

Thus, there remains a need for the development of effective strategies for the diagnosis, treatment, and prevention of dengue viral infection. The availability of nucleic acid diagnostic tests capable of efficiently detecting dengue virus in human specimens such as plasma, serum and respiratory secretions will assist the medical community in better diagnosing and treating dengue viral infections.

SUMMARY

The present ~~invention~~disclosure is based on the development of sensitive, reliable nucleic acid-based diagnostic assays for the detection of dengue viruses in biological samples from potentially infected subjects. The methods allow the rapid detection in a single assay of dengue virus infection caused by one or several dengue viruses, such as caused by one or more dengue virus serotypes 1-4. The methods can also be used to quantitate the amount of virus that is present and to determine which dengue virus serotypes are present in the biological sample. If infection is detected, the individual can be given appropriate therapy, and steps can be taken to prevent or reduce further transmission and spread of dengue virus. If infection is ruled-out, other potential causes of undifferentiated febrile illness can be further investigated.

The methods utilize primers and probes for amplifying and/or detecting target sequences of one or more dengue virus serotypes, to allow detection of a single serotype or multiple serotypes simultaneously in a single assay. In certain embodiments, the dengue virus sequences are detected using reverse transcriptase-polymerase chain reaction (RT-PCR), for example, using real-time RT-PCR and/or multiplex RT-PCR. Other nucleic-acid based detection techniques such as, but not limited to, nucleic acid sequence based amplification (NASBA), a 5' nuclease assay (e.g., TaqManTM), or transcription-mediated amplification (TMA), can also be used.

Exemplary primers (SEQ ID NOS:5-12) and probes (SEQ ID NOS:13-21) are shown in Example 1 (see Tables 1 and 2). Changes to the nucleotide sequences of these primers and probes may be introduced corresponding to genetic variations in particular Dengue strains. For example up to three nucleotide changes, including 1 nucleotide change, 2 nucleotide changes, or three nucleotide changes, may be made in a sequence selected from the group consisting of SEQ ID NOS:5-21, wherein the oligonucleotide primer or probe is capable of hybridizing to and amplifying or detecting a particular dengue virus target nucleic acid.

In one ~~aspect~~embodiment, the ~~invention~~disclosure includes a composition for detecting dengue virus in a biological sample using a nucleic acid amplification assay, wherein the composition comprises at least one set of oligonucleotide primers comprising
 5 a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, the portion comprising a 5' UTR sequence or a dengue virus capsid coding sequence, wherein the primers are not more than 40 nucleotides in length, wherein said set of primers is selected from the group consisting of:

- a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse
 10 primer comprising the sequence of SEQ ID NO:9;
- b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;
- c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;
- 15 d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;
- e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;
- f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse
 20 primer comprising the sequence of SEQ ID NO:9;
- g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;
- h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward
 25 primer and reverse primer of a primer set selected from the group consisting of (a)-(g);
- i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence,
 30 wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay; and
- j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the

forward primer and reverse primer of a primer set selected from the group consisting of (a)-(i).

In one aspect, there is provided a composition for detecting dengue virus in a biological sample using a nucleic acid amplification assay, the composition comprising at least one set of oligonucleotide primers comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, said portion comprising a 5' UTR sequence or a dengue virus capsid coding sequence, wherein said primers are not more than 40 nucleotides in length, wherein said set of primers is selected from the group consisting essentially of:

(a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;

(b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;

(c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;

(d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;

(e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;

(f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;

(g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;

(h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(g);

(i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting essentially of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay;

(j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(i); and

(k) a combination of a primer set selected from the group comprising (a)-(j).

In certain embodiments, the composition may further comprise at least one detectably labeled oligonucleotide probe sufficiently complementary to and capable of hybridizing with dengue virus RNA or an amplicon thereof. Exemplary probes include a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, a probe comprising the sequence of SEQ ID NO:16, a probe comprising the sequence of SEQ ID NO:17, a probe comprising the sequence of SEQ ID NO:18, a probe comprising the sequence of SEQ ID NO:19, a probe comprising the sequence of SEQ ID NO:20, and a probe comprising the sequence of SEQ ID NO:21. The composition may include a set of probes capable of detecting multiple serotypes of dengue virus, including two or more, three or more, or all four serotypes of dengue virus. In one embodiment, the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

The probe may be detectably labeled with a fluorophore such as, but not limited to carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'- tetrachloro-4-7-dichlorofluorescein (TET). In one embodiment, the probe comprises a 5'-fluorophore and a 3'-quencher. For example, the probe may comprises a 5'-fluorophore selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, and 2', 4', 5', 7'-tetrachloro-4-7-dichlorofluorescein (TET) and a 3'-quencher selected from the group consisting of a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and dihydrocyclopyrroloindole tripeptide minor groove binder (MGB). In one embodiment, the probe is a molecular beacon.

