DIAGNOSTIC KIT FOR RAPIDLY DIAGNOSING A DENGUE VIRUS, A METHOD OF PREPARING THE DIAGNOSTIC KIT AND A METHOD OF RAPIDLY DIAGNOSING A DENGUE VIRUS USING THE DIAGNOSTIC KIT

FIELD OF THE TECHNOLOGY

The present disclosure generally relates to the rapid diagnosis and/or rapid detection of a dengue virus. In particular, the present disclosure relates to the rapid diagnosis and/or rapid detection of a dengue virus by aggregation.

BACKGROUND

The dengue virus belongs to the virus family Flaviviridae. The dengue virus is believed to be the most common arthropod-borne disease in the world. Transmission of the dengue virus to people primarily occurs through the bite of dengue virus infected mosquitoes of the genus *Aedes* and more specifically of the *Aedes* species *Aedes aegypti* and *Aedes albopictus.* The mosquitoes require a warm climate and, therefore, the dengue virus is mainly found in the tropical and subtropical regions of Asia, Africa, Central America and South America. The dengue virus is transmitted to a mosquito of the genus *Aedes* when the mosquito bites an individual infected with the dengue virus.

It is estimated that every year one hundred (100) million people become infected with the dengue virus. Infection with the dengue virus can produce a wide spectrum of clinical manifestations. For example, manifestations can range from an asymptomatic manifestation or a non-specific febrile illness to dengue fever or the potentially fatal dengue haemorrhagic fever/dengue shock syndrome. Dengue fever and dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) are major public health problems in tropical and subtropical countries.

There are four strains of the dengue virus, which are called serotypes, and these are referred to as dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4). The four (4) serotypes of the dengue virus are closely related at the genetic level. However, exposure to more than one serotype of the dengue virus can be potentially fatal. The majority of deaths that result from dengue virus infection are from dengue haemorrhagic fever/dengue shock syndrome.

Dengue hemorrhagic fever/dengue shock syndrome occurs when an individual recovers from an initial dengue virus infection caused by a first serotype of the dengue virus and develops an immune response to that particular serotype of the dengue virus. The immune response produces specific antibodies to that particular dengue virus serotype's surface proteins. However, at the event of an infection involving a second serotype of the dengue virus, the second serotype of the dengue virus will activate the immune system and "trick" it. The immune system will attack the second serotype of the dengue virus as if it was the first serotype of the dengue virus. The immune system produces antibodies that will bind to the surface proteins of the second serotype of the dengue virus but do not inactivate the second serotype of the dengue virus. Thus, the immune system responds by attracting numerous white blood cells (macrophages) that are supposed to remove the second serotype of the dengue virus. However, instead the second serotype of the dengue virus infects the white blood cells. This makes the viral infection much more acute. The cytokines released by the body cause the endothelial tissue to become permeable, which results in hemorrhagic fever and fluid loss from blood vessels.

To date, there are no effective antiviral drugs or vaccines available for treating dengue virus infections. All the available treatments for typical dengue infections are symptomatic-based and are not very effective. With no available drugs or vaccines currently available, rapid and accurate diagnosis of dengue infections is important for better patient management, for example, in hospitals, clinics, homes and/or remote areas where access to medical care is limited.

The current gold standard for dengue virus diagnosis and identification involves viral isolation in cell cultures or Toxorhynchites species mosquito larvae followed by direct

detection using immunofluorescence (IF). However, this requires cell culture facilities and trained personnel to operate the cell culture facilities. In addition, the viral isolation usually takes longer than seven days to complete.

Two other techniques have also been shown to be useful in dengue virus diagnosis, such as, the enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcriptase polymerase chain reaction (RT-PCR). However, ELISA takes at least about 6 hours to complete while the entire real-time RT-PCR method takes about 3 hours to complete. Further, the real-time RT-PCR method requires additional post-PCR handling which increases the risks of contamination and/or cross-contamination of specimens.

As can be seen from the above discussion, the known techniques currently being used in the art are not appropriate for a rapid test kit for use at point of care. Instead, these techniques are expensive and time consuming.

Thus, there is an urgent need for a diagnostic kit for rapidly diagnosing or rapidly detecting dengue virus infections and/or a method for rapidly diagnosing or rapidly detecting dengue virus infections so that earlier patient management can be carried out.

SUMMARY

The present disclosure relates to a diagnostic kit for rapidly diagnosing a dengue virus, a method of preparing the diagnostic kit and a method of rapidly diagnosing or detecting the presence of a dengue virus in a sample using the diagnostic kit. The diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure can generate specific diagnostic results in about 15 minutes or less.

Further the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure can have an accuracy or efficiency of 98% or greater in diagnosing and/or detecting dengue virus

infections from samples. Thus, the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure enable efficient, effective and accurate diagnostic or clinical testing for dengue virus infections thereby improving patient management and improving the likelihood of recovery from dengue virus infections. In embodiments, the samples can be clinical samples and/or biological samples. In embodiments, the samples can be biological fluid samples. In embodiments, the biological fluid can be plasma, serum, spinal fluid, lymph fluid, synovial fluid, urine or tears.

A first aspect of the present disclosure provides a method of preparing a diagnostic kit for rapidly diagnosing a dengue virus that can comprise: designing a first oligonucleotide comprising a sequence that is complementary to a target sequence of the dengue virus; providing a plurality of carbon nanotubes; and providing a plurality of solid supports.

In embodiments, the method of preparing a diagnostic kit described above can further comprise designing a second oligonucleotide comprising a sequence that is complementary to the target sequence of the dengue virus.

In embodiments, the target sequence of the dengue virus can comprise a target sequence of the NS1 region of the dengue virus. In embodiments, the first oligonucleotide comprises a sequence that is complementary to the target sequence of the NS1 region of the dengue virus and the second oligonucleotide comprises a sequence that is complementary to the target sequence of the NS1 region of the dengue virus.

In embodiments, the carbon nanotubes can be single walled carbon nanotubes. In embodiments, the single walled carbon nanotubes are carboxylated single walled carbon nanotubes.

In embodiments, the solid supports can be magnetic beads. In embodiments, the plurality of magnetic beads can be streptavidin functionalized magnetic beads or carboxylic acid functionalized magnetic beads.

In embodiments, the method of preparing a diagnostic kit described above can further comprise: designing the first oligonucleotide to function as a detection probe; tagging the first oligonucleotide with a NH_2 functional group on the 5' end of the first oligonucleotide; and hybridising a plurality of the first oligonucleotide to the plurality of single walled carbon nanotubes to form a plurality of carbon nanotube based labels.

In embodiments, the method of preparing a diagnostic kit described above can further comprise: designing the second oligonucleotide to function as a capture probe; biotinylating the second oligonucleotide; tagging the second oligonucleotide with a NH_2 functional group on the 3'end of the second oligonucleotide; and immobilising a plurality of the second oligonucleotide to the plurality of magnetic beads to form a plurality of conjugated magnetic beads.

In embodiments, the method of preparing a diagnostic kit described above can further comprise: designing a third oligonucleotide comprising a sequence that is non-complementary to the target sequence of the dengue virus, wherein the third oligonucleotide functions as a negative control sequence.

