

ANTI-DENGUE VIRUS ANTIBODIES AND APPLICATIONS THEREOF

TECHNOLOGY FIELD

5 [0001] The present invention relates to anti-virus dengue virus antibodies and applications thereof. Specifically, the anti-virus dengue virus antibodies of the present invention are serotype specific and useful for specific detection and differentiation of various dengue virus serotypes in a biological sample. The present invention also provide methods and kits for detecting dengue virus and methods and
10 compositions for use in diagnosis and treatment of dengue virus disease using the anti-virus dengue virus antibodies as described herein.

BACKGROUND OF THE INVENTION

[0002] Today about 3.9 billion of the world's population live in areas where there is
15 a risk of dengue transmission. An estimated 100 million dengue infection occur yearly, including 250,000-500,000 severe illness cases and around 25,000 deaths. Dengue is a mosquito-borne viral disease that is caused by transmission of any one of the four dengue virus serotypes (DENV1, DENV2, DENV3, and DENV4). Globally, dengue fever is predominantly spread by two major mosquito vectors: *Aedes aegypti*
20 (urban-adapted) and *Aedes albopictus*. The rapid spread of dengue is attributed to urbanization in tropical and subtropical countries, increasing travel within and between endemic countries, and ineffective vector control strategies. Indeed, the disease and economic burden of dengue fever have become a global public health problem.

25 [0003] As so far, no effective antiviral agents are currently available to treat dengue infection and vaccine still in the phase of evaluation. Furthermore, infection with one serotype of dengue virus confers lifelong immunity to the same serotype but increases the risk of developing severe dengue when infected with another serotype. Therefore, the diagnosis of DENV in early stage is very important for disease
30 management. The early diagnosis of dengue virus infection would provide intervention to treat patients and control the epidemics. People infected with the dengue virus exhibit a wide range of clinical presentations, ranging from asymptomatic infections to severe dengue fever, making accurate diagnoses difficult. Current laboratory diagnostic tests for dengue include isolating the virus, viral RNA

detection, antigen detection, and serological methods. Virus isolation and viral RNA detection are effective during first five (5) days of illness; both methods can identify the serotype of dengue virus. However, virus isolation is time consuming and viral RNA detection requires special equipment and training personnel. Anti-DENV IgM or IgG antibodies merely appear in blood until 5 days post illness onset and may cross react with other flaviviruses; therefore detection of anti-DENV IgM or IgG is not useful in confirming the presence of the virus during the first 5 days of symptoms and false-positive results could occur frequently.

[0004] During the acute phase of the disease (i.e. between days 1 and 7 that follow the onset of illness), dengue virus nonstructural protein 1 (NS1), a 50 kDa glycoprotein, is secreted and accumulate to high concentrations in the blood sera of patients. The presence of NS1 in human sera can be confirmed between 1 and 9 days after infection. In addition, NS1 can be detected when viral RNA is not detectable by RT-PCR and before IgM antibodies appear. NS1 proteins are good target to be an early diagnostic marker. The NS1 antigen detection assays could be performed with simple, low cost, handle large of samples and rapid methods in early diagnosis of DENV. However, although several commercial NS1 detection tests are available to diagnose dengue during the early stages of disease, none of them can differentiate among various dengue serotypes.

[0005] There remains a need to develop a diagnostic approach that can detect and differentiate among various dengue serotypes.

SUMMARY OF THE INVENTION

[0006] The present invention is based on the identification of a number of isolated antibodies, which unexpectedly exhibit superior specificity for respective dengue virus serotypes. Each of the serotype specific antibody of the present invention is capable of detecting one specific serotype of dengue virus without substantially cross reacting with other serotypes. The present invention provides such antibodies and antigen-binding fragment thereof and also compositions and kits comprising the same and methods using the same. The present invention is useful for specific detection of one or more serotypes of dengue virus in a sample, particularly a sample from a patient suspected to be infected by the virus. The present invention also provides a cross reactive antibody specific to dengue virus (all serotypes), which can be paired with any of the serotype specific antibody as described herein for use in an

immunoassay for detecting dengue virus in a sample.

[0007] In one aspect, the present invention provides an isolated antibody or antigen-binding fragment thereof, wherein the isolated antibody is selected from the group consisting of:

- 5 (i) a first antibody specific for dengue virus serotype 1 (DENV1) comprising
 - (a) a heavy chain variable region (V_H) which comprises a heavy chain complementary determining region (HC CDR1) of SEQ ID NO: 2, a heavy chain complementary determining region 2 (HC CDR2) of SEQ ID NO: 4, and a heavy chain complementary determining region 3 (HC CDR3) of SEQ ID NO: 6; and
 - 10 (b) a light chain variable region (V_L) which comprises a light chain complementary determining region 1 (LC CDR1) of SEQ ID NO: 9, a light chain complementary determining region (LC CDR2) of SEQ ID NO: 11, and a light chain complementary determining region 3 (LC CDR3) of SEQ ID NO: 13;
- (ii) a second antibody specific for dengue virus serotype 2 (DENV2) comprising
 - 15 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 16, a HC CDR2 of SEQ ID NO: 18, and a HC CDR3 of SEQ ID NO: 20; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 23, a LC CDR2 of SEQ ID NO: 25, and a LC CDR3 of SEQ ID NO: 27;
- (iii) a third antibody specific for dengue virus serotype 3 (DENV3) comprising
 - 20 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 30, a HC CDR2 of SEQ ID NO: 32, and a HC CDR3 of SEQ ID NO: 34; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 37, a LC CDR2 of SEQ ID NO: 39, and a LC CDR3 of SEQ ID NO: 41;
- (iv) a fourth antibody specific for dengue virus serotype 4 (DENV4) comprising
 - 25 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 44, a HC CDR2 of SEQ ID NO: 46, and a HC CDR3 of SEQ ID NO: 48; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 51, a LC CDR2 of SEQ ID NO: 53, and a LC CDR3 of SEQ ID NO: 55;
- (v) a fifth antibody cross-reactive to DENV1, DENV2, DENV3 and DENV4
 - 30 comprising
 - (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 58, a HC CDR2 of SEQ ID NO: 60, and a HC CDR3 of SEQ ID NO: 62; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 65, a LC CDR2 of SEQ ID NO: 67, and a LC CDR3 of SEQ ID NO: 69; and

(vi) any combination of (i) to (v).

[0008] In some embodiments, the first antibody comprises a V_H comprising SEQ ID NO: 71 and a V_L comprising SEQ ID NO: 72.

[0009] In some embodiments, the second antibody comprises a V_H comprising
5 SEQ ID NO: 73 and a V_L comprising SEQ ID NO: 74.

[0010] In some embodiments, the third antibody comprises a V_H comprising SEQ ID NO: 75 and a V_L comprising SEQ ID NO: 76.

[0011] In some embodiments, the fourth antibody comprises a V_H comprising SEQ ID NO: 77 and a V_L comprising SEQ ID NO: 78.

10 [0012] In some embodiments, the fifth antibody comprises a V_H comprising SEQ ID NO: 79 and a V_L comprising SEQ ID NO: 80.

[0013] The dengue virus specific antibodies of the present invention can be a full-length antibody. The antigen-binding fragment of the antibodies of the present invention can be scFv, (scFv)₂, Fab, Fab', F(ab')₂.

15 [0014] In another aspect, the present invention provides a nucleic acid comprising a nucleotide sequence encoding an antibody heavy chain variable region (V_H) or an antibody light chain variable region (V_L) or both, wherein the V_H and V_L are as described herein.

[0015] The present invention also provides a vector (e.g. an expression vector)
20 comprising any of the nucleic acids described herein and a host cell comprising such a vector.

[0016] The present invention further provides a method for preparing an antibody specific for dengue virus, comprising (i) culturing the host cell as described herein under conditions allowing for expression of the antibody, and optionally (ii)
25 harvesting the antibody from the cell culture.

[0017] In a further aspect, the present invention provides a composition comprising (a) any of the antibody specific for dengue virus as described herein, any of the nucleic acids as described herein, or any of the vectors as described herein; and (b) a carrier e.g. a pharmaceutically acceptable carrier.

30 [0018] In some embodiments, the composition of the present invention is a pharmaceutical composition for use in treatment of dengue virus disease.

[0019] In some embodiments, the composition of the present invention is a diagnostic composition for use in diagnosis of dengue virus disease.

[0020] In still another aspect, the present invention provides a method for detecting

dengue virus in a sample suspected of containing said dengue virus, comprising contacting the sample with an isolated antibody or antigen-binding fragment thereof specific for dengue virus, as described herein, and assaying binding of the antibody with the sample. The binding results are to determine the presence of respective
5 dengue virus serotypes in the sample. Specifically, (i) binding of the first antibody with the sample is an indicative of the presence of DENV1 in the sample; (ii) binding of the second antibody with the sample is an indicative of the presence of DENV2 in the sample; (iii) binding of the third antibody with the sample is an indicative of the presence of DENV3 in the sample; and/or (iv) binding of the fourth antibody with the
10 sample is an indicative of the presence of ENV4 in the sample. In some embodiments, the respective serotype specific antibody of the present invention is paired with a partner antibody to perform a sandwich assay.

[00021] In further another aspect, the present invention provides a kit for detecting the presence of one or more serotypes of dengue virus in a sample, comprising one or
15 more serotype-specific antibodies to dengue virus as described herein and an optional partner antibody. The partner antibody can be a serotype cross-reactive antibody to dengue virus, for example, the fifth antibody as described herein.

[00022] In some embodiments, at least one of the antibodies in the kit comprises a detectable label.

20 **[00023]** Examples of the detectable label include but are not limited to an enzymatic label, a fluorescent label, a metal label and a radio label.

[00024] In some embodiments, the kit is an immunoassay kit.

[00025] Examples of the immunoassay include but are not limited to ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), FIA (fluorescence
25 immunoassay), LIA (luminescence immunoassay), or ILMA immunoluminometric assay.

[00026] In certain embodiments, the immunoassay is in a lateral flow assay format.

[00027] In particular, the immunoassay is a sandwich assay.

[00028] The details of one or more embodiments of the invention are set forth in the
30 description below. Other features or advantages of the present invention will be apparent from the following detailed description of several embodiments, and also from the appending claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[00029] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood,
5 however, that the invention is not limited to the precise arrangements and instrumentalities shown.

[00030] In the drawings:

[00031] Fig. 1A shows the basic design of the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention as capture antibodies, paired with a
10 serotype cross-reactive antibody to dengue virus as detection antibodies.

[00032] Fig. 1B shows the results of detection in the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention, 12-4.1, 33-7.1, 43-1.3, and 22-1.5, as capture antibodies, paired with a serotype cross-reactive antibody to dengue virus, as detection antibodies. In this assay, cell culture supernatants from Vero cells
15 infected with DENV1, DENV2, DENV3, DENV4, or JEV were used as sources of NS1 antigens, and supernatant from non-infected Vero cells was used a negative control.

[00033] Fig. 2A shows the basic design of the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention as detection antibodies, paired with a
20 serotype cross-reactive antibody to dengue virus as capture antibodies.

[00034] Fig. 2B shows the detection results of the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention, 12-4.1, 33-7.1, 43-1.3, and 22-1.5, as detection antibodies, paired with a serotype cross-reactive antibody to dengue virus, as capture antibodies. In this assay, cell culture supernatants from
25 Vero cells infected with DENV1, DENV2, DENV3, DENV4, or JEV were used as sources of NS1 antigens, and supernatant from non-infected Vero cells was used a negative control.

[00035] Fig. 3 shows the sensitivities of the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention, 12-4.1, 33-7.1, 43-1.3, and 22-1.5, as
30 detection antibodies, paired with a serotype cross-reactive antibody to dengue virus, as capture antibodies. Capture ELISA was performed with serial diluted and purified NS1 proteins of each DENV serotype to determine the detection limits of the serotype-specific mAbs of the present invention. Bovine serum albumin (BSA) was used to establish the baseline. Each data point represents the mean $\pm$ SD of three

replicate tests.

[00036] Fig. 4 shows the basic design of the sandwich strip using the serotype-specific mAbs of the present invention as capture antibodies (upper panel) or detection antibodies (lower panel), paired with a serotype cross-reactive antibody to dengue virus (mAb82-1.1).

[00037] Fig. 5 shows the specificities of the sandwich strip of the present invention. Serotype-specific mAbs 12-4.1, 33-7.1, 43-1.3, and 22-1.5, paired with a serotype cross-reactive antibody to dengue virus (mAb82-1.1), were used as capture and detection antibodies and then tested for binding specificity. In this assay, cell culture supernatants from Vero cells infected with DENV1, DENV2, DENV3, DENV4, or JEV were used as sources of NS1 antigens, and supernatant from non-infected Vero cells was used as a negative control.

[00038] Fig. 6A to Fig. 6F include charts showing the specificities of the sandwich (capture) ELISA of the present invention for testing sera of acute febrile patients.

Fig. 6A shows the results of the ELISA test for sera of 17 patients infected with DENV1. Fig. 6B shows the result of the ELISA test for sera of 19 patients infected with DENV2. Fig. 6C shows the results of the ELISA test for sera of 16 patients infected with DENV3. Fig. 6D shows the results of the ELISA test for sera of 9 patients infected with DENV4. Fig. 6E shows the results of the ELISA test for sera of 5 patients with infected with DENVs. Fig. 6F shows the results of the ELISA test for sera of 80 other febrile patients. T/N ratios were calculated by dividing the OD450 value of patient serum by the average OD450 value of all negative healthy normal sera samples (i.e., patient serum OD450/mean OD450 of negative healthy normal sera). Samples were considered to be positive if their OD450 value was at least double the mean values of the negative controls.

[00039] Fig. 7 shows the CDR sequences of heavy chain and light chain the antibodies of the present invention.

[00040] Fig. 8 shows the sequences of heavy chain and light chain of the antibodies of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[00041] The present invention relates to anti-virus dengue virus antibodies which are serotype specific. The present invention provides such antibodies and antigen-binding fragments thereof, which are useful for specific detection and

differentiation of various dengue virus serotypes in a biological sample. The present invention further provides methods and vectors for preparing the antibodies or antigen-binding fragments thereof, and also kits and compositions comprising the same and methods using the same for detection of dengue virus and diagnosis and treatment of dengue virus disease. The present invention further provides a cross reactive antibody specific to dengue virus, which can be paired with any of the serotype specific antibody as described herein for use in an immunoassay for detecting dengue virus in a sample.