In one embodiment, the composition comprises a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, a primer comprising the sequence of SEQ ID NO:12, a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14,

a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

In one embodiment, the probe comprising the sequence of SEQ ID NO:13 further comprises a 5' FAM fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:14 further comprises a 5' CAL Fluor Orange 560 fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:15 further comprises a 5' CAL Fluor Red 610 fluorophore and a 3' BHQ-2 quencher, and the probe comprising the sequence of SEQ ID NO:16 further comprises a 5' Quasar Blue 670 fluorophore and a 3' BHQ-2 quencher.

In another aspect, ~~the invention includes~~ there is provided a method for detecting dengue virus in a biological sample, the method comprising:

a) contacting a biological sample suspected of containing dengue virus with a composition, as described herein, for detecting dengue virus by nucleic acid amplification of viral RNA,

b) amplifying dengue virus nucleic acids if present, wherein the nucleic acids comprise at least one target sequence selected from the group consisting of a dengue-1 target sequence, a dengue-2 target sequence, a dengue-3 target sequence, and a dengue-4 target sequence; and

c) detecting the presence of the amplified nucleic acids using at least one detectably labeled oligonucleotide probe sufficiently complementary to and capable of hybridizing with the dengue virus RNA or amplicon thereof, if present, as an indication of the presence or absence of dengue virus in the sample.

In one embodiment, at least one probe is selected from the group consisting of a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, a probe comprising the sequence of SEQ ID NO:16, a probe comprising the sequence of SEQ ID NO:17, a probe comprising the sequence of SEQ ID NO:18, a probe comprising the sequence of SEQ ID NO:19, a probe comprising the sequence of SEQ ID NO:20, and a probe comprising the sequence of SEQ ID NO:21.

In another embodiment, a set of probes is used for detecting dengue virus in a biological sample, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16. In one embodiment, the probe comprising the sequence of SEQ ID NO:13 further comprises a 5' FAM fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:14 further comprises a 5' CAL Fluor Orange 560 fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:15 further comprises a 5' CAL Fluor Red 610 fluorophore and a 3' BHQ-2 quencher, and the probe comprising the sequence of SEQ ID NO:16 further comprises a 5' Quasar Blue 670 fluorophore and a 3' BHQ-2 quencher.

In one aspect, there is provided an isolated oligonucleotide not more than 40 nucleotides in length comprising:

(a) a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting essentially of SEQ ID NOS:5-21;

(b) a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting essentially of SEQ ID NOS:5-21 by up to three nucleotide changes, wherein the oligonucleotide is capable of hybridizing to and amplifying dengue virus nucleic acids; or

(c) complements of (a) and (b).

In another aspectembodiment, the inventiondisclosure includes an isolated oligonucleotide not more than 40 nucleotides in length comprising a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21; or variants thereof comprising a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21 by up to three nucleotide changes, wherein the oligonucleotide is capable of hybridizing to and amplifying and/or detecting dengue virus nucleic acids; or complements thereof. Oligonucleotides may further comprise a detectable label. For example, the detectable label may be a fluorophore such as, but not limited to carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'-tetrachloro-4-7-dichlorofluorescein (TET). The oligonucleotide may further comprise a quencher such as, but not limited to a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyl, and a minor groove binder (MGB). In certain embodiments, the oligonucleotide is selected from the group consisting of: an oligonucleotide consisting of a

nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21 or a complement thereof.

In one aspect, there is provided an isolated oligonucleotide selected from the group consisting essentially of:

(a) an oligonucleotide consisting essentially of a nucleotide sequence selected from the group consisting essentially of SEQ ID NOS:5-21;

(b) an oligonucleotide complementary to an oligonucleotide of (a); and

(c) a combination of an oligonucleotide selected from (a) and (b).