In embodiments, the method of preparing a diagnostic kit described above can further comprise: hybridising the plurality of carbon nanotube labels with the plurality of conjugated magnetic beads to form a mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads.

In embodiments, a plurality of the third oligonucleotide and the mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads can be provided in one test tube.

In embodiments, the dengue virus can be dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) or dengue virus serotype 4 (DENV-4).

A second aspect of the present disclosure provides a diagnostic kit for rapidly diagnosing a dengue virus, wherein the diagnostic kit can be prepared accorded to the method of preparing a diagnostic kit described above.

A third aspect of the present disclosure provides a diagnostic kit for rapidly diagnosing a dengue virus that can comprise: a mixture of a plurality of carbon nanotube based labels and a plurality of conjugated magnetic beads, wherein the plurality of carbon nanotube labels are hybridised with the plurality of conjugated magnetic beads, and wherein the mixture is provided in one test tube.

In embodiments, the plurality of carbon nanotube based labels of the mixture can comprise a plurality of a first oligonucleotide hybridised to a plurality of single walled carbon nanotubes, and the first oligonucleotide can comprise a sequence that is complementary to a target sequence of the dengue virus.

In embodiments, the first oligonucleotide functions as a detection probe, and the first oligonucleotide is tagged with a NH_2 functional group on the 5'end of the first oligonucleotide to facilitate hybridisation of the plurality of the first oligonucleotide to the plurality of single walled carbon nanotubes to form the plurality of carbon nanotube based labels.

In embodiments, the plurality of conjugated beads of the mixture can comprise a plurality of a second oligonucleotide immobilised to a plurality of magnetic beads, and the second oligonucleotide can comprise a sequence that is complementary to the target sequence of the dengue virus.

In embodiments, the second oligonucleotide functions as a capture probe, the second oligonucleotide is biotinylated, and the second oligonucleotide is tagged with a NH_2 functional group on the 3'end of the second oligonucleotide to facilitate the immobilisation of the plurality of the second oligonucleotide to the plurality of magnetic beads to form the plurality of conjugated magnetic beads.

In embodiments, the target sequence of the dengue virus can comprise a target sequence of the NS1 region of the dengue virus.

In embodiments, the plurality of magnetic beads are streptavidin functionalized magnetic beads or carboxylic acid functionalized magnetic beads.

In embodiments, the diagnostic kit described above can further comprise: a third oligonucleotide comprising a sequence that is non-complementary to the target sequence of the dengue virus, wherein the third oligonucleotide functions as a negative control sequence, and wherein a plurality of the third oligonucleotide is provided in the one test tube.

In embodiments, the dengue virus can be dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) or dengue virus serotype 4 (DENV-4).

A fourth aspect of the present disclosure provides a method of rapidly diagnosing a dengue virus that can comprise: providing the diagnostic kit described above; providing target ribonucleic acid (hereinafter 'RNA') of the dengue virus; and adding the target RNA of the dengue virus to the mixture of the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads in the one test tube.

In embodiments, the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads aggregate in the presence of the target RNA of the dengue virus. In embodiments, the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads aggregate in the presence of the target RNA of the dengue virus to form dark coloured complexes. In embodiments, the formation of dark coloured complexes allows for visual detection of the target RNA of the dengue virus. In embodiments, the dark coloured complexes are dark brown.

In embodiments, the aggregation of the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads is dependent on the concentration of the target RNA of the dengue virus added to the mixture.

In embodiments, the dengue virus can be dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) or dengue virus serotype 4 (DENV-4).

In embodiments, the formation of the dark coloured complexes can be generated in about 15 minutes or less, and the diagnosis of the dengue virus can be achieved in about 15 minutes or less. In embodiments, the formation of the dark coloured complexes can be generated in about 10 minutes or less, and the diagnosis of the dengue virus can be achieved in about 10 minutes or less. In embodiments, the formation of the dark coloured complexes can be generated in about 5 minutes or less, and the diagnosis of the dengue virus can be achieved in about 5 minutes or less. In embodiments, the dark coloured complexes are dark brown.

In embodiments, the method of rapidly diagnosing a dengue virus described above can have an accuracy of about 98% or greater for diagnosing the dengue virus. In embodiments, the method of rapidly diagnosing a dengue virus can have an accuracy of about 99% or greater for diagnosing the dengue virus. In embodiments, the method of rapidly diagnosing a dengue virus can have an accuracy of about 100% for diagnosing the dengue virus.

In embodiments, the method of rapidly diagnosing a dengue virus can have a specificity of about 98% or greater for diagnosing the dengue virus. In embodiments, the method of rapidly diagnosing a dengue virus can have a specificity of about 99% or greater for diagnosing the dengue virus. The method of rapidly diagnosing a dengue virus can have a specificity of about 100% for diagnosing the dengue virus.

A fifth aspect the present disclosure provides a method of preparing a diagnostic kit for rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

A sixth aspect of the present disclosure provides a diagnostic kit for rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

A seventh aspect of the present disclosure provides a method of rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present disclosure are described herein with reference to the drawings in which:

FIG. 1 illustrates the hybridisation or conjugation of an oligonucleotide detection probe to a single walled carbon nanotube by diimide-activated amidation in accordance with an embodiment of the present disclosure.

FIG. 2 is a schematic diagram of a carbon nanotube based label in accordance with an embodiment of the present disclosure.

FIG. 3A – 3C illustrate the aggregation of carbon nanotube based labels and conjugated magnetic beads in the presence of a target dengue virus ribonucleic acid (hereinafter 'RNA') sequence to form dark coloured complexes with the target dengue virus RNA sequence in accordance with an embodiment of the present disclosure. The formation of dark coloured complexes allows for visual detection of the target dengue virus RNA. FIG. 3A illustrates the principle of aggregation and the formation of dark coloured complexes when a high concentration (i.e., about 80 ng/μL) of the target dengue virus RNA sequence is present. FIG. 3B illustrates the principle of aggregation and the formation of dark coloured complexes when a low concentration (i.e., about 2.5 ng/μL) of the target dengue virus RNA sequence is present. FIG. 3C illustrates the use of a negative control.

FIG. 4A-4E illustrates the effect of the concentration of the target dengue virus RNA sequence present on the aggregation and the formation of dark coloured complexes. FIG. 4A illustrates the effect of the concentration of the target dengue virus serotype type 1 (DENV-1) RNA sequence present on the aggregation and the formation of dark coloured complexes. FIG. 4B illustrates the effect of the concentration of the target dengue virus serotype 2 (DENV-2) RNA sequence present on the aggregation and the formation of dark coloured complexes. FIG. 4C illustrates the effect of the concentration of the target dengue virus serotype 3 (DENV-3) RNA sequence present on the aggregation and the formation of dark coloured complexes. FIG. 4D illustrates the effect of the concentration of the target dengue virus serotype 4 (DENV-4) RNA sequence present on the aggregation and the formation of dark coloured complexes. FIG. 4E illustrates the effect of the concentration of the target pan-dengue virus RNA sequence present on the aggregation and the formation of dark coloured complexes.

DETAILED DESCRIPTION

In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments can be utilized, and other changes can be made, without departing from the spirit or scope of the subject matter presented herein.