[00042] The following description is merely intended to illustrate various embodiments of the invention. As such, specific embodiments or modifications discussed herein are not to be construed as limitations to the scope of the invention. It will be apparent to one skilled in the art that various changes or equivalents may be made without departing from the scope of the invention.

[00043] I. Definitions

[00044] In order to provide a clear and ready understanding of the present invention, certain terms are first defined. Additional definitions are set forth throughout the detailed description. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as is commonly understood by one of skill in the art to which this invention belongs.

[00045] As used herein, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a component” includes a plurality of such components and equivalents thereof known to those skilled in the art.

[00046] The term “comprise” or “comprising” is generally used in the sense of include/including which means permitting the presence of one or more features, ingredients or components. The term “comprise” or “comprising” encompasses the term “consists” or “consisting of.”

[00047] As used herein, the term “polypeptide” refers to a polymer composed of amino acid residues linked via peptide bonds. The term “protein” typically refers to relatively large polypeptides. The term “peptide” typically refers to relatively short polypeptides (e.g., containing up to 100, 90, 70, 50, 30, or 20 amino acid residues).

[00048] As used herein, the term “about” or “approximately” refers to a degree of acceptable deviation that will be understood by persons of ordinary skill in the art, which may vary to some extent depending on the context in which it is used. In

general, “about” or “approximately” may mean a numeric value having a range of $\pm$ 10% around the cited value.

[00049] As used herein, the term “substantially identical” refers to two sequences having 80% or more, preferably 85% or more, more preferably 90% or more, even
5 more preferably 95% or more homology.

[00050] As used herein, the term “antibody” (interchangeably used in plural form) means an immunoglobulin molecule having the ability to specifically bind to a particular target antigen. As used herein, the term “antibody” includes not only intact (i.e. full-length) antibody molecules but also antigen-binding fragments thereof
10 retaining antigen binding ability e.g. Fab, Fab’, F(ab’)2 and Fv. Such fragments are also well known in the art and are regularly employed both in vitro and in vivo. The term “antibody” also includes humanized antibodies, chimeric antibodies, diabodies, linear antibodies, single chain antibodies, multispecific antibodies (e.g., bispecific antibodies) and any other modified configuration of the immunoglobulin molecule
15 that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies.

[00051] An intact or complete antibody comprises two heavy chains and two light chains. Each heavy chain contains a variable region (V_H) and a first, second and
20 third constant regions (C_{H1} , C_{H2} and C_{H3}); and each light chain contains a variable region (V_L) and a constant region (C_L). The antibody has a “Y” shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and
25 constant regions of a single light chain. The variable regions of the light chains and those of heavy chains are responsible for antigen binding. The variables region in both chains generally contain three highly variable regions, called the complementarity determining regions (CDRs); namely, light (L) chain CDRs including LC CDR1, LC CDR2, and LC CDR3, and heavy (H) chain CDRs including
30 HC CDR1, HC CDR2, HC CDR3). The three CDRs are flanked by framework regions (FR1, FR2, FR3, and FR4), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable regions. The constant regions of the heavy and light chains are not responsible for antigen binding, but involved in various effector functions. Depending on the antibody amino acid sequence of the

constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that
5 correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

[00052] As used herein, the term "antigen-binding domain" or "antigen-binding fragment" refers to a portion or region of an intact antibody molecule that is responsible for antigen binding. An antigen-binding fragment is capable of binding
10 to the same antigen to which the parent antibody binds. Examples of antigen-binding fragments include, but are not limited to: (i) a Fab fragment, which can be a monovalent fragment composed of a V_H-C_H1 chain and a V_L-C_L chain; (ii) a F(ab')₂ fragment which can be a bivalent fragment composed of two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fv fragment, composed of the
15 V_H and V_L domains of an antibody molecule associated together by noncovalent interaction; (iv) a single chain Fv (scFv), which can be a single polypeptide chain composed of a V_H domain and a V_L domain through a peptide linker; and (v) a (scFv)₂, which can comprise two V_H domains linked by a peptide linker and two V_L domains, which are associated with the two V_H domains via disulfide bridges.

[00053] As used herein, the term "chimeric antibody" refers to an antibody containing polypeptides from different sources, e.g., different species. In some embodiments, in these chimeric antibodies, the variable region of both light and heavy chains may mimic the variable region of antibodies derived from one species of mammal (e.g., a non-human mammal such as mouse, rabbit and rat), while the
20 constant portions may be homologous to the sequences in antibodies derived from another mammal such as a human.

[00054] As used herein, the term "humanized antibody" refers to an antibody comprising a framework region from a human antibody and one or more CDRs from a non-human (usually a mouse or rat) immunoglobulin.

[00055] As used herein, the term "human antibody" refers to an antibody in which
30 essentially the entire sequences of the light chain and heavy chain sequences, including the complementary determining regions (CDRs), are from human genes. The human antibodies may include one or more amino acid residues not encoded by human germline immunoglobulin sequences e.g. by mutations in one or more of the

CDRs, or in one or more of the FRs, so as to, for example, decrease possible immunogenicity, increase affinity, eliminate cysteines that might cause undesirable folding, etc.

[00056] As used herein, an “isolated” substance means that it has been altered by the hand of man from the natural state. In some embodiments, the polypeptide (e.g. antibody) or nucleic acids of the present invention can be said to be “isolated” or “purified” if they are substantially free of cellular material or chemical precursors or other chemicals/components that may be involved in the process of peptides/nucleic acids preparation. It is understood that the term “isolated” or “purified” does not necessarily reflect the extent to which the peptide has been “absolutely” isolated or purified e.g. by removing all other substances (e.g., impurities or cellular components). In some cases, for example, an isolated or purified polypeptide includes a preparation containing the polypeptide having less than 50%, 40%, 30%, 20% or 10% (by weight) of other proteins (e.g. cellular proteins), having less than 50%, 40%, 30%, 20% or 10% (by volume) of culture medium, or having less than 50%, 40%, 30%, 20% or 10% (by weight) of chemical precursors or other chemicals/components involved in synthesis procedures.

[00057] As used herein, the term “specific binds” or “specifically binding” refers to a non-random binding reaction between two molecules, such as the binding of the antibody to an epitope of its target antigen. An antibody that “specifically binds” to a target antigen or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit “specific binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen or an epitope than it does with other targets/epitopes. An antibody “specifically binds” to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. In other words, it is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means specific/preferential binding. The affinity of the binding is defined in terms of a dissociation constant (K_D). Typically, specifically binding when used with respect to an antibody can refer to an antibody

that specifically binds to (recognize) its target with an K_D value less than about 10^{-7} M, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, about 10^{-n} M or less, about 10^{-12} M or less, or even less, and binds to the specific target with an affinity corresponding to a K_D that is at least ten-fold lower than its
5 affinity for binding to a non-specific antigen (such as BSA or casein), such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower.

[00058] As used herein, the term “cross-reactive” can refer to the ability of an antibody to react with similar antigenic sites on different proteins. For example, a
10 cross-reactive antibody to dengue virus serotypes may mean that such antibody can cross react with (i.e. the antibody binding to) more than one serotype of dengue virus (e.g. all the four serotypes of dengue virus) or even other flavivirus e.g. Zika virus and/or Japanese encephalitis virus, instead of specifically react with only one certain serotype of dengue virus.

[00059] Dengue virus is a single-stranded RNA virus that is a member of the family Flaviviridae, genus Flavivirus. Dengue virus, as used herein, refers to any serotype of dengue virus, including dengue virus serotype 1 (DENV1), dengue virus serotype 2 (DENV2), dengue virus serotype 3 (DENV3) and dengue virus serotype 4 (DENV4). Those distinct dengue virus serotypes are genetically related but antigenically distinct.
15 Immunity is serotype specific and there is no cross protection between the serotypes that infection with one serotype cannot protect human against the other serotypes. The serotypes can be determined by conventional methods known in the art such as RT-PCR using specific primer sets (TS1, TS2, TS3 or TS4, together with D1) to amplify serotype-specific fragments from the regions encoding the capsid and
20 membrane proteins of dengue virus, as described in, for example, Konogoi et al. Virology Journal (2016) 13:182. Typical strains of each dengue virus serotype are available in this art, for example, DENV1 Hawaii, DENV2 16681, DENV3 H87, and DENV4 H241.

[00060] As used herein, the term "dengue virus disease" or "dengue" means a
30 disease caused by dengue virus infection, typically transmitted by mosquitoes. People with dengue virus infection may experience no signs, or symptoms from mild to severe. When symptoms occur, they typically begin three to fourteen days after infection. Classic dengue fever (DF) frequently presents with fever, headache, severe muscle and joint pains, nausea and skin rash, while severe forms (DHF/DSS)

are characterized by all the symptoms of DF, in combination with hemorrhagic manifestations (bleeding from gums or nose, in urine, stools, or under the skin), severe abdominal pain, persistent vomiting, fatigue, irritability or restlessness, which can be life-threatening without prompt treatment.

5 **[00061]** The terms "NS1," "NS1 polypeptide" and "NS1 protein" are used herein interchangeably. NS1 refers to a nonstructural glycoprotein encoded by dengue virus, which associates with the membrane on the cell surface and is eventually released into the circulation as early as 1 day post-onset of symptoms and detectable at least until about 9 days after infection. Therefore, NS1 makes it a good marker for
10 diagnosis of dengue infection, especially in very early stage. The mature form of NS1 contains 352 amino acid residues, having a molecule weight of around 40 kDa or more depending on the glycosylation level. NS1 is serotype specific and the amino acid sequences of NS1 from different dengue virus serotypes are known and available in this art, for example, SEQ ID NO: 91 for NS1 from DENV1 (DENV1 NS1), SEQ
15 ID NO: 92 for NS1 from DENV2 (DENV2 NS1), SEQ ID NO: 93 for NS1 from DENV3 (DENV3 NS1), and SEQ ID NO: 94 for NS1 from DENV4 (DENV1 NS1). NS1 can be prepared by a recombinant method as known in the art, or alternatively can be produced and harvested from the culture supernatant of dengue virus e.g. DENV1 Hawaii for producing DENV1 NS1, DENV2 16681 for producing DENV2
20 NS1, DENV3 H87 for producing DENV3 NS1, and DENV4 H241 for producing DENV4 NS1.

[00062] Table 1 shows exemplified amino acid sequence of NS1 of DENV1, DENV2, DENV3 and DENV4.

Dengu e virus type 1 strain Hawai i	NS1	DSGCVINWKG	RELKCGSGIF	VTNEVHTWTE	QYKFQADSPK
		RLSAAIGKAW	EEGVCGIRSA	TRLENIMWKQ	ISNELNHILL
		ENDMKFTVVV	GDVSGILTQG	RKMIGPQPME	HKYSWKSWSGK
		AKIIGADVQN	TTFIIDGPNT	PECPDDQRAW	NIWEVEDYGF
		GIFTTNIWLK	LRDSYTQVCD	PRLMSAAIKD	SKAVHADMGY
		WIESEKNETW	KLARASFIEV	KTCVWPKSHT	LWSNGVLESE
		MIIPKIYGGP	ISQHNYPGY	STQTAGPWHL	GKLELDFDLC
		EGTTVVVDEH	CGNRGPSLRT	TTVTGKIIHE	WCCRSC TLPP
		LRFKGEDGCW	YGMEIRPVKD	KEENLVKSLV	SA (SEQ ID NO: 91)
Dengu e virus type 2 strain 16681	NS1	DSGCVVSWKN	KELKCGSGIF	ITDNVHTWTE	QYKFQPESPS
		KLASAIQKAH	EEGICGIRSV	TRLENLMWKQ	ITPELNHILS
		ENEVKLTIMT	GDIKGIMQAG	KRSLRPQPT	LKYSWK TWGK
		AKMLSTESHN	QTF LIDGPET	AEC PNTNRAW	NSLEVEDYGF
		GVFTTNIWLK	LKEKQDV FCD	SKLMSAAIKD	NRAVHADMGY

		WIESALNDTW	KIEKASFIEV	KNCHWPKSHT	LWSNGVLESE
		MIIPKNLAGP	VSQHNYPGY	HTQITGPWHL	GKLEMDDFDC
		DGTTVVVTE	CGNRGPSLRT	TTASGKLITE	WCCRSC TLPP
		LRYRGEDGCW	YGMEIRPLKE	KEENLVNSLV	TA (SEQ ID NO: 92)
Dengu e virus type 3 strain H87	NS1	DMGCVINWKG	KELKCGSGIF	VTNEVHTWTE	QYKFQADSPK
		RLATAIAGAW	ENGVCGIRST	TRMENLLWKQ	IANELNYILW
		ENNIKLTVVV	GDITGVLEQG	KRTLTPQPM	LKYSWKTWKG
		AKIVTAETQN	SSFIIDGPST	PECPSASRAW	NVWEVEDYGF
		GVFTTNIWLK	LREVTQLCD	HRLMSAAVKD	ERAVHADMGY
		WIESQKNGSW	KLEKASLIEV	KTCTWPKSHT	LWSNGVLESD
		MIIPKSLAGP	ISQHNHRPGY	HTQTAGPWHL	GKLELDFNYC
		EGTTVVISEN	CGTRGPSLRT	TTVSGKLIHE	WCCRSC TLPP
		LYRMGEDGCW	YGMEIRPINE	KEENMVKSLA	SA (SEQ ID NO: 93)
Dengu e virus type 4 strain H241	NS1	DTGCAVSWSG	KELKCGSGIF	VIDNVHTWTE	QYKFQPESPA
		RLASAILNAH	KDGVCGIRST	TRLENIMWKQ	ITNELNYVLW
		EGGHDLTVVA	GDVKGVL SKG	KRALAPPVND	LKYSWKTWKG
		AKIFTPEAKN	STFLIDGPD	SECPNERRAW	NFLEVEDYGF
		GMFTTNIWMK	FREGSSEVCD	HRLMSAAIKD	QKAVHADMGY
		WIESSKNQTW	QIEKASLIEV	KTCLWPKTHT	LWSNGVLESQ
		MLIPKAYAGP	FSQHNYPGY	ATQTVGPWHL	GKLEIDFGEC
		PGTTVTIQED	CDHRGPSLRT	TTASGKLVTQ	WCCRSC TMPP
		LRFLGEDGCW	YGMEIRPLSE	KEENMVKSQV	SA (SEQ ID NO: 94)

[00063] The term “nucleic acid” or “polynucleotide” can refer to a polymer composed of nucleotide units. Polynucleotides include naturally occurring nucleic acids, such as deoxyribonucleic acid (“DNA”) and ribonucleic acid (“RNA”) as well as nucleic acid analogs including those which have non-naturally occurring nucleotides. Polynucleotides can be synthesized, for example, using an automated DNA synthesizer. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which “U” replaces “T.” The term “cDNA” refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

[00064] The term “complementary” refers to the topological compatibility or matching together of interacting surfaces of two polynucleotides. Thus, the two molecules can be described as complementary, and furthermore the contact surface characteristics are complementary to each other. A first polynucleotide is complementary to a second polynucleotide when the nucleotide sequence of the first polynucleotide is identical to the nucleotide sequence of the polynucleotide binding partner of the second polynucleotide. Thus, the polynucleotide whose sequence

5'-TATAG-3' is complementary to a polynucleotide whose sequence is 5'-CTATA-3'."

