

CLAIMS

1. A method of simultaneously detecting, differentiating, and/or quantifying Chikungunya virus (CHIKV), Dengue virus serotype-1 (DENV1), Dengue virus serotype-2 (DENV2), Dengue virus serotype-3 (DENV3), Dengue virus serotype-4 (DENV4) and Zika virus (ZIKV) in a sample, wherein the method comprising:

determining the presence of the target regions or fragments thereof selected from the group consisting of Non Structural protein 5 (NS5) of Zika virus, NS5 of DENV1, NS5 of DENV2, NS5 of DENV3, Capsid of DENV4, and E1 glycoprotein of CHIKV.

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2. The method of claim 1, wherein the target regions or fragments thereof are encoded by the sequence SEQ ID NO: 1 (CHIKV E1 consensus sequence); SEQ ID NO: 2 (DENV1 NS5 48+22 seq consensus sequence); SEQ ID NO: 3 (DENV2 NS5 consensus sequence); SEQ ID NO: 4 (DENV3 NS5 consensus sequence); SEQ ID NO: 5 (DENV4 Capsid consensus sequence); and SEQ ID NO: 6 (ZIKV NS5 check_56seq consensus sequence).

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3. The method of any of the preceding claims, wherein the detecting comprises performing reverse transcription polymerase chain reaction (RT-PCR).

4. The method of any of the preceding claims, wherein the primers and probe are selected from the group consisting of:

a ZIKV forward primer comprising a sequence at least 90% identical to CCTTGGATTCTTGAACGAGGATCAC (NS5_ZIKV-F / SEQ ID NO: 7);

a ZIKV reverse primer comprising a sequence at least 90% identical to GCTTCATTCTCCAGATCAAACCTGC (NS5_ZIKV-R / SEQ ID NO: 9) or GCTTCATTCTCTAGATCAAACCTGC (ZIKV-R1_T / SEQ ID NO: 32);

a ZIKV probe comprising a sequence at least 90% identical to TACCAGGAGGAAGGATGTATGCAG (5'-FAM NS5_ZIKV-P/ZEN/3' IBFQ / SEQ ID NO: 8) or ACCAGGAGGAAAGATGTACGCAG (ZIKV_P1_AF / SEQ ID NO: 33);

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a DENV1 first forward primer comprising a sequence at least 90% identical to GGCTGAAGAAAGTCACAGAAG (NS5_D1-F_A/ SEQ ID NO: 10);

a DENV1 second forward primer comprising a sequence at least 90% identical to GGCTGAAGAAAGTCACTGAAG (NS5_D1-F_T/ SEQ ID NO: 11);

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a DENV1 reverse primer comprising a sequence at least 90% identical to GAGGACTCACCAATATCACACAA (NS5_D1-R / SEQ ID NO: 13);

a DENV1 probe comprising a sequence at least 90% identical to ACCTATGGATGGAACCTAGTAAAGCT (5'-HEX NS5_D1/ZEN/3' IBFQ/ SEQ ID NO: 12);

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a DENV3 forward primer comprising a sequence at least 90% identical to GCTCAGCCTCCTCCATGATAAATG (NS5_D3-F / SEQ ID NO: 14);

a DENV3 reverse primer comprising a sequence at least 90% identical to GGGTGTCTGGTGTCCACTTTCTC (NS5_D3-R / SEQ ID NO: 17);

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a DENV3 first probe comprising a sequence at least 90% identical to CATGGTGACACAGATGGCAATGAC (5'-Texas Red NS5_D3-P_T/3' IBRQ/ SEQ ID NO: 15);

a DENV3 second probe comprising a sequence at least 90% identical to CACGGTGACACAGATGGCAATGAC (5'-Texas Red NS5_D3-P_C/3' IBRQ/ SEQ ID NO: 16);

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a CHIKV forward primer comprising a sequence at least 90% identical to GGCGCCTACTGCTTCTGCGAC (E1_CHIKV-F1/ SEQ ID NO: 18);

a CHIKV reverse primer comprising a sequence at least 90% identical to TTGGTAAAGGACGCGGAGCTTAGC (E1_CHIKV-R1/ SEQ ID NO: 21);

