

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

In one aspect, there is provided a multiplex composition for detecting and serotyping dengue virus in a biological sample using a nucleic acid amplification assay, the composition comprising a combination of oligonucleotide primer pairs, each primer pair comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, wherein said forward and reverse primers are not more than 40 nucleotides in length, and wherein said combination of primer pairs 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 18 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); 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 comprise 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, 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, 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; 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 for use in a multiplex composition for detecting and serotyping dengue virus by amplification of at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, comprising:

(a) a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:7 and 9-21;

(b) a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting of SEQ ID NOS:7 and 9-21 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 embodiment, the disclosure 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), dabcyI, 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 a primer set comprising two or more isolated oligonucleotides for use in a multiplex composition for detecting and serotyping dengue virus by amplification of at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, comprising a primer selected from the group consisting of:

(a) an oligonucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS:7 and 9-12;

(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 and serotyping dengue virus in a biological sample, the kit comprising:

written instructions for identifying the presence and serotype of dengue virus; and

a combination of primer pairs, each primer pair comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, wherein said forward and reverse primers are not more than about 40 nucleotides in length, and wherein said combination of primer pairs 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 18 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,
5 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); and

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

In another embodiment, the disclosure 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
15 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

chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), or a fluorogenic 5' nuclease assay.

The methods of the disclosure can be used to detect dengue virus in biological samples such as, but not limited to, blood, plasma, serum, peripheral blood mononuclear
 5 cells, liver, lung, spleen, thymus, kidneys, lymph nodes, and bone marrow. Using the methods of the disclosure, individuals infected with dengue virus can be identified. The, dengue virus can be specifically detected even in samples containing other viruses, such as West Nile virus, Japanese encephalitis virus, tick-born encephalitis virus, HIV and/or HCV. Moreover, the assays can be used to screen mosquitoes, primates, and other hosts
 10 for dengue virus in order to determine if a particular insect or animal population is infected with the virus, thereby preventing further transmission and spread of dengue virus infection. Additionally, infected blood samples can be detected and excluded from transfusion, as well as from the preparation of blood derivatives.

These and other embodiments of the subject disclosure will readily occur to those
 15 of skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1D show the linear ranges for multiplex rRT-PCR assays for the four dengue serotypes: dengue 1 (Fig. 1A), dengue 2 (Fig. 1B), dengue 3 (Fig. 1C), and
 20 dengue 4 (Fig. 1D).

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of virology, chemistry, biochemistry, recombinant DNA techniques
 25 and immunology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Fundamental Virology* (D.M. Knipe, P.M. Howley, D.E. Griffin, R.A. Lamb, M.A. Martin, B. Roizman, S.E. Straus, eds.), Lippincott Williams & Wilkins; Fourth edition, 2001; *Dengue Virus* (Current Topics in Microbiology and Immunology, A.L. Rothman, ed.), Springer; 1st edition, 2009; *Frontiers in Dengue Virus Research* (K.A.
 30 Hanley and S.C. Weaver eds.), Caister Academic Press, 1st edition, 2010; *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., Blackwell Scientific Publications); T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and

Claims

What is claimed is:

1. A multiplex composition for detecting and serotyping dengue virus in a biological sample using a nucleic acid amplification assay, the composition comprising a combination of oligonucleotide primer pairs, each primer pair comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, wherein said forward and reverse primers are not more than 40 nucleotides in length, and wherein said combination of primer pairs 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 18 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); 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 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 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 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 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 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 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 for use in a multiplex composition for detecting and serotyping dengue virus by amplification of at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, comprising:

(a) a nucleotide sequence comprising at least 10 contiguous nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS: 7 and 9-21;

(b) a nucleotide sequence that differs from a nucleotide sequence selected from the group consisting of SEQ ID NOS: 7 and 9-21 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. A primer set comprising two or more isolated oligonucleotides for use in a multiplex composition for detecting and serotyping dengue virus by amplification of at least a portion of a dengue virus capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, comprising a primer selected from the group consisting of:

(a) an oligonucleotide consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS: 7 and 9-12;

(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 and serotyping dengue virus in a biological sample, the kit comprising:

written instructions for identifying the presence and serotype of dengue virus; and a combination of primer pairs, each primer pair comprising a forward primer and a reverse primer capable of amplifying at least a portion of a dengue capsid coding sequence corresponding to a detectable serotype of dengue virus selected from dengue-1, dengue-2, dengue-3, and dengue-4, wherein said forward and reverse primers are not more than about 40 nucleotides in length, and wherein said combination of primer pairs 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 18 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); 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 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.