In one aspect, there is provided a kit for detecting dengue virus in a biological sample, the kit comprising:

written instructions for identifying the presence of dengue virus; and

at least one set of primers comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, said portion comprising a 5' UTR sequence or a dengue capsid coding sequence, wherein said primers are not more than about 40 nucleotides in length, wherein said set of primers is selected from the group consisting essentially of:

(a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;

(b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;

(c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;

(d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;

(e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;

(f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;

(g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;

(h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(g);

(i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting essentially of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay;

(j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(i); and

(k) a combination of primers selected from the group comprising (a)-(j).

In another aspectembodiment, the inventiondisclosure includes a kit for detecting dengue virus in a biological sample by nucleic acid amplification of viral RNA. The kit may comprise a composition, as described herein, comprising at least one set of primers including a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, including a 5' UTR sequence or a dengue capsid coding sequence. The kit may further comprise written instructions for identifying the presence of the dengue virus, quantitating the virus, and/or serotyping dengue virus. The kit may also comprise reagents for performing reverse transcriptase polymerase

Claims

What is claimed is:

1. A composition for detecting dengue virus in a biological sample using a nucleic acid amplification assay, the composition comprising at least one set of oligonucleotide primers comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, said portion comprising a 5' UTR sequence or a dengue virus capsid coding sequence, wherein said primers are not more than 40 nucleotides in length, wherein said set of primers is selected from the group consisting of:

- a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;
- b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;
- c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;
- d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;
- e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;
- f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;
- g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;
- h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(g);
- i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay, and

- j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(i).
2. The composition of claim 1, further comprising at least one detectably labeled oligonucleotide probe sufficiently complementary to and capable of hybridizing with dengue virus RNA or an amplicon thereof.
3. The composition of claim 2, wherein the probe is selected from the group consisting of:
- a) a probe comprising the sequence of SEQ ID NO:13,
 - b) a probe comprising the sequence of SEQ ID NO:14,
 - c) a probe comprising the sequence of SEQ ID NO:15,
 - d) a probe comprising the sequence of SEQ ID NO:16,
 - e) a probe comprising the sequence of SEQ ID NO:17,
 - f) a probe comprising the sequence of SEQ ID NO:18,
 - g) a probe comprising the sequence of SEQ ID NO:19,
 - h) a probe comprising the sequence of SEQ ID NO:20,
 - i) a probe comprising the sequence of SEQ ID NO:21, and
 - j) a probe that differs from the corresponding nucleotide sequence of a probe selected from the group consisting of (a)-(i) in that the probe has up to three nucleotide changes compared to the corresponding sequence, wherein the probe is capable of hybridizing to and detecting the dengue virus RNA or an amplicon thereof.
4. The composition of claim 3 comprising a set of probes, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.
5. The composition of claim 2, wherein the detectably labeled probe comprises a fluorophore.
6. The composition of claim 5, wherein the fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610,

Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'- tetrachloro-4-7-dichlorofluorescein (TET).

7. The composition of claim 6, wherein the detectably labeled probe comprises a 5'-fluorophore and a 3'-quencher.

8. The composition of claim 7, wherein the 5'-fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, and 2', 4', 5', 7'-tetrachloro-4-7-dichlorofluorescein (TET).

9. The composition of claim 7, wherein the 3'-quencher is selected from the group consisting of a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and dihydrocyclopyrroloindole tripeptide minor groove binder (MGB).

10. The composition of claim 7, wherein the probe is a molecular beacon.

11. The composition of claim 2 comprising a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, a primer comprising the sequence of SEQ ID NO:12, a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

12. The composition of claim 11, wherein the probe comprising the sequence of SEQ ID NO:13 further comprises a 5' FAM fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:14 further comprises a 5' CAL Fluor Orange 560 fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:15 further comprises a 5' CAL Fluor Red 610 fluorophore and a 3' BHQ-2 quencher, and the probe comprising the sequence of SEQ ID NO:16 further comprises a 5' Quasar Blue 670 fluorophore and a 3' BHQ-2 quencher.