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a CHIKV first probe comprising a sequence at least 90% identical to AGCGAAGCACATGTGGAGAAGTCC (5'-FAM E1_CHIKV-P1_T/ZEN/3' IBFQ/ SEQ ID NO: 19);

a CHIKV second probe comprising a sequence at least 90% identical to AGCGAAGCACACGTGGAGAAGTCC (5'-FAM E1_CHIKV-P1_C/ZEN/3' IBFQ/ SEQ ID NO: 20);

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a DENV2 forward primer comprising a sequence at least 90% identical to ACACAGATGGCAATGACAGACACG (NS5_D2-F2/ SEQ ID NO: 22);

a DENV2 reverse primer comprising a sequence at least 90% identical to CCAAGGCTGCATTGCTTCTCAC (NS5_D2-R2/ SEQ ID NO: 24);

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a DENV2 probe comprising a sequence at least 90% identical to TGGAAGAAGTAGGAAAGAAAAAGACAC (5'-HEX NS5_D2-P2/ZEN/3' IBFQ / SEQ ID NO: 23);

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a DENV4 first forward primer comprising a sequence at least 90% identical to TGGTTAGACCACCTTTCAATATG (C_D4-F1.2_T/ SEQ ID NO: 25);

a DENV4 second forward primer comprising a sequence at least 90% identical to TGGCTAGACCACCTTTCAATATG (C_D4-F1.2_C/ SEQ ID NO: 26);

a DENV4 reverse primer comprising a sequence at least 90% identical to TGCTAGCACCATCCGTAA (C_D4-R1.2/ SEQ ID NO: 28);

5 a DENV4 probe comprising a sequence at least 90% identical to CCTCAAGGGTTGGTGAAGAGATTC (5'-Texas Red C_D4-P1/3' IBRQ/ SEQ ID NO: 27); and

a combination thereof.

10 5. The method of any of the preceding claims, wherein the primers and probes are conjugated with a detectable label. In some examples, the detectable label may include, but is not limited to a fluorophore, a quencher, or a combination thereof.

6. The method of any of the preceding claims, wherein the sample is selected from
15 the group consisting of whole blood, serum, plasma, cerebrospinal fluid, urine, and amniotic fluid.

7. The method of any of the preceding claims, wherein the sample is whole blood.

20 8. The method of any of the preceding claims, wherein the sample is whole blood treated with EDTA.

9. An isolated oligonucleotide for a simultaneous detection and/or differentiation and/or quantification of virus selected from the group consisting of Chikungunya virus
25 (CHIKV), Dengue virus serotype-1 (DENV1), Dengue virus serotype-2 (DENV2), Dengue virus serotype-3 (DENV3), Dengue virus serotype-4 (DENV4) and Zika virus (ZIKV) in a sample, wherein the oligonucleotide detects a nucleic acid sequence that is at least 80% identical to the sequences selected from the group consisting of:

a nucleic acid molecule that encodes a nucleotide sequence of Non Structural protein
30 5 (NS5) of Zika virus,

a nucleic acid molecule that encodes a nucleotide sequence of NS5 of DENV1,
a nucleic acid molecule that encodes a nucleotide sequence of NS5 of DENV2,
a nucleic acid molecule that encodes a nucleotide sequence of NS5 of DENV3,
a nucleic acid molecule that encodes a nucleotide sequence of Capsid of DENV4, and
35 a nucleic acid molecule that encodes a nucleotide sequence of E1 glycoprotein of CHIKV.