[00065] The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide (*e.g.*, a gene, a cDNA, or an mRNA) to serve as templates for synthesis of other polymers and macromolecules in biological processes

5 having either a given sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a given sequence of amino acids and the biological properties resulting therefrom. Therefore, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. It is understood by a skilled person that numerous different polynucleotides and nucleic
10 acids can encode the same polypeptide as a result of the degeneracy of the genetic code. It is also understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described there to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed. Therefore, unless otherwise
15 specified, a "nucleotide sequence encoding an amino acid sequence" encompasses all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence.

[00066] The term "recombinant nucleic acid" refers to a polynucleotide or nucleic acid having sequences that are not naturally joined together. A recombinant nucleic
20 acid may be present in the form of a vector. "Vectors" may contain a given nucleotide sequence of interest and a regulatory sequence. Vectors may be used for expressing the given nucleotide sequence (expression vector) or maintaining the given nucleotide sequence for replicating it, manipulating it or transferring it between different locations (*e.g.*, between different organisms). Vectors can be introduced
25 into a suitable host cell for the above-described purposes. A "recombinant cell" refers to a host cell that has had introduced into it a recombinant nucleic acid. "A transformed cell" mean a cell into which has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding a protein of interest.

[00067] Vectors may be of various types, including plasmids, cosmids, fosmids,
30 episomes, artificial chromosomes, phages, viral vectors, etc. Typically, in vectors, the given nucleotide sequence is operatively linked to the regulatory sequence such that when the vectors are introduced into a host cell, the given nucleotide sequence can be expressed in the host cell under the control of the regulatory sequence. The regulatory sequence may comprise, for example and without limitation, a promoter

sequence (*e.g.*, the cytomegalovirus (CMV) promoter, simian virus 40 (SV40) early promoter, T7 promoter, and alcohol oxidase gene (*AOXI*) promoter), a start codon, a replication origin, enhancers, a secretion signal sequence (*e.g.*, α -mating factor signal), a stop codon, and other control sequence (*e.g.*, Shine-Dalgarno sequences and
5 termination sequences). Preferably, vectors may further contain a marker sequence (*e.g.*, an antibiotic resistant marker sequence) for the subsequent screening/selection procedure. For purpose of protein production, in vectors, the given nucleotide sequence of interest may be connected to another nucleotide sequence other than the above-mentioned regulatory sequence such that a fused polypeptide is produced and
10 beneficial to the subsequent purification procedure. Said fused polypeptide includes a tag for purpose of purification *e.g.* a His-tag.

[00068] The term “individual” or “subject” used herein includes human and non-human animals such as companion animals (such as dogs, cats and the like), farm animals (such as cows, sheep, pigs, horses and the like), or laboratory animals (such
15 as rats, mice, guinea pigs and the like).

[00069] The term “treating” as used herein refers to the application or administration of a composition including one or more active agents to a subject afflicted with a disorder, a symptom or conditions of the disorder, or a progression of the disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or
20 affect the disorder, the symptoms or conditions of the disorder, the disabilities induced by the disorder, or the progression of the disorder or the symptom or condition thereof.

[00070] The term “effective amount” used herein refers to the amount of an active ingredient to confer a desired biological effect in a treated subject or cell. The
25 effective amount may change depending on various reasons, such as administration route and frequency, body weight and species of the individual receiving said pharmaceutical, and purpose of administration. Persons skilled in the art may determine the dosage in each case based on the disclosure herein, established methods, and their own experience.

30 **[00071] II. Antibodies specific to dengue virus**

[00072] The present invention is based on the identification of a number of isolated antibodies with serotype specificity to dengue virus, including mAb12-4.1 (specific to DENV1), mAb33-7.1 (specific to DENV2), mAb43-1.3 (specific to DENV3), and

mAb22-1.5 (specific to DENV4). These anti-dengue virus antibodies were found to be capable of recognizing one specific serotype of dengue virus without cross reacting with other serotypes. More specifically, these anti-dengue virus antibodies are specific for dengue virus NS1 protein of different serotypes; namely mAb12-4.1 is
5 specific to the NS1 polypeptide originating from DENV1, mAb33-7.1 is specific to the NS1 polypeptide originating from DENV2, mAb43-1.3 is specific to the NS1 polypeptide originating from DENV3, and mAb22-1.5 is specific to the NS1 polypeptide originating from DENV4. These anti-dengue virus antibodies were found effective and workable in various forms of immunoassays to detect dengue
10 virus in a sample with superior sensitivity and specificity. The present invention is also based on the identification of a cross reactive antibody specific to dengue virus, mAb 82-1.1, which is cross reactive to various serotypes of dengue virus, including DENV1, DENV2, DENV3, and DENV4, and can be paired with any of the serotype specific antibody as described herein for use in an immunoassay for detecting dengue
15 virus in a sample.

[00073] Accordingly, described herein are serotype specific antibodies to dengue virus, including mAb12-4.1 (specific to DENV1), mAb33-7.1 (specific to DENV2), mAb43-1.3 (specific to DENV3) and mAb22-1.5 (specific to DENV4), and functional variants thereof. Also described herein is a cross reactive antibody specific to
20 dengue virus, including mAb82-1.1 (cross reactive to DENV1, DENV2, DENV3, and DENV4), and functional variants thereof. The reactivity of these mAbs with DENV1-4 was determined using ELISA and Western blot. The characterizations of these mAbs are showed in Table 2. The amino acid sequences of the heavy chain variable region (V_H) and light chain variable region (V_L), and their complementary
25 determining regions (CDR1, CDR2, CDR3), of each of mAb12-4.1, mAb33-7.1, mAb43-1.3, mAb22-1.5, and mAb 82-1.1, are as shown in Table 3 below.

[00074] A functional variant of mAb12-4.1 (specific to DENV1) (also referred to "a first antibody specific for DENV1" as described herein) can comprise

(a) a heavy chain variable region (V_H) which comprises a heavy chain
30 complementary determining region (HC CDR1) of SEQ ID NO: 2, a heavy chain complementary determining region 2 (HC CDR2) of SEQ ID NO: 4, and a heavy chain complementary determining region 3 (HC CDR3) of SEQ ID NO: 6; and

(b) a light chain variable region (V_L) which comprises a light chain complementary determining region 1 (LC CDR1) of SEQ ID NO: 9, a light chain

complementary determining region (LC CDR2) of SEQ ID NO: 11, and a light chain complementary determining region 3 (LC CDR3) of SEQ ID NO: 13,

or an antigen-binding fragment thereof.

[00075] In some embodiments, the first antibody comprises a V_H comprising SEQ ID NO: 71 or an amino acid sequence substantially identical thereto and a V_L comprising SEQ ID NO: 72 or an amino acid sequence substantially identical thereto. Specifically, the first antibody includes a V_H comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO:71, and a V_L comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO:72. The first antibody also includes any recombinantly (engineered)-derived antibody encoded by the polynucleotide sequence encoding the relevant V_H or V_L amino acid sequences as described herein.

[00076] A functional variant of mAb33-7.1 (specific to DENV2) (also referred to "a second antibody specific for DENV2" as described herein) can comprise

(a) a V_H which comprises a HC CDR1 of SEQ ID NO: 16, a HC CDR2 of SEQ ID NO: 18, and a HC CDR3 of SEQ ID NO: 20; and

(b) a V_L which comprises a LC CDR1 of SEQ ID NO: 23, a LC CDR2 of SEQ ID NO: 25, and a LC CDR3 of SEQ ID NO: 27.

[00077] In some embodiments, the second antibody comprises a V_H comprising SEQ ID NO: 73 or an amino acid sequence substantially identical thereto and a V_L comprising SEQ ID NO: 74 or an amino acid sequence substantially identical thereto. Specifically, the second antibody includes a V_H comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 73, and a V_L comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 74. The second antibody also includes any recombinantly (engineered)-derived antibody encoded by the polynucleotide sequence encoding the relevant V_H or V_L amino acid sequences as described herein.

[00078] A functional variant of mAb43-1.3 (specific to DENV3) (also referred to "a third antibody specific for DENV3" as described herein) can comprise

(a) a V_H which comprises a HC CDR1 of SEQ ID NO: 30, a HC CDR2 of SEQ ID NO: 32, and a HC CDR3 of SEQ ID NO: 34; and

(b) a V_L which comprises a LC CDR1 of SEQ ID NO: 37, a LC CDR2 of SEQ

ID NO: 39, and a LC CDR3 of SEQ ID NO: 41.

[00079] In some embodiments, the third antibody comprises a V_H comprising SEQ ID NO: 75 or an amino acid sequence substantially identical thereto and a V_L comprising SEQ ID NO: 76 or an amino acid sequence substantially identical thereto.

5 Specifically, the third antibody includes a V_H comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 75, and a V_L comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 76. The third antibody also includes any
10 recombinantly (engineered)-derived antibody encoded by the polynucleotide sequence encoding the relevant V_H or V_L amino acid sequences as described herein.

[00080] A functional variant of mAb22-1.5 (specific to DENV4) (also referred to "a fourth antibody specific for DENV4" as described herein) can comprise

(a) a V_H which comprises a HC CDR1 of SEQ ID NO: 44, a HC CDR2 of SEQ
15 ID NO: 46, and a HC CDR3 of SEQ ID NO: 48; and

(b) a V_L which comprises a LC CDR1 of SEQ ID NO: 51, a LC CDR2 of SEQ ID NO: 53, and a LC CDR3 of SEQ ID NO: 55.

[00081] In some embodiments, the fourth antibody comprises a V_H comprising SEQ ID NO: 77 or an amino acid sequence substantially identical thereto and a V_L comprising SEQ ID NO: 78 or an amino acid sequence substantially identical thereto.

20 Specifically, the fourth antibody includes a V_H comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 77, and a V_L comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or
25 99%) identity to SEQ ID NO: 78. The fourth antibody also includes any recombinantly (engineered)-derived antibody encoded by the polynucleotide sequence encoding the relevant V_H or V_L amino acid sequences as described herein.

[00082] A functional variant of mAb82-1.1 (also referred to "a fifth antibody specific for DENV1, DENV2, DENV3, and DENV4" as described herein) can

30 comprise

(a) a V_H which comprises a HC CDR1 of SEQ ID NO: 58, a HC CDR2 of SEQ ID NO: 60, and a HC CDR3 of SEQ ID NO: 62; and

(b) a V_L which comprises a LC CDR1 of SEQ ID NO: 65, a LC CDR2 of SEQ ID NO: 67, and a LC CDR3 of SEQ ID NO: 69.

[00083] In some embodiments, the fifth antibody comprises a V_H comprising SEQ ID NO: 79 or an amino acid sequence substantially identical thereto and a V_L comprising SEQ ID NO: 80 or an amino acid sequence substantially identical thereto. Specifically, the fourth antibody includes a V_H comprising an amino acid sequence
5 has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 79, and a V_L comprising an amino acid sequence has at least 80% (e.g. 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%) identity to SEQ ID NO: 80. The fourth antibody also includes any recombinantly (engineered)-derived antibody encoded by the polynucleotide sequence
10 encoding the relevant V_H or V_L amino acid sequences as described herein.

[00084] The term “substantially identical” can mean that the relevant amino acid sequences (e.g., in FRs, CDRs, V_H, or V_L) of a variant differ insubstantially as compared with a reference antibody such that the variant has substantially similar binding activities (e.g., affinity, specificity, or both) and bioactivities relative to the
15 reference antibody. Such a variant may include minor amino acid changes. It is understandable that a polypeptide may have a limited number of changes or modifications that may be made within a certain portion of the polypeptide irrelevant to its activity or function and still result in a variant with an acceptable level of equivalent or similar biological activity or function. In some examples, the amino
20 acid residue changes are conservative amino acid substitution, which refers to the amino acid residue of a similar chemical structure to another amino acid residue and the polypeptide function, activity or other biological effect on the properties smaller or substantially no effect. Typically, relatively more substitutions can be made in FR regions, in contrast to CDR regions, as long as they do not adversely impact the
25 binding function and bioactivities of the antibody (such as reducing the binding affinity by more than 50% as compared to the original antibody). In some embodiments, the sequence identity can be about 80%, 82%, 84%, 85%, 86%, 88%, 90%, 92%, 94%, 95%, 96%, 98%, or 99%, or higher, between the reference antibody and the variant. Variants can be prepared according to methods for altering
30 polypeptide sequence known to one of ordinary skills in the art such as those found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. For example, conservative substitutions of amino acids include substitutions made amongst amino acids within the following

groups: (i) A, G; (ii) S, T; (iii) Q, N; (iv) E, D ; (v) M, I, L, V; (vi) F, Y, W; and (vii) K, R, H.