13. A method for detecting dengue virus in a biological sample, the method comprising:

- a) contacting a biological sample suspected of containing dengue virus with the composition of claim 1,
- b) amplifying dengue virus nucleic acids if present, said nucleic acids comprising at least one target sequence selected from the group consisting of a dengue-1 target sequence, a dengue-2 target sequence, a dengue-3 target sequence, and a dengue-4 target sequence;
- c) detecting the presence of the amplified nucleic acids using at least one detectably labeled oligonucleotide probe sufficiently complementary to and capable of hybridizing with the dengue virus RNA or amplicon thereof, if present, as an indication of the presence or absence of dengue virus in the sample.

14. The method of claim 13, wherein at least one probe is selected from the group consisting of:

- a) a probe comprising the sequence of SEQ ID NO:13,
- b) a probe comprising the sequence of SEQ ID NO:14,
- c) a probe comprising the sequence of SEQ ID NO:15,
- d) a probe comprising the sequence of SEQ ID NO:16,
- e) a probe comprising the sequence of SEQ ID NO:17,
- f) a probe comprising the sequence of SEQ ID NO:18,
- g) a probe comprising the sequence of SEQ ID NO:19,
- h) a probe comprising the sequence of SEQ ID NO:20,
- i) a probe comprising the sequence of SEQ ID NO:21, and
- j) a probe that differs from the corresponding nucleotide sequence of a probe selected from the group consisting of (a)-(i) in that the probe has up to three nucleotide changes compared to the corresponding sequence, wherein the probe is capable of hybridizing to and detecting the dengue virus RNA or amplicon thereof.

15. The method of claim 14, wherein a set of probes is used for detecting dengue virus in a biological sample, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

16. The method of claim 13, wherein the detectably labeled probe comprises a fluorophore.
17. The method of claim 16, wherein the fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'- tetrachloro-4-7-dichlorofluorescein (TET).
18. The method of claim 16, wherein the detectably labeled probe comprises a 5'-fluorophore and a 3'-quencher.
19. The method of claim 18, wherein the 5'-fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, and 2', 4', 5', 7'-tetrachloro-4-7-dichlorofluorescein (TET).
20. The method of claim 18, wherein the 3'-quencher is selected from the group consisting of a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and dihydrocyclopyrroloindole tripeptide minor groove binder (MGB).
21. The method of claim 18, wherein the probe is a molecular beacon.
22. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.
23. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID

NO:9, a primer comprising the sequence of SEQ ID NO:10, and a primer comprising the sequence of SEQ ID NO:12.

24. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

25. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

26. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, and a primer comprising the sequence of SEQ ID NO:12.

27. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

28. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, and a primer comprising the sequence of SEQ ID NO:12.

29. The method of claim 13, wherein the primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

30. The method of claim 13, wherein amplifying comprises reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), or a fluorogenic 5' nuclease assay, or a combination thereof.

31. The method of claim 30, wherein amplifying comprises real-time RT-PCR.

32. The method of claim 30, wherein amplifying comprises multiplex RT-PCR.

33. The method of claim 32, wherein the primers and probes that are used for detecting dengue virus in a biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, a primer comprising the sequence of SEQ ID NO:12, a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

34. The method of claim 33, wherein the probe comprising the sequence of SEQ ID NO:13 further comprises a 5' FAM fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:14 further comprises a 5' CAL Fluor Orange 560 fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:15 further comprises a 5' CAL Fluor Red 610 fluorophore and a 3' BHQ-2 quencher, and the probe comprising the sequence of SEQ ID NO:16 further comprises a 5' Quasar Blue 670 fluorophore and a 3' BHQ-2 quencher.

35. The method of claim 13, wherein the biological sample is selected from the group consisting of blood, plasma, serum, and peripheral blood mononuclear cells.
36. The method of claim 13, wherein the biological sample is obtained from the liver, lung, spleen, thymus, kidneys, lymph nodes, or bone marrow.
37. The method of claim 13, further comprising quantitating the amount of dengue virus present in the biological sample.
38. The method of claim 13, further comprising serotyping the dengue virus present in the biological sample by detecting the presence of at least one target sequence selected from the group consisting of a dengue-1 target sequence, a dengue-2 target sequence, a dengue-3 target sequence, and a dengue-4 target sequence.
39. The method of claim 13, further comprising isolating the dengue virus nucleic acids from the biological sample prior to amplification.
40. The method of claim 13, further comprising isolating the dengue virus nucleic acids from the biological sample after amplification.
41. An isolated oligonucleotide not more than 40 nucleotides in length comprising:
- a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21;
 - a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21 by up to three nucleotide changes, wherein the oligonucleotide is capable of hybridizing to and amplifying dengue virus nucleic acids; or
 - complements of (a) and (b).
42. The oligonucleotide of claim 41, further comprising a detectable label.
43. The oligonucleotide of claim 42, wherein the detectable label is a fluorophore.