10. The oligonucleotide of claim 1, wherein
the nucleotide sequence of E1 glycoprotein of CHIKV comprises SEQ ID NO: 1 or its
fragment thereof,
- 5 the nucleotide sequence of NS5 of DENV1 comprises SEQ ID NO: 2 or its fragment
thereof,
the nucleotide sequence of NS5 of DENV2 comprises SEQ ID NO: 3 or its fragment
thereof,
the nucleotide sequence of NS5 of DENV3 comprises SEQ ID NO: 4 or its fragment
10 thereof,
the nucleotide sequence of Capsid of DENV4 comprises SEQ ID NO: 5 or its fragment
thereof, and
the nucleotide sequence of Non Structural protein 5 (NS5) of Zika virus comprises SEQ
ID NO: 6 or its fragment thereof.
- 15 11. The oligonucleotide of claim x, wherein the oligonucleotide comprises:
- a ZIKV forward primer comprising a sequence at least 90% identical to
CCTTGGATTCTTGAACGAGGATCAC (SEQ ID NO: 7);
- 20 a ZIKV reverse primer comprising a sequence at least 90% identical to
GCTTCATTCTCCAGATCAAACCTGC (SEQ ID NO: 9) or
GCTTCATTCTCTAGATCAAACCTGC (SEQ ID NO: 32);
- a ZIKV probe comprising a sequence at least 90% identical to
TACCAGGAGGAAGGATGTATGCAG (SEQ ID NO: 8) or
25 ACCAGGAGGAAAGATGTACGCAG (SEQ ID NO: 33);
- a DENV1 first forward primer comprising a sequence at least 90% identical to
GGCTGAAGAAAGTCACAGAAG (SEQ ID NO: 10);
- a DENV1 second forward primer comprising a sequence at least 90% identical to
30 GGCTGAAGAAAGTCACTGAAG (SEQ ID NO: 11);
- a DENV1 reverse primer comprising a sequence at least 90% identical to
GAGGACTCACCAATATCACACAA (SEQ ID NO: 13);
- a DENV1 probe comprising a sequence at least 90% identical to
ACCTATGGATGGAACCTAGTAAAGCT (SEQ ID NO: 12);

a DENV3 forward primer comprising a sequence at least 90% identical to GCTCAGCCTCCTCCATGATAAATG (SEQ ID NO: 14);

a DENV3 reverse primer comprising a sequence at least 90% identical to GGGTGTCTGGTGTCCACTTTCTC (SEQ ID NO: 17);

5 a DENV3 first probe comprising a sequence at least 90% identical to CATGGTGACACAGATGGCAATGAC (SEQ ID NO: 15);

a DENV3 second probe comprising a sequence at least 90% identical to CACGGTGACACAGATGGCAATGAC (SEQ ID NO: 16);

10 a CHIKV forward primer comprising a sequence at least 90% identical to GGCGCCTACTGCTTCTGCGAC (SEQ ID NO: 18);

a CHIKV reverse primer comprising a sequence at least 90% identical to TTGGTAAAGGACGCGGAGCTTAGC (SEQ ID NO: 21);

15 a CHIKV first probe comprising a sequence at least 90% identical to AGCGAAGCACATGTGGAGAAGTCC (SEQ ID NO: 19);

a CHIKV second probe comprising a sequence at least 90% identical to AGCGAAGCACACGTGGAGAAGTCC (SEQ ID NO: 20);

20 a DENV2 forward primer comprising a sequence at least 90% identical to ACACAGATGGCAATGACAGACACG (SEQ ID NO: 22);

a DENV2 reverse primer comprising a sequence at least 90% identical to CCAAGGCTGCATTGCTTCTCAC (SEQ ID NO: 24);

a DENV2 probe comprising a sequence at least 90% identical to TGGAAGAAGTAGGAAAGAAAAAGACAC (SEQ ID NO: 23);

25 a DENV4 first forward primer comprising a sequence at least 90% identical to TGGTTAGACCACCTTTCAATATG (SEQ ID NO: 25);

a DENV4 second forward primer comprising a sequence at least 90% identical to TGGCTAGACCACCTTTCAATATG (SEQ ID NO: 26);

30 a DENV4 reverse primer comprising a sequence at least 90% identical to TGCTAGCACCATCCGTAA (SEQ ID NO: 28); and

a DENV4 probe comprising a sequence at least 90% identical to CCTCAAGGGTTGGTGAAGAGATTC (SEQ ID NO: 27).