[00085] The antibodies described herein may be animal antibodies (e.g., mouse-derived antibodies), chimeric antibodies (e.g., mouse-human chimeric antibodies), humanized antibodies, or human antibodies. The antibodies described herein may be monoclonal or polyclonal. A “monoclonal antibody” refers to a homogenous antibody population, and a “polyclonal antibody” refers to a heterogeneous antibody population. The antibodies described herein may also include their antigen-binding fragments e.g. a Fab fragment, a F(ab')₂ fragment, a Fv fragment, a single chain Fv (scFv) and a (scFv)₂. The antibodies or their antigen-binding fragments can be prepared by methods known in the art.

[00086] III. Preparation of antibodies

[00087] Numerous methods conventional in this art are available for obtaining antibodies or antigen-binding fragments thereof.

[00088] In some embodiments, the antibodies provided herein may be made by the conventional hybridoma technology. In general, a target antigen, e.g. a dengue virus NS1 protein, optionally coupled to a carrier protein, e.g. keyhole limpet hemocyanin (KLH), and/or mixed with an adjuvant, e.g. complete Freund's adjuvant, may be used to immunize a host animal for generating antibodies binding to that antigen.

Lymphocytes secreting monoclonal antibodies are harvested and fused with myeloma cells to produce hybridoma. Hybridoma clones formed in this manner are then screened to identify and select those that secrete the desired monoclonal antibodies.

[00089] In some embodiments, the antibodies provided herein may be prepared via recombinant technology. In related aspects, isolated nucleic acids that encode the disclosed amino acid sequences, together with vectors comprising such nucleic acids and host cells transformed or transfected with the nucleic acids, are also provided.

[00090] For examples, nucleic acids comprising nucleotide sequences encoding the heavy and light chain variable regions of such an antibody can be cloned into expression vectors (e.g., a bacterial vector such as an *E. coli* vector, a yeast vector, a viral vector, or a mammalian vector) via routine technology, and any of the vectors can be introduced into suitable cells (e.g., bacterial cells, yeast cells, plant cells, or mammalian cells) for expression of the antibodies. Examples of nucleotide sequences encoding the heavy and light chain variable regions of the antibodies as described herein are as shown in Table 3 below. Examples of mammalian host cell

lines are human embryonic kidney line (293 cells), baby hamster kidney cells (BHK cells), Chinese hamster ovary cells (CHO cells), African green monkey kidney cells (VERO cells), and human liver cells (Hep G2 cells). The recombinant vectors for expression the antibodies described herein typically contain a nucleic acid encoding the antibody amino acid sequences operably linked to a promoter, either constitutive or inducible. Typical vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the nucleic acid encoding the antibody. The vectors optionally contain selection markers for both prokaryotic and eukaryotic systems. In some examples, both the heavy and light chain coding sequences are included in the same expression vector. In other examples, each of the heavy and light chains of the antibody is cloned into an individual vector and produced separately, which can be then incubated under suitable conditions for antibody assembly.

[00091] The recombinant vectors for expression the antibodies described herein typically contain a nucleic acid encoding the antibody amino acid sequences operably linked to a promoter, either constitutive or inducible. The recombinant antibodies can be produced in prokaryotic or eukaryotic expression systems, such as bacteria, yeast, insect and mammalian cells. Typical vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the nucleic acid encoding the antibody. The vectors optionally contain selection markers for both prokaryotic and eukaryotic systems. The antibody protein as produced can be further isolated or purified to obtain preparations that substantially homogeneous for further assays and applications. Suitable purification procedures, for example, may include fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), high-performance liquid chromatography (HPLC), ammonium sulfate precipitation, and gel filtration.

[00092] When a full-length antibody is desired, coding sequences of any of the V_H and V_L chains described herein can be linked to the coding sequences of the Fc region of an immunoglobulin and the resultant gene encoding a full-length antibody heavy and light chains can be expressed and assembled in a suitable host cell, e.g., a plant cell, a mammalian cell, a yeast cell, or an insect cell.

[00093] Antigen-binding fragments can be prepared via routine methods. For example, $F(ab')_2$ fragments can be generated by pepsin digestion of an full-length

antibody molecule, and Fab fragments that can be made by reducing the disulfide bridges of F(ab')₂ fragments. Alternatively, such fragments can also be prepared via recombinant technology by expressing the heavy and light chain fragments in suitable host cells and have them assembled to form the desired antigen-binding fragments either *in vivo* or *in vitro*. A single-chain antibody can be prepared via recombinant technology by linking a nucleotide sequence coding for a heavy chain variable region and a nucleotide sequence coding for a light chain variable region. Preferably, a flexible linker is incorporated between the two variable regions.

[00094] IV. Use of antibodies

10 **[00095]** The serotype-specific antibodies of the present invention are specific for respective dengue virus serotypes, and each of which does not react with any other closely related viruses such as other flavivirus e.g. Japanese encephalitis (JE) virus and Zika virus (ZIKV). The present invention thus provides a method employing any of the disclosed antibodies or any combination thereof that can be effectively
15 employed to detect dengue virus in a sample.

[00096] In general, the method of the present invention comprises contacting the sample with any of the disclosed antibodies or any combination thereof and assaying binding of the antibody with said sample. Particularly, the binding of the antibody with said sample includes (i) binding of a first antibody to DENV1 NS1 and forming
20 a 1st antibody-DENV1 NS1 complex, (ii) binding of a second antibody to DENV2 NS1 and forming a 2nd antibody-DENV2 NS1 complex, (iii) binding of a third antibody to DENV3 NS1 and forming a 3rd antibody-DENV3 NS1 complex, and (iv) binding of a fourth antibody to DENV4 NS1 and forming a 4th antibody-DENV4 NS1 complex. The method of the present invention further comprises determining the
25 presence of absence of a particular serotype dengue virus based on the binding of the antibody with the sample, wherein (i) binding of a first antibody to DENV1 NS1 and forming a 1st antibody-DENV1 NS1 complex is an indicative of the presence of DENV1 in the sample, (ii) binding of a second antibody to DENV2 NS1 and forming a 2nd antibody-DENV2 NS1 complex is an indicative of the presence of DENV2 in
30 the sample, (iii) binding of a third antibody to DENV3 NS1 and forming a 3rd antibody-DENV3 NS1 complex is an indicative of the presence of DENV3 in the sample, and (iv) binding of a fourth antibody to DENV4 NS1 and forming a 4th antibody-DENV4 NS1 complex is an indicative of the presence of DENV4 in the sample.

[00097] There are various assay formats known to those of ordinary skill in the art for using antibodies to detect an antigen or pathogen in a sample. These assays that use antibodies specific to target antigens/pathogens are generally called immunoassays. Examples of immunoassays include but are not limited to ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), FIA (fluorescence immunoassay), LIA (luminescence immunoassay), or ILMA immunoluminometric assay. Such assays can be employed to detect the presence of dengue virus in biological samples including blood, serum, plasma, saliva, cerebrospinal fluid, urine, and other tissue specimens.

10 [00098] In certain embodiments, the assay is a sandwich assay.

[00099] In some embodiments, the assay is performed by first immobilizing a capture antibody on a solid support. The immobilized antibody is then incubated with the biological sample, and the dengue virus or its target antigen e.g. a NS1 polypeptide (if present in the sample) is allowed to bind to the antibody, to form an antibody-virus/antigen complex or conjugate. Unbound sample can then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.05% Tween, and a detection antibody that can bind to the immobilized antibody-virus/antigen complex and comprises a detectable label is added to the solid support. Specifically, the capture antibody and the detection antibody need to bind to the virus/antigen at different epitopes such that the virus/antigen can be "sandwiched" between the two antibodies. Preferred detectable labels include an enzymatic label (such as horseradish peroxidase), a fluorescent label, a metal label and a radio label. Some particular examples of detectable labels include gold nanoparticles, colored latex beads, magnetic particles, carbon nanoparticles and selenium nanoparticles. The detection antibody is incubated with the immobilized antibody-virus/antigen complex for a period of time sufficient to detect the bound virus/antigen. Unbound detection antibody is then removed and bound detection antibody is detected based on the detectable label. For example, an enzymatic label may be generally be detected by the addition of substrate, followed by spectroscopic or other analysis of the reaction products.

25
30 [000100] To determine the presence or absence of target dengue virus in the sample, the signal detected from the detectable label bound to the solid support is generally compared to a signal that corresponds to a cut-off value. This cut-off value is typically the average mean signal obtained when the immobilized antibody is

incubated with samples from an uninfected subject. In general, a sample generating a signal that is higher than the cut-off value is considered positive for the presence of dengue virus in the sample.

[000101] The serotype-specific antibodies of the present invention can be used as capture or detection antibodies, paired with a partner antibody, to form an antibody pair to perform a sandwich assay. A partner antibody as described herein needs to be able to also bind to the target virus/antigen but at a different epitope than any of the serotype-specific antibodies of the present invention as used in the pair for performing a sandwich assay. In some embodiments, a partner antibody for performing a sandwich assay can be a different "serotype specific" antibody to dengue virus other than any of those described herein (i.e. the first antibody, the second antibody, the third antibody and the forth antibody) which also is capable of binding to the respective serotype of dengue virus but at a different epitope. In some embodiments, a partner antibody can be a "serotype cross-reactive" antibody, either a polyclonal or monoclonal antibody, which can recognize all dengue virus serotypes but at a different epitope than any of the serotype-specific antibodies of the present invention does. One certain example of such cross-reactive antibody is the fifth antibody (e.g. mAb82-1.1) which is cross-reactive to at least four serotypes of dengue virus and recognizes a separate epitope than any of the serotype-specific antibodies of the present invention does, thus forming a matched pair therewith to perform a sandwich assay.

[000102] In some embodiments, the method of the present invention for detecting dengue virus in a sample comprises

(i) contacting the sample with a cross-reactive antibody specific for a dengue NS1 polypeptide (for four serotypes) (e.g. a fifth antibody specific to DENV1 NS1, DENV2 NS1, DENV3 NS1 and DENV4 NS1), as a capture antibody, to form a cross-reactive antibody-NS1 (any serotypes) complex;

(ii) contacting the cross-reactive antibody -NS1 polypeptide (any serotypes) complex with a serotype-specific monoclonal antibody as described herein (e.g. a first antibody specific to DENV1 NS1, a second antibody specific to DENV2 NS1, a third antibody specific to DENV3 NS1, or a fourth antibody specific to DENV4 NS1), as a detection antibody, to form a cross-reactive antibody- NS1 (any serotypes)-serotype-specific monoclonal antibody complex; and

(iii) detecting the presence of the cross-reactive antibody-NS1 (any

serotypes)-serotype-specific monoclonal antibody complex, thereby detecting the presence of respective serotype dengue virus in the sample.

[000103] In some embodiments, the method of the present invention for detecting dengue virus in a sample comprises

- 5 (i) contacting the sample with a serotype-specific monoclonal antibody as described herein (e.g. a first antibody specific to DENV1 NS1, a second antibody specific to DENV2 NS1, a third antibody specific to DENV3 NS1, or a fourth antibody specific to DENV4 NS1), as a capture antibody, to form a monoclonal antibody-serotype specific NS1 complex;
- 10 (ii) contacting the monoclonal antibody-respective serotype dengue virus complex with a cross-reactive antibody specific for a dengue NS1 polypeptide (for four serotypes) (e.g. a fifth antibody specific to DENV2 NS1, DENV2 NS1, DENV3 NS1 and DENV4 NS1), as a detection antibody, to form a monoclonal antibody-serotype specific NS1-cross-reactive antibody complex; and
- 15 (iii) detecting the presence of the serotype-specific monoclonal antibody-NS1-cross-reactive antibody complex, thereby detecting the presence of respective serotype dengue virus in the sample.

[000104] In one example, the method of the present invention is performed in an ELISA sandwich assay. In this assay, the capture antibody is coated onto ELISA
20 plates. After blocking, the plates are incubated with the biological sample, washed and then incubated with a detection antibody. For example, the capture antibody is a polyclonal or monoclonal, cross-reactive antibody specific for a dengue NS1 polypeptide (any serotypes), and the detection antibody is a first antibody specific to DENV1 NS1, a second antibody specific to DENV2 NS1, a third antibody specific to
25 DENV3 NS1, or a fourth antibody specific to DENV4 NS1, or any combination thereof, as described herein, and the plate is developed using a ELISA colorimetric TMB reagent.

[000105] In another example, the method of the present invention is performed in a flow-through or lateral flow format. In this assay, the detection antibody with a
30 detectable label such as a colorimetric label (e.g. colloidal gold) is immobilized to a membrane such as nitrocellulose (as a strip). A biological sample suspected of containing said dengue virus is applied to the membrane to which the detection antibody is present. The biological sample migrates along the membrane through a region containing the detection antibody wherein the detection antibody binds to the

NS1 of the dengue virus if present in the biological sample. The complex of the detection body with its bound antigen then migrates to the test area where a capture antibody is immobilized and also binds the NS1 of the dengue virus, thereby forming a sandwich of the detection antibody, antigen and capture antibody.

- 5 Concentration/aggregation of detection antibody at the test (capture) area indicates the presence of specific dengue NS1 in the sample. Such tests can typically be performed with a very small amount of biological sample.

[000106] In a related aspect, the present invention also provides a kit for performing the method of the invention, which comprises any of the antibody or its combination
10 thereof as described herein. The kit can further comprise instructions for using the kit to detect the dengue virus in a sample.

[000107] In some embodiments, the immunoassay is in a sandwich format.

[000108] In particular, the kit comprises a pair of a dengue virus serotype-specific antibody selected from the group consisting of a first antibody specific to DENV1
15 NS1, a second antibody specific to DENV2 NS1, a third antibody specific to DENV3 NS1, and a fourth antibody specific to DENV4 NS1, and any combination thereof, each antibody paired with a partner antibody for performing a sandwich assay

[000109] In certain embodiments, at least one of the serotype-specific antibodies is performed as a capture antibody, paired with a partner antibody as a detection
20 antibody.