44. The oligonucleotide of claim 43, wherein the fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'- tetrachloro-4-7-dichlorofluorescein (TET).
45. The oligonucleotide of claim 43, further comprising a quencher.
46. The oligonucleotide of claim 45, wherein the quencher is selected from the group consisting of a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and a minor groove binder (MGB).
47. An isolated oligonucleotide selected from the group consisting of:
- a) an oligonucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS:5-21; and
 - b) an oligonucleotide complementary to an oligonucleotide of (a).
48. The oligonucleotide of claim 47, further comprising a detectable label.
49. The oligonucleotide of claim 48, wherein the detectable label is a fluorophore.
50. The oligonucleotide of claim 49, wherein the fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'- tetrachloro-4-7-dichlorofluorescein (TET).
51. The oligonucleotide of claim 49, further comprising a quencher.
52. The oligonucleotide of claim 51, wherein the quencher is selected from the group consisting of a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and a minor groove binder (MGB).
53. A kit for detecting dengue virus in a biological sample, the kit comprising:
written instructions for identifying the presence of dengue virus; and

at least one set of primers comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, said portion comprising a 5' UTR sequence or a dengue capsid coding sequence, wherein said primers are not more than about 40 nucleotides in length, wherein said set of primers is selected from the group consisting of:

- a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;
- b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;
- c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;
- d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;
- e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;
- f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;
- g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;
- h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(g);
- i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay; and
- j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting of (a)-(i).

54. The kit of claim 53, further comprising at least one probe for detecting dengue virus in a biological sample, wherein the at least one probe is selected from the group

consisting of a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, a probe comprising the sequence of SEQ ID NO:16, a probe comprising the sequence of SEQ ID NO:17, a probe comprising the sequence of SEQ ID NO:18, a probe comprising the sequence of SEQ ID NO:19, a probe comprising the sequence of SEQ ID NO:20, and a probe comprising the sequence of SEQ ID NO:21.

55. The kit of claim 54 comprising a set of probes for detecting dengue virus in a biological sample, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

56. The kit of claim 54, wherein at least one probe comprises a detectable label.

57. The kit of claim 56, wherein the detectable label comprises a fluorophore.

58. The kit of claim 57, wherein the fluorophore is selected from the group consisting of carboxyfluorescein (FAM), CAL Fluor Orange 560, CAL Fluor Red 610, Quasar Blue 670, 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7'-tetrachloro-4'-chloro-6'-fluorescein (TET).

59. The kit of claim 58, wherein at least one probe comprises a 5'-fluorophore and a 3'-quencher.

60. The kit of claim 59, wherein the 3'-quencher is selected from the group consisting of a black hole quencher (BHQ), tetramethyl rhodamine (TAMRA), dabcyI, and dihydrocyclopyrroloindole tripeptide minor groove binder (MGB).

61. The kit of claim 53 comprising a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

62. The kit of claim 61 further comprising a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

63. The kit of claim 62, wherein the probe comprising the sequence of SEQ ID NO:13 further comprises a 5' FAM fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:14 further comprises a 5' CAL Fluor Orange 560 fluorophore and a 3' BHQ-1 quencher, the probe comprising the sequence of SEQ ID NO:15 further comprises a 5' CAL Fluor Red 610 fluorophore and a 3' BHQ-2 quencher, and the probe comprising the sequence of SEQ ID NO:16 further comprises a 5' Quasar Blue 670 fluorophore and a 3' BHQ-2 quencher.

64. The kit of claim 53, further comprising reagents for performing reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA) or a fluorogenic 5' nuclease assay.

~~65. The kit of claim 53, further comprising a polymerase and buffers.~~

Claims

What is claimed is:

1. A composition for detecting dengue virus in a biological sample using a nucleic acid amplification assay, the composition comprising at least one set of oligonucleotide primers comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, said portion comprising a 5' UTR sequence or a dengue virus capsid coding sequence, wherein said primers are not more than 40 nucleotides in length, wherein said set of primers is selected from the group consisting essentially of:
 - (a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;
 - (b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;
 - (c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;
 - (d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;
 - (e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;
 - (f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;
 - (g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;
 - (h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(g);
 - (i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting essentially of (a)-(g) in that the primer has up to three nucleotide changes compared to

the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay;

(j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(i); and

(k) a combination of a primer set selected from the group comprising (a)-(j).