35 12. A method for detecting and/or differentiating and/or quantifying virus selected from the group consisting of Chikungunya virus (CHIKV), Dengue virus serotype-1 (DENV1),

Dengue virus serotype-2 (DENV2), Dengue virus serotype-3 (DENV3), Dengue virus serotype-4 (DENV4) and Zika virus (ZIKV) in a sample, the method comprising:

subjecting the sample to a reverse transcription polymerase chain reaction (RT-PCR) using primers and a probe specific for CHIKV E1 glycoprotein, primers and a probe specific for DENV1 Non Structural protein 5 (NS5), primers and a probe specific for DENV2 NS5, primers and a probe specific for DENV3 NS5, primers and a probe specific for DENV4 capsid, and primers and a probe specific for ZIKV NS5.

13. The method of claim 12, wherein the primers and probes comprise:

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a ZIKV forward primer comprising a sequence at least 90% identical to CCTTGGATTCTTGAACGAGGATCAC (SEQ ID NO: 7);

a ZIKV reverse primer comprising a sequence at least 90% identical to GCTTCATTCTCCAGATCAAACCTGC (SEQ ID NO: 9) or
15 GCTTCATTCTCTAGATCAAACCTGC (SEQ ID NO: 32);

a ZIKV probe comprising a sequence at least 90% identical to TACCAGGAGGAAGGATGTATGCAG (SEQ ID NO: 8) or
ACCAGGAGGAAAGATGTACGCAG (SEQ ID NO: 33);

20 a DENV1 first forward primer comprising a sequence at least 90% identical to GGCTGAAGAAAGTCACAGAAG (SEQ ID NO: 10);

a DENV1 second forward primer comprising a sequence at least 90% identical to GGCTGAAGAAAGTCACTGAAG (SEQ ID NO: 11);

a DENV1 reverse primer comprising a sequence at least 90% identical to
25 GAGGACTCACCAATATCACACAA (SEQ ID NO: 13);

a DENV1 probe comprising a sequence at least 90% identical to ACCTATGGATGGAACCTAGTAAAGCT (SEQ ID NO: 12);

a DENV3 forward primer comprising a sequence at least 90% identical to
30 GCTCAGCCTCCTCCATGATAAATG (SEQ ID NO: 14);

a DENV3 reverse primer comprising a sequence at least 90% identical to GGGTGTCTCTGGTGTCCACTTTCTC (SEQ ID NO: 17);

a DENV3 first probe comprising a sequence at least 90% identical to CATGGTGACACAGATGGCAATGAC (SEQ ID NO: 15);

35 a DENV3 second probe comprising a sequence at least 90% identical to CACGGTGACACAGATGGCAATGAC (SEQ ID NO: 16);

a CHIKV forward primer comprising a sequence at least 90% identical to GGCGCCTACTGCTTCTGCGAC (SEQ ID NO: 18);

5 a CHIKV reverse primer comprising a sequence at least 90% identical to TTGGTAAAGGACGCGGAGCTTAGC (SEQ ID NO: 21);

a CHIKV first probe comprising a sequence at least 90% identical to AGCGAAGCACATGTGGAGAAGTCC (SEQ ID NO: 19);

a CHIKV second probe comprising a sequence at least 90% identical to AGCGAAGCACACGTGGAGAAGTCC (SEQ ID NO: 20);

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a DENV2 forward primer comprising a sequence at least 90% identical to ACACAGATGGCAATGACAGACACG (SEQ ID NO: 22);

a DENV2 reverse primer comprising a sequence at least 90% identical to CCAAGGCTGCATTGCTTCTCAC (SEQ ID NO: 24);

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a DENV2 probe comprising a sequence at least 90% identical to TGGAAAGAACTAGGAAAGAAAAAGACAC (SEQ ID NO: 23);

a DENV4 first forward primer comprising a sequence at least 90% identical to TGGTTAGACCACCTTTCAATATG (SEQ ID NO: 25);

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a DENV4 second forward primer comprising a sequence at least 90% identical to TGGCTAGACCACCTTTCAATATG (SEQ ID NO: 26);

a DENV4 reverse primer comprising a sequence at least 90% identical to TGCTAGCACCATCCGTAA (SEQ ID NO: 28); and

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a DENV4 probe comprising a sequence at least 90% identical to CCTCAAGGGTTGGTGAAGAGATTC (SEQ ID NO: 27).