[000110] In other embodiments, at least one of the serotype-specific antibodies is performed as a detection antibody, paired with a partner antibody as a capture antibody.

[000111] As a detection antibody, the antibody can comprise a detectable label such
25 as an enzymatic label, a fluorescent label, a metal label and a radio label.

[000112] In some examples, in a lateral flow format, the kit comprises an assay strip (e.g. a nitrocellulose membrane); a detection antibody may be bound to a reaction zone of the strip, and a capture antibody may be bound in a test zone of the strip. The strip also contains a sample pad where a body fluid sample is placed to and then
30 the sample migrates towards an opposite end of the strip by capillary action, through which the sample first engages the detection antibody in the reaction zone where the antigen in the sample binds to the detection antibody forming an antigen-detection antibody complex and then the antigen-detection antibody complex engages the capture antibody in the test (capture) zone. Using colloidal gold as the detectable

label of the detection antibody, for example, concentration/aggregation of detection label at the test (capture) area reveals red color, indicating the presence of specific antigen in the sample. Alternatively, the test zone may have a chromogenic substrate and when the antigen-detection antibody complex engage the capture antibody, the chromogenic substrate is converted to a visible colored product.

[000113] In some examples, in an ELISA format, the kit comprises a microtiter plate with wells to which a capture antibody has been immobilized; a solution containing a detection antibody; and a color developing reagent.

[000114] Particularly, the kit may further comprise additional reagents or buffers, a medical device for collecting a biological sample from a subject, and/or a container for holding and/or storing the sample.

[000115] In further embodiments, the present invention provides compositions comprising one or more antibodies as described herein and a pharmaceutically acceptable carrier.

[000116] In some embodiments, the composition of the present invention is a pharmaceutical composition for use in treatment of dengue virus disease.

[000117] In some embodiments, the composition of the present invention is a diagnostic composition for use in diagnosis of dengue virus disease.

[000118] As used herein, “pharmaceutically acceptable” means that the carrier is compatible with the active ingredient in the composition, and preferably can stabilize said active ingredient and is safe to the individual receiving the treatment. Said carrier may be a diluent, vehicle, excipient, or matrix to the active ingredient. Some examples of appropriate excipients include lactose, dextrose, sucrose, sorbose, mannose, starch, Arabic gum, calcium phosphate, alginates, tragacanth gum, gelatin, calcium silicate, microcrystalline cellulose, polyvinyl pyrrolidone, cellulose, sterilized water, syrup, and methylcellulose. The composition may additionally comprise lubricants, such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preservatives, such as methyl and propyl hydroxybenzoates; sweeteners; and flavoring agents. The composition of the present invention can provide the effect of rapid, continued, or delayed release of the active ingredient after administration to the patient.

[000119] According to the present invention, the form of said composition may be tablets, pills, powder, lozenges, packets, troches, elixers, suspensions, lotions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterilized injection

fluid, and packaged powder.

[000120] The composition of the present invention may be delivered via any physiologically acceptable route, such as oral, parenteral (such as intramuscular, intravenous, subcutaneous, and intraperitoneal), transdermal, suppository, and intranasal methods. Regarding parenteral administration, it is preferably used in the form of a sterile water solution, which may comprise other substances, such as salts or glucose sufficient to make the solution isotonic to blood. The water solution may be appropriately buffered (preferably with a pH value of 3 to 9) as needed. Preparation of an appropriate parenteral composition under sterile conditions may be accomplished with standard pharmacological techniques well known to persons skilled in the art, and no extra creative labor is required.

[000121] The present invention is further illustrated by the following examples, which are provided for the purpose of demonstration rather than limitation. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[000122] Examples

[000123] 1.1 Viral strains

[000124] Dengue viral strains DENV1 Hawaii, DENV2 16681, DENV3 H87, and DENV4 H241 were propagated in C6/36 *Aedes albopictus* in RPMI 1640 medium supplemented with 2% FBS and incubated at 28°C until cytopathic effects were observed. As a control, the viral strain JEV SA14-14-2 was propagated in BHK-21 cells in RPMI 1640 medium containing 2% FBS. The titers of each virus were determined via plaque assays in which BHK-21 cells were inoculated.

[000125] 1.2 Preparation of NS1 proteins

[000126] Cell culture supernatants of Vero cells infected with viruses (DENV1 Hawaii, DENV2 16681, DENV3 H87, and DENV4 H241) were harvested after five days and inactivated by UV irradiation. To purify the soluble form of NS1 proteins in cell culture supernatants, we performed immunoaffinity chromatography column according to manufacturer's instructions. For this, anti-NS1 monoclonal antibodies (for all serotype) that had been immobilized onto a HiTrap™ NHS-activated HP column (GE Healthcare, Uppsala, Sweden) were used to purify NS1 proteins from each of the dengue serotypes. Equilibrate the column with 5 volumes of PBS, viral

cell culture supernatants were passed over the immunoaffinity column, washing out unbound proteins with ten volumes of PBS and bound NS1 were eluted using glycine solution with a pH of 2.8, then buffered to neutral pH. The purified DENV NS1 proteins were then detected by Western blot analysis, and NS1 protein concentrations were determined using a Pierce BCA protein assay kit (Thermo Fisher Scientific, Illinois, USA).

[000127] 1.3 Generation and characterization of serotype-specific mAbs against DENV1-4 NS1

[000128] All experiments used BALB/c mice that were purchased from the National Laboratory Animal Center and maintained at the Institute of Preventative Medicine's animal housing facility. Experiments using animals were licensed by the Association for Assessment and Accreditation of Laboratory Animal Care International (IACUC no AN-104-12, AN-105-05). Four-week-old BALB/c mice were immunized i.p. with 15 µg of immunoaffinity-purified NS1 proteins in complete Freund's adjuvant for the first inoculation and the mice were then immunized against their respective serotypes with 15 µg of NS1 proteins in incomplete Freund's adjuvant for the subsequent boosting. DENV-specific antisera were obtained from the mice after consecutive challenge. Briefly, spleen of an immunized mouse was removed. Splenocytes were fused with NS1/1-Ab4-1 myeloma cells to generate hybridoma cells, which were selected according to standard procedures (Kohler & Milstein, 1975). After fused cells were wash twice with RPMI then mixed in a 15ml conical tube and 1 ml 50% (w/v) PEG 1500 (Roche, Penzberg, Germany) was added over 1 min with gentle stirring. The mixture was diluted by slow (1min) addition of 1 ml RPMI, twice, followed by the slow addition (2 min) of 8 ml serum-free RPMI. The mixture was then centrifuged at 400g for 5 min. The fused cell pellet was re-suspended in RPMI supplemented with 20% FBS, HAT medium (Life technologies, Burlington, ONT Canada) and HFCS solution (Roche, Mannheim, Germany). Next, 200µl per well of the resuspension mixture was distributed in 96-well plates. The hybridoma cell lines that secreted specific antibodies against NS1 were identified by indirect ELISA (using purified DENV NS1 as the coating antigen for each serotype). Single clone cells were generated by limiting dilution. Western blotting of lysates from C6/36 cells infected with DENVs was performed to determine (a) the specificity of anti-NS1 mAbs and (b) whether the epitopes recognized by the antibodies were conformation or linear. The mAbs were isotyped using a commercially available mouse monoclonal antibody

isotyping kit (IsoStrip™, Roche, Mannheim, Germany). The hybridoma cells were injected into pristane-primed BALB/c mice for ascitic fluid production. mAbs were then purified from ascitic fluids using a protein G-sepharose column (HiTrap protein G, GE Healthcare, Uppsala, Sweden) according to the manufacturer's instructions.

5 **[000129] 1.4 HRP conjugation**

[000130] To conjugate mAbs with HRP (Innova Biosciences, Cambridge, UK), 100 µg of HRP and a 20 µl aliquot of modifier reagent were mixed with 200 µl of 1 mg/ml mAb. After incubating the mixture for 3 hours at room temperature (20-25°C), the reaction was stopped with a 20 µl aliquot of quencher. Following incubation for an additional 30 min at room temperature, 260 µl of glycerol was added, and the final solution was stored at -20°C. The final concentration of mAb-HRP was 400 µg/ml.

[000131] 1.5 Development of four serotype-specific NS1 capture ELISAs

[000132] Several serotype-specific and highly reactive mAbs were selected based on the characteristics of anti-NS1 mAbs. The serotype-specific antibodies in a capture ELISA format of the four serotype-specific mAbs were tested by for compatibility with serotype-cross-reactive mAbs. To determine the best combinations for the capture ELISA with respect to assay sensitivity, the serotype-specific mAbs and serotype-cross-reactive mAbs were used as either capture or detection antibodies.

Specifically, serotype-cross-reactive mAbs were selected as a capture antibody, and optimal pairing to the respective four serotype-specific mAbs (mAb12-4.1, mAb33-7.1, mAb43-1.3, and mAb22-1.5) as detection antibodies was determined, the Dengue four serotype NS1 ELISA was assembled, as shown in Fig. 1A, Fig. 1B, Fig. 2A and Fig. 2B. The most appropriate experimental condition such as coating concentration and the dilution of mAb-HRP were determined by checkerboard titration. Microwell plates (Costar Corning Inc., Corning, NY) were coated with 100 µl of 10 µg /ml of capture cross-reactive mAb and incubated overnight at 4°C. Wells were subsequently blocked with blocking buffer (PBS, 0.05% Tween, 5% skim milk) for 1 h at 37°C and washed with wash buffer (PBS, 0.05% Tween). Viral culture supernatant or NS1 proteins were then serially diluted with blocking buffer and incubated for 1 h at 37°C. After the plates were washed, 100 µl of 0.8 µg/ml of serotype-specific mAb-HRP was added, and the plates were incubated for 1 h at 37°C. Following this, the plates were washed again, 100 µl of TMB reagent was added, and the plates were incubated for 10 min at room temperature. The reaction was then

stopped with 1 N sulfuric acid, and the absorbance was read at 450 nm using a microplate autoreader.

[000133] 1.6 Clinical samples

[000134] A total of 146 clinical serum samples were used in this study; 85 samples
5 comprised confirmed dengue cases that had been reported to the Centers for Disease Control, Department of Health, Taipei, and 61 samples were collected from three hospitals during 2016-2017 (Kaohsiung Armed Forces General Hospital, Zuoying Branch of Kaohsiung Armed Forces General Hospital, and Tangshan Branch of Kaohsiung Armed Forces General Hospital). All clinical serum samples were
10 collected during the acute phase (1-7 days after onset of illness) and tested with serotype-specific one-step SYBR green I real time RT-PCR, dengue virus specific IgM/IgG capture ELISA and commercially available Platelia Dengue NS1 Ag ELISA kit (Bio-Rad, Marnes-la-Coquette, France). The study protocol was approved by the Kaohsiung Armed Forces General Hospital Institutional Review Board (IRB no.
15 KAFGH 104-048).

[000135] 1.7 Testing clinical sera for NS1 using serotype-specific NS1 capture ELISA

[000136] Microwell plates were coated with 100 μ l of 10 μ g /ml of a cross-reactive mAb and incubated overnight at 4°C . Wells were subsequently blocked with blocking
20 buffer (PBS, 0.05% Tween, 5% skim milk) and incubated with 50 μ l of sera diluted in blocking buffer at a 1:1 ratio for 1 h at 37°C . The plates were then washed four times with 0.05% PBS/T, incubated with 100 μ l of 0.8 μ g/ml of serotype-specific mAb-HRP (mAb12-4.1, mAb33-1.7, mAb43-1.3, and mAb22-1.5) for 1 h at 37°C , and washed four times again. Subsequent steps involved in the testing of clinical sera were as
25 described in the previous section. Normal human serum samples (Sigma-Aldrich, Saint Louis, USA) were used as a negative control. For each serotype, samples that exceeded the reference cut-off value, calculated as the two-fold mean of the negative control, were considered positive in the NS1 capture ELISA.

[000137] 1.8 Detection of NS1 antigens by a commercially available Platelia Dengue NS1 Ag ELISA kit (Bio-Rad, Marnes-la-Coquette, France)

[000138] DENV NS1 proteins were detected in clinical serum samples using a commercially available Platelia Dengue NS1 Ag ELISA kit (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's instructions. Briefly,

dilutions of positive, negative, calibration, and samples were incubated on well plates with HRP-conjugated mAbs for 90 min at 37°C. After washing the plates six times, 160 µl of TMB was added into each well, and plates were incubated for 30 min at room temperature away from light. The enzymic reaction was stopped by adding 100
5 µl of stop solution, and optical densities were measured at OD 450/620nm.

[000139] 1.9 Reproducibility

[000140] A total of 58 DENV positive serum samples (16 serum samples for DENV1, 17 serum samples for DENV2, 16 serum samples for DENV3, and 9 serum samples for DENV4) and 50 negative serum samples were tested by the four-serotype NS1
10 capture ELISAs by a second operator on a different day.

[000141] 1.10 Statistical analysis

[000142] Diagnosis accuracy, sensitivity, specificity, and the corresponding 95% confidence intervals (CI95) for each ELISA were performed using GraphPad Prism version 6.0 (GraphPad software, San Diego, CA), and the significance level was set at
15 a P value of <0.05.

[000143] 1.11 Preparation of colloidal gold probe

[000144] Colloid gold 35± 5nm (TANBead NanoGold-40, Taiwan Advanced Nanotech) was used for conjugation of IgG. The colloid gold solution (1% w/v) was adjusted pH with 0.2 N NaOH and anti-dengue NS1 mAb82-1.1 (in PBS, PH 7.4) was
20 added to pH-adjusted colloid gold solution. The most appropriate pH value for each serotype conjugation were PH 7.8 for D1 strip; PH 7.4 for D2, D3, and D4 strip. The optimized antibody concentration for conjugation was 1 µg/strip. The antibody/colloid gold mixture was gently mixed for 90 min, blocked by 2% BSA solution for 30 min and centrifuged at 7000 rpm for 15 min. After centrifugation and
25 wash once by 2% BSA (20 mM Tris/HCl buffer [pH7.2] containing 2% [w/v] BSA), the gold pellets were suspended in 2% BSA. Make this anti-gengue NS1 mAb82-1.1 coated colloidal gold probe to release pad and dried then stored at 4°C over night.