2. The composition of claim 1, further comprising at least one detectably labeled oligonucleotide probe sufficiently complementary to and capable of hybridizing with dengue virus RNA or an amplicon thereof.

3. The composition of claim 2, wherein the probe is selected from the group consisting essentially of:

- (a) a probe comprising the sequence of SEQ ID NO: 13,
- (b) a probe comprising the sequence of SEQ ID NO: 14,
- (c) a probe comprising the sequence of SEQ ID NO: 15,
- (d) a probe comprising the sequence of SEQ ID NO: 16,
- (e) a probe comprising the sequence of SEQ ID NO: 17,
- (f) a probe comprising the sequence of SEQ ID NO: 18,
- (g) a probe comprising the sequence of SEQ ID NO: 19,
- (h) a probe comprising the sequence of SEQ ID NO: 20,
- (i) a probe comprising the sequence of SEQ ID NO: 21,
- (j) a probe that differs from the corresponding nucleotide sequence

of a probe selected from the group consisting essentially of (a)-(i) in that the probe has up to three nucleotide changes compared to the corresponding sequence, wherein the probe is capable of hybridizing to and detecting the dengue virus RNA or an amplicon thereof, and

(k) a combination of probes selected from the group comprising (a)-(j).

4. The composition of claim 3 comprising a set of probes, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe

comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

5. The composition of claim 4, wherein the set of primers comprises a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

6. A method for detecting dengue virus in a biological sample, the method comprising:

(a) contacting a biological sample suspected of containing dengue virus with the composition of claim 1,

(b) amplifying dengue virus nucleic acids if present, said nucleic acids comprising at least one target sequence selected from the group consisting essentially of a dengue-1 target sequence, a dengue-2 target sequence, a dengue-3 target sequence, and a dengue-4 target sequence;

(c) detecting the presence of the amplified nucleic acids using at least one detectably labeled oligonucleotide probe sufficiently complementary to and capable of hybridizing with the dengue virus RNA or amplicon thereof, if present, as an indication of the presence or absence of dengue virus in the sample.

7. The method of claim 6, wherein at least one probe is selected from the group consisting essentially of:

- (a) a probe comprising the sequence of SEQ ID NO:13,
- (b) a probe comprising the sequence of SEQ ID NO:14,
- (c) a probe comprising the sequence of SEQ ID NO:15,
- (d) a probe comprising the sequence of SEQ ID NO:16,
- (e) a probe comprising the sequence of SEQ ID NO:17,
- (f) a probe comprising the sequence of SEQ ID NO:18,
- (g) a probe comprising the sequence of SEQ ID NO:19,
- (h) a probe comprising the sequence of SEQ ID NO:20,
- (i) a probe comprising the sequence of SEQ ID NO:21,

(j) a probe that differs from the corresponding nucleotide sequence of a probe selected from the group consisting essentially of (a)-(i) in that the probe has up to three nucleotide changes compared to the corresponding sequence, wherein the probe is capable of hybridizing to and detecting the dengue virus RNA or amplicon thereof, and

(k) a combination of probes selected from the group comprising (a)-(j).

8. The method of claim 6, wherein a set of probes is used for detecting dengue virus in a biological sample, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

9. The method of claim 6, wherein the set of primers used for detecting dengue virus in the biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.

10. The method of claim 6, wherein the set of primers used for detecting dengue virus in the biological sample comprise

a) a primer comprising the sequence of SEQ ID NO:5,
b) a primer comprising the sequence of SEQ ID NO:8,
c) a primer comprising the sequence of SEQ ID NO:9,
d) a primer comprising the sequence of SEQ ID NO:12, and
e) a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, or a primer comprising the sequence of SEQ ID NO:10 and a primer comprising the sequence of SEQ ID NO:11.

11. The method of claim 10, wherein the set of primers used for detecting dengue virus in the biological sample further comprise a primer comprising the sequence of SEQ ID NO:6 or a primer comprising the sequence of SEQ ID NO:7.