14. The method of claim 12 or 13, wherein:

30 the ZIKV forward primer comprising CCTTGGATTCTTGAACGAGGATCAC (SEQ ID NO: 7);

the ZIKV reverse primer comprising GCTTCATTCTCCAGATCAAACCTGC (SEQ ID NO: 9) or GCTTCATTCTCTAGATCAAACCTGC (SEQ ID NO: 32);

the ZIKV probe comprising TACCAGGAGGAAGGATGTATGCAG (SEQ ID NO: 8) or ACCAGGAGGAAAGATGTACGCAG (SEQ ID NO: 33);

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the DENV1 first forward primer comprising GGCTGAAGAAAGTCACAGAAG (SEQ ID NO: 10);

the DENV1 second forward primer comprising GGCTGAAGAAAGTCACTGAAG (SEQ ID NO: 11);

5 the DENV1 reverse primer comprising GAGGACTCACCAATATCACACAA (SEQ ID NO: 13);

the DENV1 probe comprising ACCTATGGATGGAACCTAGTAAAGCT (SEQ ID NO: 12);

10 the DENV3 forward primer comprising GCTCAGCCTCCTCCATGATAAATG (SEQ ID NO: 14);

the DENV3 reverse primer comprising GGGTGTCTGGTGTCCACTTTCTC (SEQ ID NO: 17);

15 the DENV3 first probe comprising CATGGTGACACAGATGGCAATGAC (SEQ ID NO: 15);

the DENV3 second probe comprising CACGGTGACACAGATGGCAATGAC (SEQ ID NO: 16);

20 the CHIKV forward primer comprising GGCGCCTACTGCTTCTGCGAC (SEQ ID NO: 18);

the CHIKV reverse primer comprising TTGGTAAAGGACGCGGAGCTTAGC (SEQ ID NO: 21);

the CHIKV first probe comprising AGCGAAGCACATGTGGAGAAGTCC (SEQ ID NO: 19);

25 the CHIKV second probe comprising AGCGAAGCACACGTGGAGAAGTCC (SEQ ID NO: 20);

the DENV2 forward primer comprising ACACAGATGGCAATGACAGACACG (SEQ ID NO: 22);

30 the DENV2 reverse primer comprising CCAAGGCTGCATTGCTTCTCAC (SEQ ID NO: 24);

the DENV2 probe comprising TGGAAAGAACTAGGAAAGAAAAAGACAC (SEQ ID NO: 23);

35 the DENV4 first forward primer comprising TGGTTAGACCACCTTTCAATATG (SEQ ID NO: 25);

the DENV4 second forward primer comprising TGGCTAGACCACCTTTCAATATG (SEQ ID NO: 26);

the DENV4 reverse primer comprising TGCTAGCACCATCCGTAA (SEQ ID NO: 28);
and

5 the DENV4 probe comprising CCTCAAGGGTTGGTGAAGAGATTC (SEQ ID NO: 27).

15. The method of any one of claims 12 to 14, wherein the primers and probes comprise a detectable label.

10 16. The method of claim 15, wherein the detectable label comprises a fluorophore, a quencher, or a combination thereof.

17. A kit for detecting Chikungunya virus (CHIKV), Dengue virus serotype-1 (DENV1), Dengue virus serotype-2 (DENV2), Dengue virus serotype-3 (DENV3), Dengue virus
15 serotype-4 (DENV4) and Zika virus (ZIKV) in a sample, comprising:

an agent specific for detecting CHIKV E1 glycoprotein, an agent specific for detecting DENV1 Non Structural protein 5 (NS5), an agent specific for detecting DENV2 NS5, an agent specific for detecting DENV3 NS5, an agent specific for detecting DENV4 capsid, and an agent specific for detecting ZIKV NS5.