[000145] 1.12 Preparation of lateral flow test strips

[000146] The control lines were prepared as follows: 0.2 (for D1, D3, and D4) to 0.32
30 (for D2) µg/strip of goat anti mouse IgG (Jackson ImmunoResearch, PA, USA). Test lines (capture antibodies) were prepared as follows: mAb12-4.1, mAb43-1.3, mAb22-1.5 working strength were 0.75 µg/strip (for D1, D3, D4 strip) in PBS (pH 7.4), and mAb33-7.1 working strength was 0.8 µg/strip (for D2 strip) in PBS (pH 7.4)

were separately applied near to the top end of a cellulose acetate supported strip of nitrocellulose membrane (Pore size: 12µm-diameter) and dried for 1 h at room temperature. After treatment of components, the Dengue serotype NS1 lateral flow test device was assembled.

5 **[000147] 1.13 Assay of NS1 antigens on strip and the detection sensitivity**

[000148] The assay was carried out by applying a sample (50µl) of the appropriate test DENV NS1 protein solution and 50µl running buffer (0.1% Tween-20/0.85% NaCl) to the sample pad of the device. The combined solution of test DENV NS1 and detection reagent rose up the membrane and colloidal gold was deposited at the site of the solid-phase antibody after 20 min at room temperature.

10 **[000149] 1.14 Cross-reactivity of DENV serotype NS1 strip**

[000150] Cell culture supernatants of Vero cells infected with viruses (DENV1 Hawaii, DENV2 16681, DENV3 H87, DENV4 H241, JEV, and ZIKV) were assayed by DENV serotype NS1 strip for evaluating cross-reactivity.

15 **[000151] 1.15 Sequence analysis of monoclonal antibodies variable domains**

[000152] The hybridoma cells from hybridomas 12-4.1, 33-7.1, 43-1.3, 22-1.5, and 82-1.1. Total RNA was isolated from hybridoma cell lines using RNeasy mini kit (Qiagen, Valencia, Ca. USA) according to manufacturer's protocol, followed by reverse transcription using oligo dT primer to generate cDNA. The heavy chain and light variable chains of the antibody are amplified using PCR and confirmed the sequence is functional variable domain. The VH and VL genes of four hybridoma cells, see attached file for sequence.

20 **[000153] 2. Results**

[000154] 2.1 Generation and characterization of mAbs against DENV

25 **[000155]** NS1 proteins from various serotypes of DENV were prepared and injected into the BALB/c mice for immunization. Spleen was removed from the immunized mice and splenocytes were isolated and fused with myeloma cells to generate hybridoma cells. The hybridoma cell lines that secreted specific antibodies against NS1 were identified by ELISA using purified DENV NS1 as the coating antigen for each serotype. In addition, western blotting of lysates from C6/36 cells infected with DENVs was performed to determine (a) the specificity of anti-NS1 mAbs and (b) whether the epitopes recognized by the antibodies were conformation or linear. Further, the mAbs were isotyped using a commercial kit.

30 **[000156]** Table 2. Characterization of mAb reactions with NS1 proteins of DENV

serotypes (D1-4)

Hybridoma cell strain	Isotype	Type of epitope	Reactivity of the four DENV serotypes (D1-4)								Specificity
			Western blot ^a				ELISA ^b				
			D1	D2	D3	D4	D1	D2	D3	D4	
mAb12-4.1	IgG1,κ	conformational	+	-	-	-	+	-	-	-	NS1
mAb33-7.1	IgG1,κ	conformational	-	+	-	-	-	+	-	-	NS1
mAb43-1.3	IgG1,κ	conformational	-	-	+	-	-	-	+	-	NS1
mAb22-1.5	IgG1,κ	conformational	-	-	-	+	-	-	-	+	NS1
mAb82-1.1	IgG1,κ	linear	+	+	+	+	+	+	+	+	NS1

^a The lysates of C6/36 cells infected with different dengue virus serotypes were treated with SDS-PAGE sample buffer and blotted with each mAb.

^b Different NS1 antigens were immunoaffinity-purified from cell culture supernatants of Vero cells infected with different serotypes of DENV. Microwell plates were coated with specific NS1 antigens and reacted with each mAb.

[000157] As shown in Table 2, the 1st to 4th clones, 12-4.1, 33-7.1, 43-1.3, and 22-1.5, are serotype specific to DENV1, DENV2, DENV3 and DENV4, respectively, while the 5th clone, mAb82-1.1, is cross-reactive to DENV1, DENV2, DENV3 and DENV4.

[000158] The amino acid sequences of the four serotype-specific mAbs (12-4.1, 33-7.1, 43-1.3, and 22-1.5) and the cross-reactive mAb (82-1.1), including the complementary determining regions 1-3 (CDR 1-3) and framework regions 1-4 (FW1-4) for both the VH and VL domains and corresponding nucleic acid sequences were determined and provided in Table 3 below.

[000159] Table 3. The amino acid sequences of the four serotype-specific mAbs (12-4.1, 33-7.1, 43-1.3, and 22-1.5) and the cross-reactive mAb (82-1.1) and corresponding nucleic acid sequences.

V_H domain				
	FW1	CDR1	FW2	CDR2
12-4.1	EVKLQESGAELAKP GASVKMSCRAS (SEQ ID NO: 1)	GYTFTSYWIH (SEQ ID NO: 2)	WVKERPGQGLEWIG (SEQ ID NO: 3)	YITPNTGYNENSQKFRG (SEQ ID NO: 4)
33-7.1	EVQLQESGAELAKP GASVKMSCKAS (SEQ ID NO: 15)	GYTFTSYWMH (SEQ ID NO: 16)	WVKQRPGQVLEWIG (SEQ ID NO: 17)	YINPNTGFTEYSQKFKD (SEQ ID NO: 18)
43-1.3	GELQESGPQVVRPG TSVKISCKAS (SEQ ID NO: 29)	GYFSFSNYWMH (SEQ ID NO: 30)	WVKQRPGQGLEWIG (SEQ ID NO: 31)	VIDPSDSETRLNQKFKD (SEQ ID NO: 32)
22-1.5	EVQLQQSGPELVKP GASVKISCKAS (SEQ ID NO: 43)	GYTFTDYNIH (SEQ ID NO: 44)	WVKQSPGKSLEWIG (SEQ ID NO: 45)	YIYPDNGDTGYNQIFKN (SEQ ID NO: 46)

82-1.2	EVQLQQSGGGLVQP GGSLKLSAAS (SEQ ID NO: 57)	GFTFSNYDMS (SEQ ID NO: 58)	WIRQTPDKRLEMVA (SEQ ID NO: 59)	AINSGGSTYYPDsvKG (SEQ ID NO: 60)
	FW3	CDR3	FW4	
12-4.1	KATLTADKSSNTAY MQLSSLTSEDSAVYF CVR (SEQ ID NO: 5)	RTYEGYLDV (SEQ ID NO: 6)	WGAGTTVTVSS (SEQ ID NO: 7)	
33-7.1	KATLTAVKSSSTAYIQ LTSLTSDSAVYYCA R (SEQ ID NO: 19)	ENRYDGAMDY (SEQ ID NO: 20)	WGQGTsvTVSS (SEQ ID NO: 21)	
43-1.3	KATLTVDKSSSTAYM QLSSPTSEDSAIYYC AR (SEQ ID NO: 33)	SQFGLRFAY (SEQ ID NO: 34)	WGQGTlVTVSA (SEQ ID NO: 35)	
22-1.5	KATLTVDTSSSAAY MELRSLTSEDSAVYY CVR (SEQ ID NO: 47)	RVLLDS (SEQ ID NO: 48)	WGQGTsvTVSS (SEQ ID NO: 49)	
82-1.2	RFTISRDKAKNTLYL QMSSLKSEDTAMYY CAS (SEQ ID NO: 61)	PNGYGAMDY (SEQ ID NO: 62)	WGQGTsvTVSS (SEQ ID NO: 63)	
V_L domain				
	FW1	CDR1	FW2	CDR2
12-4.1	DIVMTQTPLSLPVSL GDQASISC (SEQ ID NO: 8)	RSSQNLVHNNGNT YLE (SEQ ID NO: 9)	WYLQKPGQSPKLLIY (SEQ ID NO: 10)	KVSNRLS (SEQ ID NO: 11)
33-7.1	DIVLTQSPALMAAFP GDRVITTC (SEQ ID NO: 22)	SVSSSVSSSNLH (SEQ ID NO: 23)	WYQQKSETSPKPWIY (SEQ ID NO: 24)	GTSTLAS (SEQ ID NO: 25)
43-1.3	DIVMTQSPSSMTVTA GEKVTMSC (SEQ ID NO: 36)	QSSQSLFNSGTQKN YLT (SEQ ID NO: 37)	WYQQKPGQPPKLLIS (SEQ ID NO: 38)	WASTRDS (SEQ ID NO: 39)
22-1.5	DIQMTQTTSLSASL GDRVITSC (SEQ ID NO: 50)	SASQDINNFLN (SEQ ID NO: 51)	WYQQKPDGTIKLLIY (SEQ ID NO: 52)	YTSSLQS (SEQ ID NO: 53)
82-1.2	DIVITQTPLTSLVTIG QPASISC (SEQ ID NO: 64)	KSRQSLDSDGKTY LN (SEQ ID NO: 65)	WLLQRPGESPKLLIY (SEQ ID NO: 66)	LVSKLDS (SEQ ID NO: 67)
	FW3	CDR3	FW4	
12-4.1	GVPDRFSGSGSGTDF TLNISRVEAEDLGIY YC (SEQ ID NO: 12)	FQASHVPRT (SEQ ID NO: 13)	FGGGTKLEIKR (SEQ ID NO: 14)	

33-7.1	GVPVRFSGSGSGTSY SLTISSMEAEDAATY YC (SEQ ID NO: 26)	QQWSSYPLT (SEQ ID NO: 27)	FGAGTKLELKR (SEQ ID NO: 28)
43-1.3	GVPDRFTGSGSGTD FTLTINGVQAEDLAV YFC (SEQ ID NO: 40)	QNDYDSPYT (SEQ ID NO: 41)	FGGGTKLEIKR (SEQ ID NO: 42)
22-1.5	GVPSRFSGSGSGTDY SLTISNLEPEDIATYY C (SEQ ID NO: 54)	QQYSKLPRT (SEQ ID NO: 55)	FGGGTKLEIKR (SEQ ID NO: 56)
82-1.2	GVPDRFTGSGSGTD FTLKISRVEAEDLGV YYC (SEQ ID NO: 68)	LQATHFPWT (SEQ ID NO: 69)	FGGGTKLEIKRA (SEQ ID NO: 70)

Full-length amino acid sequences of heavy chain and light chain

12-4.1	heavy chain	EVKLQESGAELAKPGASVKMSCRASGYTFTSYWIHWKERPGQG LEWIGYITPNTGYNENSQKFRGKATLTADKSSNTAYMQLSSLTS EDSAVYFCVRRRTYEGYLDVWGAGTTVTVSS (SEQ ID NO: 71)	
	light chain	DIVMTQTPLSLPVS LGDQASISCRSSQNLVHNNGNTYLEWYLQK PGQSPKLLIYKVS NRLSGVPDRFSGSGSGTDFTLNISRVEAEDL GIYYCFQASHVPRTFGGGTKLEIKR (SEQ ID NO: 72)	
33-7.1	heavy chain	EVQLQESGAELAKPGASVKMSCKASGYTFTSYWMHWVKQRPGQV LEWIGYINPNTGFTEYSQKFKDKATLTAVKSSSTAYIQLTSLTS DDSAVYYCARENYRYDGAMDYWGQGTSTVTVSS (SEQ ID NO: 73)	
	light chain	DIVLTQSPALMAAFPGDRVITITCSVSSSVSSSNLHWYQQKSETS PKPWIYGTSTLASGVPVRFSGSGSGTSYSLTISSMEAEDAATYY CQQWSSYPLTFGAGTKLELKR (SEQ ID NO: 74)	
43-1.3	heavy chain	G ELQESGPQVVRPGTSVKISCKASGYSF SNYWMHWVKQRPGQGL EWIGVIDPSDSETRLNQKFKDKATLTVDKSSSTAYMQLSSPTSE DSAIYYCARSQFGLRFAYWGQGTLVTVSA (SEQ ID NO: 75)	
	light chain	DIVMTQSPSSMTVTAGEKVTMSCQSSQSLFNSTQKNYLTWYQQ KPGQPPKLLISWASTRDSGVPDRFTGSGSGTDFTLTINGVQAED LAVYFCQNDYDSPYTFGGGTKLEIKR (SEQ ID NO: 76)	
22-1.5	heavy chain	EVQLQQSGPELVKPGASVKISCKASGYFTDYNIHVWKQSPGKS LEWIGYIYPDNGDTGYNQIFKNKATLTVDTSSSAAYMELRSLTS EDSAVYYCVRRVLLDSWGQGTSTVTVSS (SEQ ID NO: 77)	
	light chain	DIQMTQTTSSLSASLGDRVITISCSASQDINNFLNHWYQQKPDGTI KLLIYYTSSLQSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYC QQYSKLPRTFGGGTKLEIKR (SEQ ID NO: 78)	
82-1.2	heavy chain	EVQLQQSGGGLVQPGGSLKLSAASGFTFSNYDMSWIRQTPDKR LEMVAAINSNGGSTYYPDSVKGRFTISRDKAKNTLYLQMSSLKS EDTAMYYCASPNGYGAMDYWGQGTSTVTVSS (SEQ ID NO: 79)	