Unless specified otherwise, the terms "comprising" and "comprise" as used herein, and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, un-recited elements.

As used herein, the term "about", in the context of concentrations of components, conditions, other measurement values, etc., means +/- 5% of the stated value, or +/- 4% of

the stated value, or +/- 3% of the stated value, or +/- 2% of the stated value, or +/- 1% of the stated value, or +/- 0.5% of the stated value, or +/- 0% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

As used herein, unless specified otherwise, the term “diagnosis” includes detection. Additionally, as used herein, unless specified otherwise, the term “diagnosing” includes detecting.

The present disclosure relates to a diagnostic kit for rapidly diagnosing dengue viruses, a method of preparing the diagnostic kit and a method of rapidly diagnosing or detecting the presence of dengue viruses in samples using the diagnostic kit.

The diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure can generate specific diagnostic results in about 15 minutes or less, which is about one-twelfth (1/12) of the time required by the presently known method of real-time PCR. Additionally, the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure includes a one step process and a fast single test-tube reaction that does not require sophisticated and expensive instruments/machines.

Further the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure can have an accuracy or efficiency of 98% or greater in diagnosing and/or detecting dengue virus

infections from samples. In embodiments, the samples can be clinical samples and/or biological samples. In embodiments, the samples can be biological fluid samples. In embodiments, the biological fluid can be plasma, serum, spinal fluid, lymph fluid, synovial fluid, urine or tears.

Thus, the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure enable efficient, effective and accurate diagnostic or clinical testing for dengue virus infections thereby improving patient management and improving the likelihood of recovery from dengue virus infections.

The method of rapidly diagnosing a dengue virus of the present disclosure via the use of the diagnostic kit for rapidly diagnosing a dengue virus of the present disclosure including carbon nanotubes and magnetic beads is by far the quickest and most accurate method for diagnosing dengue viruses. As such, the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure readily meets the current market needs.

The development of the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure is based on the protocol of the diagnosis of dengue virus infections using carbon nanotubes and magnetic beads, as well as sequences of dengue virus specific oligonucleotides. Although the viral genomes of the Dengue viruses are available on the World Wide Web, it is no small task or feat to design sequences of dengue virus specific oligonucleotides for efficient and accurate diagnosis of dengue virus infections, as well as establishing the protocol for a one step process and fast single test-tube reaction.

One advantage of the diagnostic kit and method of using thereof of the present disclosure is the ability to rapidly diagnose a dengue virus without complicated user protocols. Another advantage is the cost of the diagnostic kit of the present disclosure, which substantially reduces the costs associated with screening. The diagnostic kit of the present disclosure is inexpensively produced and provided at a low cost, which is especially necessary for use in

remote locations and doctor's offices. Yet another advantage of the diagnostic kit and method of using thereof of the present disclosure is the rapid diagnostic result provided, the excellent accuracy provided, and the excellent specificity provided.

The present disclosure relates to a method of preparing a diagnostic kit for rapidly diagnosing a dengue virus that can comprise: designing a first oligonucleotide comprising a genetic sequence that is complementary to a target genetic sequence of the dengue virus; providing a plurality of carbon nanotubes; and providing a plurality of solid supports.

In embodiments, the method of preparing a diagnostic kit of the present disclosure can further comprise designing a second oligonucleotide comprising a genetic sequence that is complementary to the target genetic sequence of the dengue virus.

In embodiments, the target genetic sequence of the dengue virus can comprise a target genetic sequence of the NS1 region of the dengue virus.

In embodiments, the carbon nanotubes can be single walled carbon nanotubes. In embodiments, the single walled carbon nanotubes are carboxylated single walled carbon nanotubes.

In embodiments, the solid supports can be magnetic beads. In embodiments, the plurality of magnetic beads can be streptavidin functionalized magnetic beads or carboxylic acid functionalized magnetic beads.

In embodiments, the method of preparing a diagnostic kit of the present disclosure can further comprise: designing the first oligonucleotide to function as a detection probe; tagging the first oligonucleotide with a NH_2 functional group on the 5' end of the first oligonucleotide; and hybridising a plurality of the first oligonucleotide to the plurality of single walled carbon nanotubes to form a plurality of carbon nanotube based labels.

In embodiments, the method of preparing a diagnostic kit of the present disclosure can further comprise: designing the second oligonucleotide to function as a capture probe;

biotinylating the second oligonucleotide; tagging the second oligonucleotide with a NH_2 functional group on the 3'end of the second oligonucleotide; and immobilising a plurality of the second oligonucleotide to the plurality of magnetic beads to form a plurality of conjugated magnetic beads.

In embodiments, the method of preparing a diagnostic kit of the present disclosure can further comprise: designing a third oligonucleotide comprising a genetic sequence that is non-complementary to the target genetic sequence of the dengue virus, wherein the third oligonucleotide functions as a negative control sequence.

In embodiments, the method of preparing a diagnostic kit of the present disclosure can further comprise: hybridising the plurality of carbon nanotube labels with the plurality of conjugated magnetic beads to form a mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads.

In embodiments, a plurality of the third oligonucleotide and the mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads can be provided in one test tube.

In embodiments, the dengue virus can be dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) or dengue virus serotype 4 (DENV-4).

The present disclosure also relates to a diagnostic kit for rapidly diagnosing a dengue virus, wherein the diagnostic kit can be prepared according to a method of preparing a diagnostic kit of the present disclosure.

The present disclosure relates to a diagnostic kit for rapidly diagnosing a dengue virus that can comprise: a mixture of a plurality of carbon nanotube based labels and a plurality of conjugated magnetic beads, wherein the plurality of carbon nanotube labels are hybridised with the plurality of conjugated magnetic beads, and wherein the mixture is provided in one test tube.

In embodiments, the plurality of carbon nanotube based labels of the mixture can comprise a plurality of a first oligonucleotide hybridised to a plurality of single walled carbon nanotubes, and the first oligonucleotide can comprise a genetic sequence that is complementary to a target genetic sequence of the dengue virus.

In embodiments, the first oligonucleotide functions as a detection probe, and the first oligonucleotide is tagged with a NH_2 functional group on the 5'end of the first oligonucleotide to facilitate hybridisation of the plurality of the first oligonucleotide to the plurality of single walled carbon nanotubes to form the plurality of carbon nanotube based labels.

In embodiments, the plurality of conjugated beads of the mixture can comprise a plurality of a second oligonucleotide immobilised to a plurality of magnetic beads, and the second oligonucleotide can comprise a genetic sequence that is complementary to the target genetic sequence of the dengue virus.

In embodiments, the second oligonucleotide functions as a capture probe, the second oligonucleotide is biotinylated, and the second oligonucleotide is tagged with a NH_2 functional group on the 3'end of the second oligonucleotide to facilitate the immobilisation of the plurality of the second oligonucleotide to the plurality of magnetic beads to form the plurality of conjugated magnetic beads.

In embodiments, the target genetic sequence of the dengue virus can comprise a target genetic sequence of the NS1 region of the dengue virus.

In embodiments, the plurality of magnetic beads are streptavidin functionalized magnetic beads or carboxylic acid functionalized magnetic beads.

