, preferably all reactions carried out for the purpose of detecting the presence or absence of an antibody are carried out in the same reaction mixture in one reaction vessel, without physical barriers between the reactions, by contrast to experimental settings contemplating that at least two reactions are carried out in separate solutions and reaction vessels.

The disclosure provides a pharmaceutical composition or a vaccine, which composition or immunogenic composition such as a vaccine comprises a polypeptide comprising SEQ ID NO1 or a variant thereof, optionally in combination with one or more further antigens such as one or more selected from the group comprising SEQ ID NO2, SEQ ID NO3, SEQ ID NO4,

SEQ ID NO5, SEQ ID NO6, SEQ ID NO7, SEQ ID NO8, SEQ ID NO19 and/or SEQ ID NO9, preferably in addition to previously used antigens such as an Zika virus Envelope glycoprotein, preferably comprising the sequence AHL16749.1 (UNIPROT data base as online on priority date) or a variant thereof or SEQ ID NO 11, preferably SEQ ID NO 11 or a variant thereof, and/or a Zika virus. Moreover, the composition or immunogenic composition may comprise an antigen comprising a sequence selected from the group comprising SEQ ID NO12, SEQ ID NO13, SEQ ID NO14, SEQ ID NO15, SEQ ID NO16, SEQ ID NO17, SEQ ID NO18, SEQ ID NO19 and SEQ ID NO27 and a variant thereof or a variant thereof. An immunogenic composition or vaccine may comprise components to inactivate a virus or bacteria and stabilize the vaccine, helping to preserve the vaccine and prevent it from losing its potency over time. Adjuvants are added to vaccines to simulate the production of antibodies against the vaccine to make it more effective. An adjuvant could be organic or inorganic. The most common inorganic adjuvants for human vaccines include aluminum phosphate and aluminum hydroxide. Organic adjuvants could be based on the organic compound squalene and an oil [squalene] in water adjuvant can be used. An immunogenic composition may comprise stabilizers that help the vaccine to maintain its effectiveness during storage, e.g., $MgCl_2$, $MgSO_4$, lactose-sorbitol, or sorbitol-gelatin, and preservatives to prevent bacterial and fungal growth, e.g., thiomersal, formaldehyde, or phenol derivatives, antibiotics. The composition is preferably suitable for administration to a subject, preferably a mammalian subject, more preferably to a human. Such a pharmaceutical composition may comprise a pharmaceutically acceptable carrier. The pharmaceutical composition may, for example, be administered orally, parenterally, by inhalation spray, topically, by eyedrops, rectally, nasally, buccally, vaginally or via an implanted reservoir, wherein the term "parentally", as used herein, comprises subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, instrasternal, intrathecal, intralesional and intracranial injection or infusion techniques. The pharmaceutical composition may be provided in suitable dosage forms, for example capsules, tablets and aqueous suspensions and solutions, preferably in sterile form. It may be used in a method of treatment of a disease, which method comprises administering an effective amount of the inventive polypeptide to a subject.

The inventive method for diagnosing a Flavivirus infection, preferably for distinguishing a primary from a secondary Flavivirus infection, may comprise the step detecting in a first sample from a subject an IgA class antibody to NS1 of a Flavivirus, optionally further comprising detecting in said first sample an IgM and/or IgG class, preferably IgM class

antibody to NS1 of a Flavivirus, more preferably SEQ ID NO1. In a preferred example, the method further comprises the step detecting in a second sample obtained from said subject an IgA class antibody to NS1 of said Flavivirus, optionally further comprising detecting in said second sample an IgM and/or IgG class, preferably IgM class antibody to NS1 of a Flavivirus such as SEQ ID NO1, optionally further comprising detecting in said first sample an IgM and/or IgG class, preferably IgM class antibody to NS1 of a Flavivirus, more preferably SEQ ID NO1.

A dynamic titer of IgA or IgM, preferably IgA class antibodies to NS1 of a Flavivirus, preferably to SEQ ID NO1, increasing and decreasing significantly relatively to the background before emergence of IgG class antibodies (i.e. seroconversion), may indicate an acute Zika infection, which is a primary Flavivirus infection. By contrast, a parallel increase of IgA and IgG (albeit the latter at higher levels) may indicate an acute Zika infection, which is a secondary Flavivirus infection. In a preferred example, the first sample is obtained at least 3, 4, 5, 6 days, 1, 2 3 or 4 weeks following the subject's exposure or suspected exposure to a Flavivirus. In a preferred example, the first sample is taken in the two weeks after onset of symptoms. The presence or absence of antibodies may be determined as well as their relative levels over time. The second sample may be obtained at least 3, 4, 5, 6 days, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28 or 32 weeks later than the first sample, preferably at least 3 days, more preferably at least 7 days. A total number of at least 2, 3, 4, 5 or six samples may be taken, preferably at least 2 samples, optionally each sample at least 1 day, 3 days, one week, preferably one week after the previous sample. The total concentrations of IgG, IgM and/or IgA, preferably IgM, may be determined in addition, for example to rule out insufficiencies. This way, the titers of the respective antibodies may be monitored over time.