		<div>light chain</div> <div>DIVITQTPLTLSVTIGQPASISCKSRQSLDSDGKTYLNWLLQR PGESPKLLIYLVSKLDSGVPDRFTGSGSGTDFTLKI SRVEAEDL GVYYCLQATHFPWTFGGGTKLEIKRA (SEQ ID NO: 80)</div>
Nucleotide sequence		
12-4.1	heavy chain	<div>GAGGTCAAACCTGCAGGAGTCTGGGGCTGAACTGGCAAACCTGGGGCCTC AGTGAAGATGTCCTGCAGGGCTTCTGGCTACACCTTTACCAGCTATTGGA TACACTGGGTGAAAGAGAGGCCTGGACAGGGTCTGGAATGGATTGGATAC ATTACTCCTAATACAGGTTATAATGAGAACAGTCAGAAGTTCAGGGGCAA GGCCACATTGACTGCAGACAAATCCTCCAACACAGCTTATATGCAACTAA GCAGCCTGACATCTGAGGACTCTGCAGTCTATTTCTGTGTAAGAAGGACG TATGAGGGGTACCTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTC CTCA (SEQ ID NO: 81)</div>
	light chain	<div>GACATTGTGATGACCCAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGA TCAAGCCTCCATCTCTTGCAGATCTAGTCAGAACCTTGTACATAATAATG GAAACACCTATTTAGAGTGGTACCTGCAGAAACCAGGCCAGTCTCCAAAG CTCCTGATCTACAAAGTTTCCAACCGATTGTCTGGGGTCCAGACAGGTT CAGTGGCAGTGGATCCGGGACAGACTTCACACTCAATATCAGCAGAGTGG AGGCTGAGGATCTGGGAATTTATTACTGCTTTCAGGCTTCACATGTTCT CGGACGTTCCGGTGGAGGCACCAAGCTGGAAATCAAACGG (SEQ ID NO: 82)</div>
33-7.1	heavy chain	<div>GAGGTGCAGCTGCAGGAGTCAGGGGCTGAACTGGCAAACCTGGGGCCTC AGTGAAGATGTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACTGGA TGCCTGGGTAAAACAGAGGCCTGGACAGGTTCTGGAATGGATTGGATAC ATTAATCCTAATACTGGTTTTACTGAATATAGTCAGAAATCAAGGACAA GGCCACATTGACTGCAGTCAAGTCTCCAGCACAGCCTATATACAACCTGA CCAGCCTGACATCTGATGACTCTGCAGTCTATTACTGTGCAAGAGAGAAC TATAGGTACGACGGGGCTATGGACTACTGGGGTCAAGGAACCTCAGTCAC CGTCTCTCTCA (SEQ ID NO: 83)</div>
	light chain	<div>GATATTGTGCTAACTCAGTCTCCAGCACTCATGGCTGCATTTCCAGGGGA CAGGGTCACCATCACCTGCAGTGTGAGCTCAAGTGTAAGTTCCAGCAACT TGCCTGGTACCAACAGAAGTCAGAAACCTCCCCCAAACCTGGATTTAT GGCACATCCACCCTGGCTTCTGGAGTCCCTGTTCTGCTTCAGTGGCAGTGG ATCTGGGACCTCTTATTCTCTCACAATCAGCAGCATGGAGGCTGAAGATG CTGCCACTTATTACTGTCAACAGTGGAGTAGTTACCCACTCACGTTCCGGT GCTGGGACCAAGCTGGAGCTGAAACGG (SEQ ID NO: 84)</div>
43-1.3	heavy chain	<div>GGTGAAGTGCAGGAGTCAGGGCCTCAGGTGGTTAGGCCTGGGACTTCAGT GAAGATATCCTGCAAGGCTTCTGGTTATTCATTTTCCAACTACTGGATGC ACTGGGTGAAGCAGAGGCCTGGACAAGGTCTTGAGTGGATTGGCGTGATT GATCCTTCCGATAGTGAACTAGATTAAATCAGAAGTTCAAGGACAAGGC CACATTGACTGTAGACAAATCCTCCAGTACAGCCTACATGCAACTCAGCA GCCCCACATCTGAGGACTCTGCGATCTATTATTGTGCAAGATCACAGTTC GGGCTACGTTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGC A (SEQ ID NO: 85)</div>
	light chain	<div>GACATTGTGATGACCCAGTCTCCATCCTCCATGACTGTGACAGCAGGAGA GAAGGTCACTATGAGCTGCCAGTCCAGTCAGAGTCTATTCAACAGTGGAA CTCAAAAGAACTACTTGACCTGGTACCAGCAGAAACCAGGACAGCCTCCT AAACTGTTGATCTCCTGGGCATCCACTAGGGATTCTGGGGTCCCTGATCG CTTCACAGGCAGTGGATCTGGAACAGATTTCACTCTCACCATTAACGGTG TGCAGGCTGAAGACCTGGCAGTTTATTTCTGTGCAAGATGATTATGATTCT CCGTACACGTTCCGGAGGGGGGACGAAGCTGGAAATAAAACGG (SEQ ID NO: 86)</div>
22-1.5	heavy chain	<div>GAGGTGCAAACCTGCAGCAGTCAGGACCTGAGCTGGTGAAACCTGGGGCC TCAGTGAAGATATCCTGCAAGGCTTCTGGATACACATTCACTGACTACAA CATACACTGGGTGAAACAGAGCCCTGGAAAGAGCCTTGAGTGGATTGGAT</div>

		ATATTTATCCTGACAATGGTGATACTGGCTACAACCAGATTTCAAGAAC AAGGCCACATTGACTGTAGACACTTCGTCCAGCGCAGCCTACATGGAAC CCGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGTAAGACGGG TCCTTTTGGACTCCTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 87)
light chain		GATATCCAGATGACACAGACTACATCTTCCCTGTCTGCCTCTCTGGGAGA CAGAGTCACCATCAGTTGCAGTGCAAGTCAGGACATTAACAATTTTTTAA ACTGGTATCAGCAGAAACCAGATGGAACTATTAAACTCCTGATCTATTAC ACATCAAGTTTACAGTCAGGAGTCCCGTCAAGGTTTCAGTGGCAGTGGGTC TGGGACAGATTATTCTCTCACCATCAGCAACCTGGAACCTGAAGATATTG CCACTTACTATTGTCAACAATATAGTAAACTTCCTCGGACGTTCCGTGGA GGCACCAAGCTGGAAATCAAACGG (SEQ ID NO: 88)
82-1.1	heavy chain	GAGGTGCAGCTGCAGCAGTCAGGGGGAGGCTTAGTGCAGCCTGGAGGGTC CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTTCAGTAACTATGACA TGTCTTGGATTCCGACAGTCCAGACAAGAGGCTGGAGATGGTCGCAGCC ATTAATAGTAATGGTGGTAGCACCTATTATCCAGACAGTGTGAAGGGCCG ATTCACCATCTCCAGAGACAAAGCCAAGAACCCTATACCTGCAAATGA GCAGTCTGAAGTCTGAGGACACAGCCATGTATTACTGTGCGAGCCCTAAT GGTTACGGAGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTC CTCA (SEQ ID NO: 89)
light chain		GATATTGTGATAACCCAGACTCCACTCACTTTGTTCGGTTACCATTGGACA ACCAGCTTCCATCTCTTGCAAGTCACGTCAGAGCCTCTTAGATAGTGATG GAAAAACCTATTTAAATTGGTTATTACAGAGGCCAGGCGAGTCTCCAAAG CTCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTT CACTGGCAGTGGATCAGGGACAGATTTCACTGAAAATCAGCAGAGTGG AGGCTGAGGATTTGGGAGTTTATTACTGCTTGCAAGCTACACATTTCCG TGGACGTTCCGTGGAGGCACCAAGCTGGAAATCAAACGG (SEQ ID NO: 90)

[000160] 2.2 Sandwich (capture) ELISA

[000161] 2.2.1 Serotype-specific mAbs as capture antibodies

[000162] The four serotype-specific mAbs of the present invention were used as capture antibodies in sandwich (capture) ELISA. Fig. 1A shows the basic design of the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention as capture antibodies, paired with cross-reactive mAbs as detection antibodies.

[000163] Microwell plates were coated with serotype-specific mAbs of the present invention as capture antibodies and incubated overnight at 4°C. The wells were then blocked and washed. The cell culture supernatants of Vero cells infected with different serotype DENVs (DENV1 Hawaii, DENV2 16681, DENV3 H87 and DENV4 H241) or Japanese encephalitis (JE) virus or the cell culture supernatants of non-infected Vero cells (a negative control) were separately added to the wells and incubated at 37°C for one hour. After washing, cross-reactive mAbs labelled with horseradish peroxidase (HRP), were added to the wells and incubated at 37°C for one

hour. The wells were washed again and 3,3',5,5'- tetramethylbenzidine (TMB), was added as a substrate of HRP to the wells. The absorbance was read at 450 nm. As shown in Fig. 1B, the serotype-specific mAbs of the present invention were demonstrated to successfully act as capture antibodies in sandwich (capture) ELISA, exhibiting superior serotype specificity without cross-reaction with other flavivirus (Japanese encephalitis (JE) virus).

[000164] 2.2.2 Serotype-specific mAbs as detection antibodies

[000165] The four serotype-specific mAbs of the present invention were used as detection antibodies in sandwich (capture) ELISA. Fig. 2A shows the basic design of the sandwich (capture) ELISA using the serotype-specific mAbs of the present invention as detection antibodies, paired with cross-reactive mAbs as capture antibodies.

[000166] Microwell plates were coated with cross-reactive mAbs as capture antibodies and incubated overnight at 4°C. The wells were then blocked and washed. The cell culture supernatants of Vero cells infected with different serotype DENVs (DENV1 Hawaii, DENV2 16681, DENV3 H87 and DENV4 H241) or Japanese encephalitis (JE) virus or the cell culture supernatants of non-infected Vero cells (a negative control) were separately added to the wells and incubated at 37°C for one hour. After washing, serotype-specific mAbs of the present invention labelled with horseradish peroxidase (HRP), were added to the wells and incubated at 37°C for one hour. The wells were washed again and 3,3',5,5'- tetramethylbenzidine (TMB), was added as a substrate of HRP to the wells. The absorbance was read at 450 nm. As shown in Fig. 2B, the serotype-specific mAbs of the present invention were demonstrated to successfully act as detection antibodies in sandwich (capture) ELISA, exhibiting superior serotype specificity without cross-reaction with other flavivirus (Japanese encephalitis (JE) virus).

[000167] 2.2.3 Detection limits

[000168] Capture ELISA was performed with serial diluted and purified NS1 proteins of each DENV serotype to determine the detection limits of the serotype-specific mAbs of the present invention. Table 4 and Fig. 3 shows the results.

[000169] Table 4. Detection limits of four serotype-specific NS1 capture ELISA of the present invention and commercially available Platelia Dengue NS1 AG ELISA

NS1 detection assays	Minimum detectable concentration of NS1 proteins (ng/ml)
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	D1	D2	D3	D4
Four serotype-specific NS1 capture ELISA	1.953	3.906	3.906	0.977
Platelia Dengue NS1 AG ELISA	3.906	31.250	0.977	7.813

Sensitivity comparison between the four serotype-specific NS1 capture ELISA and the commercially available Platelia Dengue NS1 AG ELISA in detecting NS1. Each NS1 protein serotype was immunoaffinity-purified and serially diluted prior to performing this analysis.

5

[000170] The results show that the capture ELISA using the serotype-specific mAbs of the present invention achieves superior sensitivity, ranging from 1 to 4 ng/ml of DENV1-4 NS1 proteins, comparable to the commercially available DENV ELISA kit (Platelia NS1 Ag ELISA, Bio-Rad) while the commercial kit cannot distinguish the serotypes.

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[000171] 2.3 Strip

[000172] The four serotype-specific mAbs of the present invention were also used to develop strips for DENV detection. Fig. 4 shows the basic design of serotyping NS1 strip using the serotype-specific mAbs of the present invention as capture antibodies (upper panel) or detection antibodies (detection panel), paired with cross reactive mAbs.

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[000173] As shown in Fig. 5, the serotype-specific mAbs of the present invention (12-4.1, 33-7.1, 43-1.3, and 22-1.5) were demonstrated to successfully act as capture antibodies, pairing with the cross-reactive mAb of the present invention (82.1.1) as detection antibodies, in serotyping NS1 strip, exhibiting superior serotype specificity to DENV1, DENV2, DENV3 and DENV4 (from top to bottom), respectively, without cross-reaction with other flavivirus (Japanese encephalitis (JE) virus and Zika virus (ZIKV)).

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[000174] In addition, the detection limits of the serotype-specific mAbs of the present invention in strips were determined. Table 5 shows the results.

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[000175] Table 5 Detection limits of four serotype-specific NS1 strips, Bio-Rad Dengue NS1 AG rapid test, and SD dengue NS1 Ag rapid test

Minimum detectable concentration of NS1 proteins (ng/ml)

	D1	D2	D3	D4
four serotype-specific NS1 strips	125	62.5	62.5	62.5
Bio-Rad dengue NS1 Ag	31.25	125~250	31.25	62.5
SD dengue NS1 Ag	62.5	125~250	31.25	125
Sensitivity of the four serotype NS1 strips in detecting NS1 protein. Each NS1 protein serotype was immunoaffinity-purified and serially diluted prior to performing this analysis.				

[000176] The results show that the NS1 strips using the serotype-specific mAbs of the present invention achieves superior sensitivity, ranging from 62.5 to 125 ng/ml of DENV1-4 NS1 proteins, comparable to the commercially available DENV strip kits, while these commercial kits cannot distinguish the serotypes.

[000177] 2.4 Clinical tests

[000178] A total of 146 clinical serum samples were collected to determine the sensitivity and specificity of sandwich (capture) ELISA using the four serotype-specific mAbs of the present invention.

[000179] The 146 clinical serum samples were collected between one (1) and seven (7) days after the onset of illness and analyzed by conventional serotyping RT-PCR, dengue specific IgM/IgG capture ELISA, commercial dengue NS1 Ag ELISA methods, and serotype-specific NS1 capture ELISA of the present invention. Table 6 shows the results. Fig. 6 shows the specificities of the sandwich (capture) ELISA of the present invention for testing sera of acute febrile patients.