In embodiments, the diagnostic kit of the present disclosure can further comprise: a third oligonucleotide comprising a genetic sequence that is non-complementary to the target genetic sequence of the dengue virus, wherein the third oligonucleotide functions as a

negative control sequence, and wherein a plurality of the third oligonucleotide is provided in the one test tube.

In embodiments, the dengue virus can be dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) or dengue virus serotype 4 (DENV-4).

In embodiments, the diagnostic kit of the present disclosure can be a rapid diagnostic kit for use at point of care.

The present disclosure also relates to a method of rapidly diagnosing a dengue virus that can comprise: providing a diagnostic kit of the present disclosure; providing target ribonucleic acid (hereinafter 'RNA') of the dengue virus; and adding the target RNA of the dengue virus to the mixture of the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads in the one test tube.

In embodiments, the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads aggregate in the presence of the target RNA of the dengue virus. In embodiments, the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads aggregate in the presence of the target RNA of the dengue virus and form dark coloured complexes with the target RNA of the dengue virus. In embodiments, the formation of dark coloured complexes allows for visual detection of the target RNA of the dengue virus. In embodiments, the dark coloured complexes are dark brown.

In embodiments, the aggregation of the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads in the presence of the target RNA of the dengue virus is dependent on the concentration of the target RNA of the dengue virus added to the mixture.

In embodiments, the dengue virus can be dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) or dengue virus serotype 4 (DENV-4).

In embodiments, the formation of the dark coloured complexes can be generated in about 15 minutes or less, and the diagnosis of the dengue virus can be achieved in about 15 minutes or less. In embodiments, the formation of the dark coloured complexes can be generated in about 10 minutes or less, and the diagnosis of the dengue virus can be achieved in about 10 minutes or less. In embodiments, the formation of the dark coloured complexes can be generated in about 5 minutes or less, and the diagnosis of the dengue virus can be achieved in about 5 minutes or less.

In embodiments, the method of rapidly diagnosing a dengue virus described above can have an accuracy of about 98% or greater for diagnosing the dengue virus. In embodiments, the method of rapidly diagnosing a dengue virus can have an accuracy of about 99% or greater for diagnosing the dengue virus. In embodiments, the method of rapidly diagnosing a dengue virus can have an accuracy of about 100% for diagnosing the dengue virus.

In embodiments, the method of rapidly diagnosing a dengue virus can have a specificity of about 98% or greater for diagnosing the dengue virus. In embodiments, the method of rapidly diagnosing a dengue virus can have a specificity of about 99% or greater for diagnosing the dengue virus. The method of rapidly diagnosing a dengue virus can have a specificity of about 100% for diagnosing the dengue virus.

The present disclosure also relates to a method of preparing a diagnostic kit for rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

The present disclosure also relates to a diagnostic kit for rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4). In embodiments, all four serotypes of the dengue

virus (i.e., DENV-1, DENV-2, DENV-3 and DENV-4) can be diagnosed using the diagnostic kit of the present disclosure.

The present disclosure also relates to a method of rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4). In embodiments, all four serotypes of the dengue virus (i.e., DENV-1, DENV-2, DENV-3 and DENV-4) can be diagnosed using the method of rapid dengue virus diagnosis of the present disclosure.

In accordance with embodiments of the present disclosure, the diagnostic kit for rapidly diagnosing dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and/or dengue virus serotype 4 (DENV-4) can be a rapid diagnostic kit for use at point of care.

EXAMPLE

Preparation of Oligonucleotides for Diagnosing Dengue Virus Serotype 1 (DENV-1)

A total of three oligonucleotides were designed to target a specific region of the NS1 region of dengue virus serotype 1 (DENV-1). A first oligonucleotide was designed having a genetic nucleotide sequence that is complementary to a target genetic sequence of the NS1 region of DENV-1. The first oligonucleotide was designed to function as detection probe (DP). The genetic nucleotide sequence of the first oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-1. The first oligonucleotide was tagged with a NH_2 functional group on the 5' end of the first oligonucleotide to facilitate hybridisation of a plurality of the first oligonucleotide to a plurality of single walled carbon nanotubes (SWNTs) to form a plurality of carbon nanotube based labels.

A second oligonucleotide was designed having a genetic nucleotide sequence that is complementary to the target genetic sequence of the NS1 region of DENV-1. The second oligonucleotide was designed to function as a capture probe (CP). The genetic nucleotide sequence of the second oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-1. The second oligonucleotide was biotinylated and tagged with a NH_2 functional group on the 3' end of the second oligonucleotide to facilitate the immobilisation of a plurality of the second oligonucleotide to a plurality of magnetic beads to form a plurality of conjugated magnetic beads.

In one example, the plurality of magnetic beads were functionalised with carboxylic acid to form carboxylic acid functionalised magnetic beads (i.e., COOH-beads). In another example, the plurality of magnetic beads were functionalised with streptavidin to form streptavidin functionalised magnetic beads. The inventors found that the carboxylic acid functionalised magnetic beads were able to form larger aggregates as compared to streptavidin functionalised magnetic beads while the streptavidin functionalised magnetic beads were more sensitive than the carboxylic acid functionalised magnetic beads.

A third oligonucleotide was designed having a genetic nucleotide sequence that is non-complementary to the target genetic sequence of the NS1 region of DENV-1. The third oligonucleotide was designed to function as a negative control sequence.

<u>Preparation of Oligonucleotides for Diagnosing Dengue Virus Serotype 2 (DENV-2)</u>

A total of three oligonucleotides were designed to target a specific region of the NS1 region of dengue virus serotype 2 (DENV-2). A first oligonucleotide was designed having a genetic nucleotide sequence that is complementary to a target genetic sequence of the NS1 region of DENV-2. The first oligonucleotide was designed to function as detection probe (DP). The genetic nucleotide sequence of the first oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-2. The first oligonucleotide was tagged with a NH_2 functional

group on the 5’ end of the first oligonucleotide to facilitate hybridisation of a plurality of the first oligonucleotide to a plurality of single walled carbon nanotubes (SWNTs) to form a plurality of carbon nanotube based labels.

A second oligonucleotide was designed having a genetic nucleotide sequence that is complementary to the target genetic sequence of the NS1 region of DENV-2. The second oligonucleotide was designed to function as a capture probe (CP). The genetic nucleotide sequence of the second oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-2. The second oligonucleotide was biotinylated and tagged with a NH_2 functional group on the 3’ end of the second oligonucleotide to facilitate the immobilisation of a plurality of the second oligonucleotide to a plurality of magnetic beads to form a plurality of conjugated magnetic beads.

In one example, the plurality of magnetic beads were functionalised with carboxylic acid to form carboxylic acid functionalised magnetic beads (i.e., COOH-beads). In another example, the plurality of magnetic beads were functionalised with streptavidin to form streptavidin functionalised magnetic beads. The inventors found that the carboxylic acid functionalised magnetic beads were able to form larger aggregates as compared to streptavidin functionalised magnetic beads while the streptavidin functionalised magnetic beads were more sensitive than the carboxylic acid functionalised magnetic beads.