12. The method of claim 6, wherein amplifying comprises reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), or a fluorogenic 5' nuclease assay, or a combination thereof.

13. The method of claim 7, wherein the primers and probes that are used for detecting dengue virus in a biological sample comprise a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, a primer comprising the sequence of SEQ ID NO:12, a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

14. The method of claim 6, further comprising serotyping the dengue virus present in the biological sample by detecting the presence of at least one target sequence selected from the group consisting essentially of a dengue-1 target sequence, a dengue-2 target sequence, a dengue-3 target sequence, and a dengue-4 target sequence.

15. An isolated oligonucleotide not more than 40 nucleotides in length comprising:

(a) a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting essentially of SEQ ID NOS:5-21;

(b) a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting essentially of SEQ ID NOS:5-21 by up to three nucleotide changes, wherein the oligonucleotide is capable of hybridizing to and amplifying dengue virus nucleic acids; or

(c) complements of (a) and (b).

16. An isolated oligonucleotide selected from the group consisting essentially of:

- (a) an oligonucleotide consisting essentially of a nucleotide sequence selected from the group consisting essentially of SEQ ID NOS:5-21;
 - (b) an oligonucleotide complementary to an oligonucleotide of (a);
- and
- (c) a combination of an oligonucleotide selected from (a) and (b).

17. A kit for detecting dengue virus in a biological sample, the kit comprising:

written instructions for identifying the presence of dengue virus; and
at least one set of primers comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus genome, said portion comprising a 5' UTR sequence or a dengue capsid coding sequence, wherein said primers are not more than about 40 nucleotides in length, wherein said set of primers is selected from the group consisting essentially of:

- (a) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:9;
- (b) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:10;
- (c) a forward primer comprising the sequence of SEQ ID NO:5 and a reverse primer comprising the sequence of SEQ ID NO:11;
- (d) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:10;
- (e) a forward primer comprising the sequence of SEQ ID NO:6 and a reverse primer comprising the sequence of SEQ ID NO:11;
- (f) a forward primer comprising the sequence of SEQ ID NO:7 and a reverse primer comprising the sequence of SEQ ID NO:9;
- (g) a forward primer comprising the sequence of SEQ ID NO:8 and a reverse primer comprising the sequence of SEQ ID NO:12;
- (h) a forward primer and a reverse primer each comprising at least 10 contiguous nucleotides from the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(g);

(i) a forward primer and a reverse primer comprising at least one nucleotide sequence that differs from the corresponding nucleotide sequence of the forward primer or reverse primer of a primer set selected from the group consisting essentially of (a)-(g) in that the primer has up to three nucleotide changes compared to the corresponding sequence, wherein the primer is capable of hybridizing to and amplifying dengue virus nucleic acids in the nucleic acid amplification assay;

(j) a forward primer and a reverse primer comprising nucleotide sequences that are complements of the corresponding nucleotide sequences of the forward primer and reverse primer of a primer set selected from the group consisting essentially of (a)-(i); and

(k) a combination of primers selected from the group comprising (a)-(j).

18. The kit of claim 17, further comprising at least one probe for detecting dengue virus in a biological sample, wherein the at least one probe is selected from the group consisting essentially of a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, a probe comprising the sequence of SEQ ID NO:16, a probe comprising the sequence of SEQ ID NO:17, a probe comprising the sequence of SEQ ID NO:18, a probe comprising the sequence of SEQ ID NO:19, a probe comprising the sequence of SEQ ID NO:20, and a probe comprising the sequence of SEQ ID NO:21.

19. The kit of claim 18 comprising a set of probes for detecting dengue virus in a biological sample, wherein the set of probes comprises a probe comprising the sequence of SEQ ID NO:13, a probe comprising the sequence of SEQ ID NO:14, a probe comprising the sequence of SEQ ID NO:15, and a probe comprising the sequence of SEQ ID NO:16.

20. The kit of claim 17 comprising a primer comprising the sequence of SEQ ID NO:5, a primer comprising the sequence of SEQ ID NO:6, a primer comprising the sequence of SEQ ID NO:7, a primer comprising the sequence of SEQ ID NO:8, a primer comprising the sequence of SEQ ID NO:9, a primer comprising the sequence of SEQ ID NO:10, a primer comprising the sequence of SEQ ID NO:11, and a primer comprising the sequence of SEQ ID NO:12.