In a preferred example, the titer of IgM, IgG and/or IgA, preferably IgA and IgG, more preferably IgA to NS1, preferably to SEQ ID NO1, is monitored by detecting the presence or absence or, preferably relative level over time for a period of at least 3, 4, 5, 6, 10, 14, 21, 28, 35 or 42 days, preferably at least 6 days, with the first sample being taken at least five days, preferably at least 7 days following onset of the symptoms. Seroconversion may be detected by monitoring the presence or absence or relative level over time of IgG class antibodies to NS1, preferably SEQ ID NO1. This may help identify the time window in which the increase and decrease of IgM and/or IgA class antibodies would be expected or concluding that this time window has passed.

The inventive method, kit and carriers may be used to distinguish between a primary and a secondary Flavivirus infection. In a preferred example, the term “primary infection”, as used herein, refers to an infection of a person who has never had an infection with said Flavivirus or another Flavivirus, preferably said Flavivirus, more preferably the Zika virus, by contrast to a secondary infection in a patient who has been exposed to a virus or immunogenic compositions derived thereof before. In a preferred example, this may involve distinguishing a primary Zika virus infection from a secondary infection with another Flavivirus, preferably selected from the group comprising dengue virus, Yellow fever virus, Tick-borne encephalitis virus, Usutu virus, West Nile virus and Japanese encephalitis virus, preferably dengue virus.

Claims

1. A kit comprising a diagnostically useful carrier, wherein the kit further comprises a means for specifically detecting a captured antibody, wherein the diagnostically useful carrier is a carrier comprising a means for specifically capturing an antibody to SEQ ID NO1 in a sample from a subject, and wherein the means for specifically detecting a captured antibody is a secondary antibody binding to the constant region of IgA class antibodies.
2. A kit comprising a diagnostically useful carrier and a polypeptide, wherein the diagnostically useful carrier comprises a means for specifically capturing all IgA class antibodies, which is an antibody binding to all IgA class antibodies, wherein the polypeptide is a recombinant polypeptide comprising SEQ ID NO1 or a variant thereof.
3. A method comprising the step detecting in a sample from a subject, the presence or absence of an antibody to SEQ ID NO1, wherein the antibody is an IgA class antibody.
4. The method according to claim 3, wherein the sample is a blood or CSF sample.
5. The method according to claim 3 or 4, further comprising the step detecting the presence or absence of an antibody to SEQ ID NO1, wherein the antibody is an IgM and/or IgG class antibody.
6. The method according to any of claims 3 to 5, comprising the step contacting a diagnostically useful carrier with a sample from the subject.
7. The kit or method according to any of claims 1 to 6, wherein the carrier is selected from the group comprising a bead; a test strip, a microtiter plate, a blot; lateral flow test, a glass surface, a slide, a biochip and a membrane.

8. The method according to any of claims 3 to 7, wherein the presence or absence of an antibody to SEQ ID NO1 and the presence or absence of an antibody to one or more further antigens is detected in spatially separate binding reactions.
- 5 9. The method according to any of claims 3 to 8, comprising the step monitoring an IgA, IgM and/or IgG, for a time period of at least three days.
- 10 10. The method according to any of claims 3 to 9, wherein the method further comprises detecting the total concentration of IgM to SEQ ID NO1.
- 10 11. The kit or method according to any of claims 1 to 10, wherein the antibody is a mammalian antibody.
12. The kit according to any of claims 1, 2, and 11, wherein the kit comprises a secondary antibody binding to the constant region of IgG class antibodies.
- 15 13. The kit according to any of claims 1, 2, and 11 to 12, wherein the kit comprises a secondary antibody binding to the constant region of IgM class antibodies.
- 20 14. The kit according to any of claims 1, 2, and 11 to 13, further comprising one or more from the group comprising a sample dilution buffer, a washing buffer and a buffer comprising a means for detecting any specifically captured antibody, a chemical solution for carrying out a detection reaction, a positive control a negative control and a standard solution comprising a SEQ ID NO1-binding antibody for preparing a calibration curve.
- 25 15. An *in vitro* use of a polypeptide comprising SEQ ID NO1 or a variant thereof, a diagnostically useful carrier comprising said polypeptide or a kit according to any of claims 1, 2, and 11 to 14 or an IgA class antibody to SEQ ID NO1 for the diagnosis of a Zika virus infection.
- 30 16. An *in vitro* use of a polypeptide comprising SEQ ID NO1 or a variant thereof, a diagnostically useful carrier comprising said polypeptide or a kit according to any of claims 1, 2, and 11 to 14 or an IgA class antibody to SEQ ID NO1 for distinguishing a primary from a secondary Zika virus infection, wherein the use comprises detecting an IgA or IgM class antibody to SEQ ID NO1, and an IgG class antibody to SEQ ID NO1.
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17. An *in vitro* use of a polypeptide comprising SEQ ID NO1 or a variant thereof, a diagnostically useful carrier comprising said polypeptide or a kit according to any of claims 1, 2, and 11 to 14 or an IgA class antibody to SEQ ID NO1 for increasing the reliability of the diagnosis.
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18. The kit, method or use according to any of claims 1 to 17, wherein the antibody is detected using a method selected from the group comprising immunodiffusion techniques, immunoelectrophoretic techniques, light scattering immunoassays, agglutination techniques, labeled immunoassays such as those from the group
- 10 comprising radiolabeled immunoassay, enzyme immunoassays such as colourimetric assays, chemiluminescence, immunoassays and immunofluorescence techniques.
19. The kit or use according to any of claims 2, 7 to 18, wherein the variant of SEQ ID NO1 has the ability to bind specifically to an antibody to SEQ ID NO1.

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