A third oligonucleotide was designed having a genetic nucleotide sequence that is non-complementary to the target genetic sequence of the NS1 region of DENV-2. The third oligonucleotide was designed to function as a negative control sequence.

<u>Preparation of Oligonucleotides for Diagnosing Dengue Virus Serotype 3 (DENV-3)</u>

A total of three oligonucleotides were designed to target a specific region of the NS1 region of dengue virus serotype 3 (DENV-3). A first oligonucleotide was designed having a genetic nucleotide sequence that is complementary to a target genetic

sequence of the NS1 region of DENV-3. The first oligonucleotide was designed to function as detection probe (DP). The genetic nucleotide sequence of the first oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-3. The first oligonucleotide was tagged with a NH_2 functional group on the 5' end of the first oligonucleotide to facilitate hybridisation of a plurality of the first oligonucleotide to a plurality of single walled carbon nanotubes (SWNTs) to form a plurality of carbon nanotube based labels.

A second oligonucleotide was designed having a genetic nucleotide sequence that is complementary to the target genetic sequence of the NS1 region of DENV-3. The second oligonucleotide was designed to function as a capture probe (CP). The genetic nucleotide sequence of the second oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-3. The second oligonucleotide was biotinylated and tagged with a NH_2 functional group on the 3' end of the second oligonucleotide to facilitate the immobilisation of a plurality of the second oligonucleotide to a plurality of magnetic beads to form a plurality of conjugated magnetic beads.

In one example, the plurality of magnetic beads were functionalised with carboxylic acid to form carboxylic acid functionalised magnetic beads (i.e., COOH-beads). In another example, the plurality of magnetic beads were functionalised with streptavidin to form streptavidin functionalised magnetic beads. The inventors found that the carboxylic acid functionalised magnetic beads were able to form larger aggregates as compared to streptavidin functionalised magnetic beads while the streptavidin functionalised magnetic beads were more sensitive than the carboxylic acid functionalised magnetic beads.

A third oligonucleotide was designed having a genetic nucleotide sequence that is non-complementary to the target genetic sequence of the NS1 region of DENV-3. The third oligonucleotide was designed to function as a negative control sequence.

<u>Preparation of Oligonucleotides for Diagnosing Dengue Virus Serotype 4 (DENV-4)</u>

A total of three oligonucleotides were designed to target a specific region of the NS1 region of dengue virus serotype 4 (DENV-4). A first oligonucleotide was designed having a genetic nucleotide sequence that is complementary to a target genetic sequence of the NS1 region of DENV-4. The first oligonucleotide was designed to function as detection probe (DP). The genetic nucleotide sequence of the first oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-4. The first oligonucleotide was tagged with a NH_2 functional group on the 5' end of the first oligonucleotide to facilitate hybridisation of a plurality of the first oligonucleotide to a plurality of single walled carbon nanotubes (SWNTs) to form a plurality of carbon nanotube based labels.

A second oligonucleotide was designed having a genetic nucleotide sequence that is complementary to the target genetic sequence of the NS1 region of DENV-4. The second oligonucleotide was designed to function as a capture probe (CP). The genetic nucleotide sequence of the second oligonucleotide is designed to form a complex with the target genetic sequence of the target RNA of DENV-4. The second oligonucleotide was biotinylated and tagged with a NH_2 functional group on the 3' end of the second oligonucleotide to facilitate the immobilisation of a plurality of the second oligonucleotide to a plurality of magnetic beads to form a plurality of conjugated magnetic beads.

In one example, the plurality of magnetic beads were functionalised with carboxylic acid to form carboxylic acid functionalised magnetic beads (i.e., COOH-beads). In another example, the plurality of magnetic beads were functionalised with streptavidin to form streptavidin functionalised magnetic beads. The inventors found that the carboxylic acid functionalised magnetic beads were able to form larger aggregates as compared to streptavidin functionalised magnetic beads while the streptavidin functionalised magnetic beads were more sensitive than the carboxylic acid functionalised magnetic beads.

A third oligonucleotide was designed having a genetic nucleotide sequence that is non-complementary to the target genetic sequence of the NS1 region of DENV-4. The third oligonucleotide was designed to function as a negative control sequence.

Table 1 seen directly below shows the specific nucleotide sequence of each of the oligonucleotides designed for diagnosing DENV-1, DENV-2, DENV-3 and DENV-4. Each oligonucleotide Sequence 1 designed for each dengue virus serotype was used as a detection probe, while each oligonucleotide Sequence 2 designed for each dengue virus serotype was used as a capture probe.

In an unexpected and surprising finding, the inventors discovered that the selection of the oligonucleotide sequence as a detection probe and the selection of the oligonucleotide sequence as a capture probe were critical in achieving a faster and more accurate method of rapidly diagnosing a dengue virus.

For example, if the oligonucleotide Sequence 1 that is complementary to a target sequence of the NS1 region of DENV-1 is used as a detection probe and if the oligonucleotide Sequence 2 that is complementary to the target sequence of the NS1 region of DENV-1 is used as a capture probe, the method of rapidly diagnosing a dengue virus of the present disclosure can generate diagnostic results in about 5 minutes or less and can have an accuracy of about 100% in diagnosing the dengue virus.

However, if the oligonucleotide Sequence 1 that is complementary to a target sequence of the NS1 region of DENV-1 is used as a capture probe and if the oligonucleotide Sequence 2 that is complementary to the target sequence of the NS1 region of DENV-1 is used as a detection probe, the method of rapidly diagnosing a dengue virus of the present disclosure will not generate diagnostic results in an efficient manner and will not be accurate in diagnosing the dengue virus.

Accordingly, surprisingly and unexpectedly, the selection and use of nucleotide sequences as a detection probe to be hybridised or conjugated to single walled carbon

nanotubes or a capture probe to be conjugated to streptavidin functionalised magnetic beads is critical to rapidly and accurately diagnosing a dengue virus.

Surprisingly and unexpectedly, the selection and use of nucleotide sequences as a detection probe to be hybridised or conjugated to single walled carbon nanotubes or a capture probe to be conjugated to streptavidin functionalised magnetic beads is critical to the aggregation of the carbon nanotube labels and conjugated magnetic beads and the formation of the dark coloured complexes with the target dengue virus RNA.

Table 1: Nucleotide sequence of each of the oligonucleotides designed for diagnosing Dengue Virus type 1 to 4.