[000180]

Table 6. Examination of acute clinical sera by RT-PCR, IgM/IgG, Platelina Dengue NS1 AG ELISA, and four serotype-specific NS1 capture ELISA of the present invention

Day that sera was collected (post onset of illness)	Total number of samples	RT-PCR serotyping ^b				IgM/IgG ^c	Platelina NS1 Ag ELISA	Negative ^d	Four serotype-specific NS1 capture ELISA of the present invention			
		D1	D2	D3	D4				D1	D2	D3	D4
1	45	6	4	4	4	0	14	27	5	4	4	3
2	46	4	7	8	1	2	19	24	3	9	6	0
3	21	3	1	1	2	0	4	14	3	1	0	2
4	12	2	3	2	1	0	8	4	2	3	1	1
5	7	0	2	0	0	1	2	4	0	2	0	0
6	6	1	1	1	0	1	4	2	1	2	1	0
7	1	0	0	0	0	0	0	1	0	0	0	0
Unknown ^a	8	1	1	0	1	1	4	4	1	2	0	0
total	146	17	19	16	9	5	55	80	15	23	12	6

^a No information pertaining to sera collection date was available.

^b Serotype-specific one-step SYBR Green I-based RT-PCR

^c Negative result by RT-PCR but positive result both by dengue virus specific IgM/IgG capture ELISA and Platelina Dengue NS1 AG ELISA

^d Negative result by RT-PCR, dengue virus specific IgM/IgG capture ELISA, and Platelina Dengue NS1 AG ELISA

[000181] The results show that the 146 clinical serum samples were confirmed to include seventeen (17) samples of DENV1 serotype, nineteen (19) samples of DENV2 serotype, sixteen (16) samples of DENV3 serotype, and nine (9) samples of DENV4 serotype; five (5) samples were determined negative by RT-PCR but positive by dengue virus specific IgM/IgG capture ELISA; fifty-five (55) samples were determined positive by Platelia NS1 Ag ELISA; and eighty (80) samples were determined negative by all the tested methods.

[000182] The results show that sandwich (capture) ELISA using the four serotype-specific mAbs of the present invention exhibit excellent sensitivity and specificity, wherein D1 ELISA (mAb12-4.1) capable of detecting 15 DENV1 samples from 17 DENV1 samples, D2 ELISA (mAb33-7.) capable of detecting 18 DENV2 samples from 19 DENV2 samples, D3 ELISA (mAb43-1.3) capable of detecting 12 DENV3 samples from 16 DENV3 samples, and D4 ELISA (mAb22-1.5) capable of detecting 6 DENV4 samples from 9 DENV1 samples; the five (5) samples that were not detected by serotype RT-PCR were determined positive and identified as DENV2; and all the eighty (80) negative samples were determined as negative as well without false positive results. Fig. 6A to Fig. 6F show the specificities of the sandwich (capture) ELISA of the present invention for testing sera of acute febrile patients.

[000183] Tables 8.1 and 8.2 summarize the results of sensitivity and specificity of sandwich (capture) ELISA using the four serotype-specific mAbs of the present invention.

[000184] Table 8.1

ELISA test	D1	D2	D3	D4
No. of all positive ^a	17	19	16	9
No. of true positive	15	18	12	6
No. of true negative	80	80	80	80
No. of false positive	0	0	0	0
No. of false negative	2	1	4	3
Serotype specificity	100	100	100	100
Serotype sensitivity	0.882	0.947	0.75	0.666

^a Serotype by serotype-specific one-step SYBR Green I-based RT-PCR

[000185] Table 8.2

No. of sera with NS1 result of positive	True status: Disease (66); No disease (80)				
	% sensitivity (95% CI)	% specificity (95% CI)	% accuracy (95% CI)	% PPV (95% CI)	% NPV (95% CI)
56	84.85 (73.9 - 92.49)	100 (95.49 – 100)	93.15 (87.76 -96.67)	100 (93.63 – 100)	88.89 (80.51 – 94.54)

PPV= positive predictive value; NPV= negative predictive value

[000186] The results show the sandwich (capture) ELISA based on the four serotype-specific mAbs of the present invention is capable to detect and distinguish the four dengue virus serotypes in serum samples even after the early five(5) days post onset of illness, exhibiting overall 100% specificity and 84.8% sensitivity.

CLAIMS

What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof specific for dengue virus, wherein the isolated antibody is selected from the group consisting of:
 - 5 (i) a first antibody specific for dengue virus serotype 1 (DENV1) comprising
 - (a) a heavy chain variable region (V_H) which comprises a heavy chain complementary determining region (HC CDR1) of SEQ ID NO: 2, a heavy chain complementary determining region 2 (HC CDR2) of SEQ ID NO: 4, and a heavy chain complementary determining region 3 (HC CDR3) of SEQ ID NO: 6; and
 - 10 (b) a light chain variable region (V_L) which comprises a light chain complementary determining region 1 (LC CDR1) of SEQ ID NO: 9, a light chain complementary determining region (LC CDR2) of SEQ ID NO: 11, and a light chain complementary determining region 3 (LC CDR3) of SEQ ID NO: 13;
 - (ii) a second antibody specific for dengue virus serotype 2 (DENV2) comprising
 - 15 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 16, a HC CDR2 of SEQ ID NO: 18, and a HC CDR3 of SEQ ID NO: 20; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 23, a LC CDR2 of SEQ ID NO: 25, and a LC CDR3 of SEQ ID NO: 27;
 - (iii) a third antibody specific for dengue virus serotype 3 (DENV3) comprising
 - 20 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 30, a HC CDR2 of SEQ ID NO: 32, and a HC CDR3 of SEQ ID NO: 34; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 37, a LC CDR2 of SEQ ID NO: 39, and a LC CDR3 of SEQ ID NO: 41;
 - (iv) a fourth antibody specific for dengue virus serotype 4 (DENV4) comprising
 - 25 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 44, a HC CDR2 of SEQ ID NO: 46, and a HC CDR3 of SEQ ID NO: 48; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 51, a LC CDR2 of SEQ ID NO: 53, and a LC CDR3 of SEQ ID NO: 55; and
 - (v) a fifth antibody cross-reactive to DENV1, DENV2, DENV3 and DENV4
 - 30 comprising
 - (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 58, a HC CDR2 of SEQ ID NO: 60, and a HC CDR3 of SEQ ID NO: 62; and
 - (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 65, a LC CDR2 of

SEQ ID NO: 67, and a LC CDR3 of SEQ ID NO: 69; and

(vi) any combination of (i) to (v).

2. The isolated antibody or antigen-binding fragment of claim 1, wherein

5 (i) the first antibody comprises a V_H that comprises SEQ ID NO: 71 and a V_L comprising SEQ ID NO: 72;

(ii) the second antibody comprises a V_H comprising SEQ ID NO: 73 and a V_L comprising SEQ ID NO: 74;

10 (iii) the third antibody comprises a V_H comprising SEQ ID NO: 75 and a V_L comprising SEQ ID NO: 76;

(iv) the fourth antibody comprises a V_H comprising SEQ ID NO: 77 and a V_L comprising SEQ ID NO: 78; and/or

(v) the fifth antibody comprises a V_H comprising SEQ ID NO: 79 and a V_L comprising SEQ ID NO: 80.

15

3. The isolated antibody or antigen-binding fragment of claim 1, wherein the antigen-binding fragment is selected from the group consisting of scFv, (scFv)₂, Fab, Fab', and F(ab')₂ of the isolated antibody specific for dengue virus.

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4. A composition comprising the isolated antibody or antigen-binding fragment thereof of claim 1.

5. The composition of claim 4, which is a pharmaceutical or diagnostic composition for use in treatment or diagnosis of dengue virus disease.

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6. The composition of claim 4, which comprises a pharmaceutically acceptable carrier.

7. A method for detecting dengue virus in a sample suspected of containing
30 said dengue virus, comprising contacting the sample with an isolated antibody or antigen-binding fragment thereof specific for dengue virus, and assaying binding of the antibody with said sample, wherein the isolated antibody is selected from the group consisting of:

(i) a first antibody specific for dengue virus serotype 1 (DENV1) that comprises

a V_H comprising a HC CDR1 of SEQ ID NO: 2, a HC CDR2 of SEQ ID NO: 4, and a HC CDR3 of SEQ ID NO: 6; and a V_L comprising a LC CDR1 of SEQ ID NO: 9, a LC CDR2 of SEQ ID NO: 11, and a LC CDR3 of SEQ ID NO: 13;

(ii) a second antibody specific for dengue virus serotype 2 (DENV2) that
5 comprises a V_H comprising a HC CDR1 of SEQ ID NO: 16, a HC CDR2 of SEQ ID NO: 18, and a HC CDR3 of SEQ ID NO: 20; and a V_L comprising a LC CDR1 of SEQ ID NO: 23, a LC CDR2 of SEQ ID NO: 25, and a LC CDR3 of SEQ ID NO: 27;

(iii) a third antibody specific for dengue virus serotype 3 (DENV3) that
10 comprises a V_H comprising a HC CDR1 of SEQ ID NO: 30, a HC CDR2 of SEQ ID NO: 32, and a HC CDR3 of SEQ ID NO: 34; and a V_L comprising a LC CDR1 of SEQ ID NO: 37, a LC CDR2 of SEQ ID NO: 39, and a LC CDR3 of SEQ ID NO: 41;

(iv) a forth antibody specific for dengue virus serotype 4 (DENV4) that
comprises a V_H comprising a HC CDR1 of SEQ ID NO: 44, a HC CDR2 of SEQ ID NO: 46, and a HC CDR3 of SEQ ID NO: 48; and a V_L comprising a LC CDR1 of
15 SEQ ID NO: 51, a LC CDR2 of SEQ ID NO: 53, and a LC CDR3 of SEQ ID NO: 55;
and

(v) any combination of (i) to (iv).

8. The method of claim 7, wherein each of the first antibody, the second antibody,
20 the third antibody and the fourth antibody, is paired with a partner antibody.

9. The method of claim 8, wherein the partner antibody is a serotype cross-reactive antibody to dengue virus which comprises

(a) a V_H which comprises a HC CDR1 of SEQ ID NO: 58, a HC CDR2 of
25 SEQ ID NO: 60, and a HC CDR3 of SEQ ID NO: 62; and

(b) a V_L which comprises a LC CDR1 of SEQ ID NO: 65, a LC CDR2 of SEQ ID NO: 67, and a LC CDR3 of SEQ ID NO: 69.

10. The method of claim 7, wherein

30 (i) binding of the first antibody with the sample is an indicative of the presence of DENV1 in the sample;

(ii) binding of the second antibody to the dengue virus is an indicative of the presence of DENV2 in the sample;

(iii) binding of the third antibody to the dengue virus is an indicative of the

presence of DENV3 in the sample; and/or

(iv) binding of the fourth antibody to the dengue virus is an indicative of the presence of DENV4 in the sample.

5 11. A kit for detecting the presence of dengue virus in a sample, wherein the kit comprises one or more antibodies specific to dengue virus, selected from the group consisting of:

 (i) a first antibody specific for dengue virus serotype 1 (DENV1) that comprises a V_H comprising a HC CDR1 of SEQ ID NO: 2, a HC CDR2 of SEQ ID NO: 4, and a
10 HC CDR3 of SEQ ID NO: 6; and a V_L comprising a LC CDR1 of SEQ ID NO: 9, a LC CDR2 of SEQ ID NO: 11, and a LC CDR3 of SEQ ID NO: 13;

 (ii) a second antibody specific for dengue virus serotype 2 (DENV2) that comprises a V_H comprising a HC CDR1 of SEQ ID NO: 16, a HC CDR2 of SEQ ID NO: 18, and a HC CDR3 of SEQ ID NO: 20; and a V_L comprising a LC CDR1 of
15 SEQ ID NO: 23, a LC CDR2 of SEQ ID NO: 25, and a LC CDR3 of SEQ ID NO: 27;

 (iii) a third antibody specific for dengue virus serotype 3 (DENV3) that comprises a V_H comprising a HC CDR1 of SEQ ID NO: 30, a HC CDR2 of SEQ ID NO: 32, and a HC CDR3 of SEQ ID NO: 34; and a V_L comprising a LC CDR1 of
SEQ ID NO: 37, a LC CDR2 of SEQ ID NO: 39, and a LC CDR3 of SEQ ID NO: 41;

20 (iv) a forth antibody specific for dengue virus serotype 4 (DENV4) that comprises a V_H comprising a HC CDR1 of SEQ ID NO: 44, a HC CDR2 of SEQ ID NO: 46, and a HC CDR3 of SEQ ID NO: 48; and a V_L comprising a LC CDR1 of
SEQ ID NO: 51, a LC CDR2 of SEQ ID NO: 53, and a LC CDR3 of SEQ ID NO: 55;

 (v) any combination of (i) to (iv); and

25 (vi) an optional partner antibody.

 12. The kit of claim 11, wherein the partner antibody is a serotype cross-reactive antibody to dengue virus which comprises

 (a) a V_H which comprises a HC CDR1 of SEQ ID NO: 58, a HC CDR2 of
30 SEQ ID NO: 60, and a HC CDR3 of SEQ ID NO: 62; and

 (b) a V_L which comprises a LC CDR1 of SEQ ID NO: 65, a LC CDR2 of
SEQ ID NO: 67, and a LC CDR3 of SEQ ID NO: 69.

 13. The kit of claim 11, wherein at least one of the serotype-specific antibodies

or the partner antibody comprises a detectable label.

14. The kit of claim 13, wherein the detectable label is selected from the group consisting of an enzymatic label, a fluorescent label, a metal label and a radio label.

5

15. The kit of claim 13, wherein the detectable label is selected from the group consisting of gold nanoparticles, colored latex beads, magnetic particles, carbon nanoparticles, selenium nanoparticles.

10 16. The kit of claim 11, wherein the kit is an immunoassay kit.

17. The kit of claim 16, wherein the immunoassay is selected from the group consisting of ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), FIA (fluorescence immunoassay), LIA (luminescence immunoassay), or ILMA
15 immunoluminometric assay.

18. The kit of claim 16, wherein the immunoassay is in a lateral flow assay format.

20 19. The kit of claim 16, wherein the immunoassay is a sandwich assay.

20. A nucleic acid comprising a nucleotide sequence encoding a heavy chain variable region (V_H), a light chain variable region (V_L) or both, wherein the V_H and V_L are as set forth in claim 1.

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21. A host cell comprising the nucleic acid of claim 20.

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