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18. The kit of claim 17, comprising

an agent for detecting a region or fragment thereof having 80% sequence identity to SEQ ID NO: 1; an agent for detecting a region or fragment thereof having 80% sequence identity to SEQ ID NO: 2; an agent for detecting a region or fragment thereof having 80%
25 sequence identity to SEQ ID NO: 3; an agent for detecting a region or fragment thereof having 80% sequence identity to SEQ ID NO: 4; an agent for detecting a region or fragment thereof having 80% sequence identity to SEQ ID NO: 5; and an agent for detecting a region or fragment thereof having 80% sequence identity to SEQ ID NO: 6.

30 19. The kit of claim 17 or 18, wherein the agent comprises primers and probes comprising:

a ZIKV forward primer comprising a sequence at least 90% identical to CCTTGGATTCTTGAACGAGGATCAC (SEQ ID NO: 7);

a ZIKV reverse primer comprising a sequence at least 90% identical to
35 GCTTCATTCTCCAGATCAAACCTGC (SEQ ID NO: 9) or
GCTTCATTCTCTAGATCAAACCTGC (SEQ ID NO: 32);

a ZIKV probe comprising a sequence at least 90% identical to TACCAGGAGGAAGGATGTATGCAG (SEQ ID NO: 8) or ACCAGGAGGAAAGATGTACGCAG (SEQ ID NO: 33);

5 a DENV1 first forward primer comprising a sequence at least 90% identical to GGCTGAAGAAAGTCACAGAAG (SEQ ID NO: 10);

a DENV1 second forward primer comprising a sequence at least 90% identical to GGCTGAAGAAAGTCACTGAAG (SEQ ID NO: 11);

a DENV1 reverse primer comprising a sequence at least 90% identical to
10 GAGGACTCACCAATATCACACAA (SEQ ID NO: 13);

a DENV1 probe comprising a sequence at least 90% identical to ACCTATGGATGGAACCTAGTAAAGCT (SEQ ID NO: 12);

a DENV3 forward primer comprising a sequence at least 90% identical to
15 GCTCAGCCTCCTCCATGATAAATG (SEQ ID NO: 14);

a DENV3 reverse primer comprising a sequence at least 90% identical to GGGTGTCTGGTGTCCACTTTCTC (SEQ ID NO: 17);

a DENV3 first probe comprising a sequence at least 90% identical to CATGGTGACACAGATGGCAATGAC (SEQ ID NO: 15);

20 a DENV3 second probe comprising a sequence at least 90% identical to CACGGTGACACAGATGGCAATGAC (SEQ ID NO: 16);

a CHIKV forward primer comprising a sequence at least 90% identical to GGCGCCTACTGCTTCTGCGAC (SEQ ID NO: 18);

25 a CHIKV reverse primer comprising a sequence at least 90% identical to TTGGTAAAGGACGCGGAGCTTAGC (SEQ ID NO: 21);

a CHIKV first probe comprising a sequence at least 90% identical to AGCGAAGCACATGTGGAGAAGTCC (SEQ ID NO: 19);

a CHIKV second probe comprising a sequence at least 90% identical to
30 AGCGAAGCACACGTGGAGAAGTCC (SEQ ID NO: 20);

a DENV2 forward primer comprising a sequence at least 90% identical to ACACAGATGGCAATGACAGACACG (SEQ ID NO: 22);

a DENV2 reverse primer comprising a sequence at least 90% identical to
35 CCAAGGCTGCATTGCTTCTCAC (SEQ ID NO: 24);

a DENV2 probe comprising a sequence at least 90% identical to TGGAAAGAACTAGGAAAGAAAAAGACAC (SEQ ID NO: 23);

5 a DENV4 first forward primer comprising a sequence at least 90% identical to TGGTTAGACCACCTTTCAATATG (SEQ ID NO: 25);

a DENV4 second forward primer comprising a sequence at least 90% identical to TGGCTAGACCACCTTTCAATATG (SEQ ID NO: 26);

a DENV4 reverse primer comprising a sequence at least 90% identical to TGCTAGCACCATCCGTAA (SEQ ID NO: 28); and

10 a DENV4 probe comprising a sequence at least 90% identical to CCTCAAGGGTTGGTGAAGAGATTG (SEQ ID NO: 27).