Primer/Probe	Nucleotide Sequence (5' - 3')
DENV-1: Sequence 1	CA GCT TCC AGG TCT CGT TCT
DENV-1: Sequence 2	CA TAG AGT GTG GGA TTT TGG CCA GAT ACA TGT TTT GAC TTC TAT/3Bio
DENV-2: Sequence 1	CTG TTT CAG GGC CAT TAA
DENV-2: Sequence 2	C AAC TTC CAG CGA GTT CCA GGC TCT GTT TGT GTT/3Bio
DENV-3: Sequence 1	C TTT CCC CAC GTT TTC CAT
DENV-3: Sequence 2	ACT CCG GTG TGT TTG GTC CAT CTA TTA/3Bio
DENV-4: Sequence 1	TC CAC AGA CCC CAT CTT TGT
DENV-4: Sequence 2	TCA ACA CCC CCT TCA CAT CCC CAG CCA CTA CAG TGA GAT/3Bio

Preparation of Carbon Nanotube (CNT) Based Labels for Diagnosing DENV-1, DENV-2, DENV-3 and DENV-4

As shown in FIG. 1, in accordance with one embodiment of the present disclosure, carbon nanotube based labels were synthesized based on diimide-activated amidation. FIG. 1 illustrates the hybridisation or conjugation of an oligonucleotide

detection probe (DP) to a single walled carbon nanotube (SWNT) by diimide-activated amidation in accordance with an embodiment of the present disclosure. A plurality of an oligonucleotide detection probe was hybridised or conjugated to a plurality of single walled carbon nanotubes by diimide-activated amidation. In short, the plurality of the oligonucleotide detection probe was labelled for hybridisation assays.
First, a plurality of single walled carbon nanotubes were carboxylated to form carboxylated single walled carbon nanotubes. The covalent hybridisation or conjugation of the carboxylated single walled carbon nanotubes with the plurality of an oligonucleotide detection probe was carried out by sonicating the carboxylated single walled carbon nanotubes at a final concentration of 0.72 µg/µl in 50 mM (millimolar) phosphate buffer having a pH of 7.0 containing 50 mM of *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride and 2 g/L of sodium deoxycholate for 2 hours. An equal volume of 1.2 µM of the plurality of the oligonucleotide detection probe was added to the carboxylated single walled carbon nanotubes to form a mixture of a plurality of carbon nanotube based labels. The resulting mixture of a plurality of carbon nanotube based labels was incubated overnight at room temperature.

FIG. 2 is a schematic diagram of a carbon nanotube based label in accordance with embodiments of the present disclosure.

<u>Immobilisation of Oligonucleotide Capture Probes with Streptavidin Functionalised Magnetic Beads</u>

100 µl of a plurality of as-supplied streptavidin functionalised magnetic beads were washed twice with 100 µl of 25 mM (millimolar) B&W buffer. The plurality of streptavidin functionalised magnetic beads were then re-suspended in 100 µl of B&W buffer. A plurality of an oligonucleotide capture probe was biotinylated. 25 µl of 10 µM of the plurality of the biotinylated capture probe was added to the plurality of streptavidin functionalised magnetic beads to form a plurality of conjugated magnetic beads. The resulting mixture of the plurality of conjugated magnetic beads was not incubated. The resulting mixture of the plurality of conjugated magnetic beads was

then washed once and re-suspended in B&W buffer. In one embodiment, the resulting mixture of the plurality of conjugated magnetic beads is incubated at room temperature for one (1) hour with gentle shaking and after incubation the resulting mixture of the plurality of conjugated magnetic beads is washed twice and re-suspended in B&W buffer.

Hybridisation of the Carbon Nanotube Based Labels with the Conjugated Magnetic Beads

20 µl of the mixture of the plurality of the carbon nanotube based labels was topped up with hybridisation buffer to a total volume of 120 µl. The mixture of the plurality of the carbon nanotube labels was then added to the mixture of the plurality of conjugated magnetic beads that had been washed and incubated at room temperature for one (1) hour to hybridise the plurality of carbon nanotube labels and the plurality of conjugated magnetic beads. After the hybridisation step, the resulting mixture of the plurality of carbon nanotube labels and the plurality of conjugated beads was washed once with 100 µl of secondary wash buffer (i.e., 0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0).

The above steps were carried out to prepare a first mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-1. The above steps were carried out to prepare a second mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-2. The above steps were carried out to prepare a third mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-3. The above steps were carried out to prepare a fourth mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-4.

In embodiments, the first mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-1 is provided in a first test tube. In embodiments, the second mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-2 is provided in a

second test tube. In embodiments, the third mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-3 is provided in a third test tube. In embodiments, the fourth mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-4 is provided in a fourth test tube.

In embodiments, a sample may be tested for each of the serotypes of the dengue virus. In embodiments, the samples can be clinical samples and/or biological samples. In embodiments, the samples can be biological fluid samples. In embodiments, the biological fluid can be plasma, serum, spinal fluid, lymph fluid, synovial fluid, urine or tears.

Diagnosis or Detection of a Dengue Virus

A dengue virus was first lysed by adding lysis buffer (Qiagen, USA) and the released ribonucleic acid (RNA) was incubated. Lysis of the dengue virus, release of the RNA and incubation of the released RNA took 10 minutes. Target RNA was extracted and lysed from each of the four serotypes of the dengue virus using the same process. The released target RNA was added to a mixture of a plurality of carbon nanotube based labels and a plurality of conjugated magnetic beads for diagnosing a dengue virus provided in a test tube. The test tube was gently shaken. After the addition of the released target RNA, diagnostic results were obtained in 5 minutes with an accuracy of about 100% in diagnosing the dengue virus. Within 5 minutes the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads aggregated in the presence of the target RNA to form dark coloured complexes with the target RNA. The dark coloured complexes were dark brown.

The diagnosis or detection principle of the mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads is illustrated in FIG. 2.

The use of a plurality of carbon nanotube based labels increases the amount of the signal per target unit. As shown in FIG. 3A - FIG. 3C, critically, the hybridisation assay

of the present disclosure also induces aggregation of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads in the presence of target dengue virus RNA or a target dengue virus RNA sequence thereby leading to the formation of dark coloured complexes with the target dengue virus RNA or target dengue virus RNA sequence. The successful aggregation of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads induces the formation of the dark coloured complexes. The formation of the dark coloured complex allows for visible diagnosis or visible detection of the target dengue virus RNA or target dengue virus RNA sequence.

The aggregation of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads in the presence of target dengue virus RNA sequence to form dark coloured complexes with the target dengue virus RNA sequence can be dependent on the concentration of the target dengue virus RNA sequence present. For example, as illustrated in FIG. 3A – FIG. 3C and FIG. 4A – FIG. 4E, the higher the target dengue virus RNA sequence concentration the greater the aggregation and the darker the complex formed. As such, the aggregation and dark coloured complexes formed can be even more visibly detectable when a higher concentration of the target dengue virus RNA sequence is present. In embodiments, the concentration of the target dengue virus RNA sequence can be about 2.5 ng/µL or more, about 5 ng/µL or more, about 10 ng/µL or more, about 20 ng/µL or more, about 40 ng/µL or more or about 80 ng/µL or more.

The first mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-1 provided in the first test tube generated diagnostic results in 5 minutes with an accuracy of 100% in diagnosing DENV-1. Further, first mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-1 provided in the first test tube was tested for its specificity for DENV-1 by using other viruses such as Herpes Simplex Virus (HSV), Cytomegalovirus (CMV) and Enterovirus 71 (EV71). The first mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for

diagnosing DENV-1 provided in the first test tube exhibited 100% specificity for DENV-1.

The second mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-2 provided in the second test tube generated diagnostic results in 5 minutes with an accuracy of 100% in diagnosing DENV-2. Further, the second mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-2 provided in the second test tube was tested for its specificity for DENV-2 by using other viruses such as Herpes Simplex Virus (HSV), Cytomegalovirus (CMV) and Enterovirus 71 (EV71). The second mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-2 provided in the second test tube exhibited 100% specificity for DENV-2.

The third mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-3 provided in the third test tube generated diagnostic results in 5 minutes with an accuracy of 100% in diagnosing DENV-3. Further, the third mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-3 provided in the third test tube was tested for its specificity for DENV-3 by using other viruses such as Herpes Simplex Virus (HSV), Cytomegalovirus (CMV) and Enterovirus 71 (EV71). The third mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-3 provided in the third test tube exhibited 100% specificity for DENV-3.

The fourth mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-4 provided in the fourth test tube generated diagnostic results in 5 minutes with an accuracy of 100% in diagnosing DENV-4. Further, the fourth mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-4 provided in the fourth test tube was tested for its specificity for DENV-4 by using other viruses such as Herpes Simplex Virus (HSV), Cytomegalovirus (CMV) and Enterovirus 71 (EV71). The

fourth mixture of a plurality of carbon nanotube labels and a plurality of conjugated magnetic beads for diagnosing DENV-4 provided in the fourth test tube exhibited 100% specificity for DENV-4.

Twenty (20) clinical samples from Kandang Kerbau Women and Children Hospital were tested using the diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus of the present disclosure. All twenty clinical samples were found to be positive for pan-dengue. Four (4) positives were found for DENV-1, nine (9) positives were found for DENV-2, eleven positives (11) were found for DENV-3, and two (2) positives were found for DENV-4. The diagnostic results were generated in 5 minutes with an accuracy of 100% for each of pan-dengue, DENV-1, DENV-2, DENV-3 and DENV-4.

The diagnostic kit for rapidly diagnosing a dengue virus and the method of rapidly diagnosing a dengue virus using the diagnostic kit of the present disclosure prove to be more useful in allowing for the rapid and accurate diagnosis of DENV-1, DENV-2, DENV-3 and DENV-4 compared to the known methods being used presently. The diagnostic kit for rapidly diagnosing a dengue virus and the method for rapidly diagnosing a dengue virus of the present disclosure are able to reduce the amount of time required for generating diagnostic results from about 3 hours using real-time RT-PCR to only about 15 minutes or less, about 10 minutes or less, or about 5 minutes or less.

Additionally, the method for rapidly diagnosing a dengue virus of the present disclosure can be performed without the need for pre-assay processing, such as, RNA extraction before real-time RT-PCR.

The diagnostic kit for rapidly diagnosing a dengue virus and the method for rapidly diagnosing a dengue virus of the present disclosure eliminate the use of sophisticated and costly detection systems. The diagnostic kit for rapidly diagnosing a dengue virus and the method for rapidly diagnosing a dengue virus of the present disclosure eliminate the use of wasteful downstream signal development steps such as the

qualitative detection of the presence or absence of target analytes. Instead, the diagnostic kit for rapidly diagnosing a dengue virus and the method for rapidly diagnosing a dengue virus of the present disclosure involve mere visualisation of the formation dark coloured complexes formed via the aggregation of carbon nanotube based labels and conjugated magnetic beads in the presence of target dengue virus RNA. The aggregation and the formation of dark coloured complexes occur without the need of any enzymatic reaction.

The diagnostic kit for rapidly diagnosing a dengue virus and the method for rapidly diagnosing a dengue virus of the present disclosure eliminate the use of large and expensive equipment and instrumentation unlike real-time RT-PCR. Additionally, the diagnostic kit for rapidly diagnosing a dengue virus and the method for rapidly diagnosing a dengue virus of the present disclosure can be used as a field diagnostic kit and a field method for rapidly diagnosing a dengue virus as the diagnostic kit and the method only require simple equipment such as test tubes and pipettes. This is especially critical if there is a lack of healthcare facilities or laboratory facilities, which can be common in certain parts of the world. The simple, user friendly, and low cost diagnostic kit for rapidly diagnosing a dengue virus and method for rapidly diagnosing a dengue virus is more suitable for point-of-care testing and/or clinical diagnosis compared to other assay systems.

The hybridisation assay of the present disclosure utilises the simple principle of aggregation. Specifically designed dengue specific oligonucleotides are hybridised or conjugated to carboxylated single walled carbon nanotubes as one component (i.e., carbon nanotube based labels) of the assay and conjugated to streptavidin functionalised magnetic beads as another component (i.e., conjugated magnetic beads) of the assay. With the addition of target dengue virus RNA or a sample that contains target dengue virus RNA, the dengue specific oligonucleotides come together to tag the target dengue virus RNA, hence causing the formation of an aggregate and the formation of dark coloured complexes with the target dengue virus RNA. In other words, the carbon nanotube based labels (i.e., dengue specific oligonucleotides hybridised or conjugated to carboxylated single walled carbon nanotubes) and the

conjugated magnetic beads (i.e., dengue specific oligonucleotides conjugated to streptavidin functionalised magnetic beads) aggregate in the presence of the target dengue virus RNA to form dark coloured complexes with the target dengue virus RNA.

On the other hand, if a negative control sample is being added in replacement of the dengue virus RNA, the specifically designed dengue specific oligonucleotides will not come together to bind to the negative control sample. Therefore, the carbon nanotube based labels and streptavidin functionalised magnetic beads will not be able to form an aggregate or a dark coloured complex thereby resulting in a negative reaction or negative result.

In an unexpected and surprising finding, the inventors discovered that the selection of the oligonucleotide sequence as a detection probe and the selection of the oligonucleotide sequence as a capture probe were critical in achieving a faster and more accurate method of rapidly diagnosing a dengue virus.

For example, referring to Table 1 discussed above, if the oligonucleotide Sequence 1 that is complementary to a target sequence of the NS1 region of DENV-1 is used as a detection probe and if the oligonucleotide Sequence 2 that is complementary to the target sequence of the NS1 region of DENV-1 is used as a capture probe, the method of rapidly diagnosing a dengue virus of the present disclosure can generate diagnostic results in about 15 minutes or less, about 10 minutes or less, or about 5 minutes or less and can have an accuracy of about 98% or greater, about 99% or greater, or about 100% in diagnosing the dengue virus.

However, if the oligonucleotide Sequence 1 that is complementary to a target sequence of the NS1 region of DENV-1 is used as a capture probe and if the oligonucleotide Sequence 2 that is complementary to the target sequence of the NS1 region of DENV-1 is used as a detection probe, the method of rapidly diagnosing a dengue virus of the present disclosure will not generate diagnostic results in an efficient manner and will not be accurate in diagnosing the dengue virus. For

example, in some cases no formation of an aggregate or a dark coloured complex occurs.

Accordingly, surprisingly and unexpectedly, the selection and use of nucleotide sequences as a detection probe to be hybridised or conjugated to single walled carbon nanotubes or a capture probe to be conjugated to streptavidin functionalised magnetic beads is critical to rapidly and accurately diagnosing a dengue virus.

Surprisingly and unexpectedly, the selection and use of nucleotide sequences as a detection probe to be hybridised or conjugated to single walled carbon nanotubes or a capture probe to be conjugated to streptavidin functionalised magnetic beads is critical to the aggregation of the carbon nanotube labels and conjugated magnetic beads and the formation of the dark coloured complexes with the target dengue virus RNA.

While various aspects and embodiments have been disclosed herein, it will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit of the invention being indicated by the appended claims.

CLAIMS

We Claim:

1. A method of preparing a diagnostic kit for rapidly diagnosing a dengue virus comprising:

designing a first oligonucleotide comprising a sequence that is complementary to a target sequence of the dengue virus;

providing a plurality of carbon nanotubes; and

providing a plurality of solid supports.

2. The method of claim 1, further comprising:

designing a second oligonucleotide comprising a sequence that is complementary to the target sequence of the dengue virus.

3. The method of claim 1 or 2, wherein the target sequence of the dengue virus comprises a target sequence of the NS1 region of the dengue virus.

4. The method of any one of claims 1-3, wherein the carbon nanotubes are single walled carbon nanotubes.

5. The method any one of claims 1-4, wherein the solid supports are magnetic beads.

6. The method of claim 5, wherein the plurality of magnetic beads are streptavidin functionalized magnetic beads or carboxylic acid functionalized magnetic beads.

7. The method of any one of claims 4-6, further comprising:

designing the first oligonucleotide to function as a detection probe;

tagging the first oligonucleotide with a NH_2 functional group on the 5' end of the first oligonucleotide; and

hybridising a plurality of the first oligonucleotide to the plurality of single walled carbon nanotubes to form a plurality of carbon nanotube based labels.

8. The method of any one of claims 5-7, further comprising:

designing the second oligonucleotide to function as a capture probe;

biotinylating the second oligonucleotide;

tagging the second oligonucleotide with a NH_2 functional group on the 3'end of the second oligonucleotide; and

immobilising a plurality of the second oligonucleotide to the plurality of magnetic beads to form a plurality of conjugated magnetic beads.

9. The method of any one of claims 1-8, further comprising:

designing a third oligonucleotide comprising a sequence that is non-complementary to the target sequence of the dengue virus;

wherein the third oligonucleotide functions as a negative control sequence.

10. The method of any one of claims 7-9, further comprising:

hybridising the plurality of carbon nanotube labels with the plurality of conjugated magnetic beads to form a mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads.

11. The method of claim 10, wherein a plurality of the third oligonucleotide and the mixture of the plurality of carbon nanotube based labels and the plurality of conjugated magnetic beads are provided in one test tube.

12. The method of any one of claims 1-11, wherein the dengue virus is selected from the group consisting of dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

13. A diagnostic kit for rapidly diagnosing a dengue virus, wherein the diagnostic kit is prepared accorded to the method of any one of claims 1-12.

14. A diagnostic kit for rapidly diagnosing a dengue virus comprising:

a mixture of a plurality of carbon nanotube based labels and a plurality of conjugated magnetic beads,

wherein the plurality of carbon nanotube labels are hybridised with the plurality of conjugated magnetic beads, and

wherein the mixture is provided in one test tube.

15. The diagnostic kit of claim 14,

wherein the plurality of carbon nanotube based labels of the mixture comprises a plurality of a first oligonucleotide hybridised to a plurality of single walled carbon nanotubes, and

wherein the first oligonucleotide comprises a sequence that is complementary to a target sequence of the dengue virus.

16. The diagnostic kit of claim 15,

wherein the first oligonucleotide functions as a detection probe, and

wherein the first oligonucleotide is tagged with a NH_2 functional group on the 5'end of the first oligonucleotide to facilitate hybridisation of the plurality of the first oligonucleotide to the plurality of single walled carbon nanotubes to form the plurality of carbon nanotube based labels.

17. The diagnostic kit of any one of claims 15-16,

wherein the plurality of conjugated beads of the mixture comprises a plurality of a second oligonucleotide immobilised to a plurality of magnetic beads, and

wherein the second oligonucleotide comprises a sequence that is complementary to the target sequence of the dengue virus.

18. The diagnostic kit of claim 17,

wherein the second oligonucleotide functions as a capture probe,

wherein the second oligonucleotide is biotinylated, and

wherein the second oligonucleotide is tagged with a NH_2 functional group on the 3'end of the second oligonucleotide to facilitate the immobilisation of the plurality of the

second oligonucleotide to the plurality of magnetic beads to form the plurality of conjugated magnetic beads.

19. The diagnostic kit of any one of claims 15-18, wherein the target sequence of the dengue virus comprises a target sequence of the NS1 region of the dengue virus.

20. The diagnostic kit of any one of claims 17-19, wherein the plurality of magnetic beads are streptavidin functionalized magnetic beads or carboxylic acid functionalized magnetic beads.

21. The diagnostic kit of any one of claims 15-20, further comprising:

a third oligonucleotide comprising a sequence that is non-complementary to the target sequence of the dengue virus,

wherein the third oligonucleotide functions as a negative control sequence, and

wherein a plurality of the third oligonucleotide is provided in the one test tube.

22. The diagnostic kit of any one of claims 14-21, wherein the dengue virus is selected from the group consisting of dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

23. A method of rapidly diagnosing a dengue virus comprising:

providing the diagnostic kit of any one of claims 13-22;

providing target RNA of the dengue virus;

adding the target RNA of the dengue virus to the mixture of the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads in the one test tube.

24. The method of claim 23, wherein the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads aggregate in the presence of the target RNA of the dengue virus.

25. The method of claim 24, wherein the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads aggregate in the presence of the target RNA of the dengue virus to form dark coloured complexes.

26. The method of claim 25, wherein the formation of dark coloured complexes allows for visual detection of the target RNA of the dengue virus.

27. The method of any one of claims 24-26, wherein the aggregation of the plurality of carbon nanotube-based labels and the plurality of conjugated magnetic beads is dependent on the concentration of the target RNA of the dengue virus added to the mixture.

28. The method of any one of claims 23-27, wherein the dengue virus is selected from the group consisting of dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3) and dengue virus serotype 4 (DENV-4).

29. The method of any one of claims 25-28, wherein the formation of the dark coloured complexes is generated in about 15 minutes or less, and wherein diagnosis of the dengue virus is achieved in about 15 minutes or less.

30. The method of any one of claims 25-29, wherein the formation of the dark coloured complexes is generated in about 10 minutes or less, and wherein diagnosis of the dengue virus is achieved in about 10 minutes or less.

31. The method of any one of claims 25-30, wherein the formation of the dark coloured complexes is generated in about 5 minutes or less, and wherein diagnosis of the dengue virus is achieved in about 5 minutes or less.

32. The method of any one of claims 23-31, wherein the method has an accuracy of about 98% or greater for diagnosing the dengue virus.

33. The method of any one of claims 23-32, wherein the method has an accuracy of about 99% or greater for diagnosing the dengue virus.

34. The method of any one of claims 23-33, wherein the method has an accuracy of about 100% for diagnosing the dengue virus.

35. The method of any one of claims 23-34, wherein the method has a specificity of about 98% or greater for diagnosing the dengue virus.

36. The method of any one of claims 23-35, wherein the method has a specificity of about 99% or greater for diagnosing the dengue virus.

37. The method of any one of claims 23-36, wherein the method has a specificity of about 100% for diagnosing the dengue virus.