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(54) COMPOSITIONS AND METHODS FOR DETECTING ZIKA VIRUS NUCLEIC ACID

ZUSAMMENSETZUNGEN UND VERFAHREN FÜR DEN NACHWEIS VON ZIKAVIRUS-NUKLEINSÄURE

COMPOSITIONS ET PROCÉDÉS DE DÉTECTION D'ACIDE NUCLÉIQUE DU VIRUS ZIKA

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Description

REFERENCE TO SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web.

BACKGROUND

[0002] Zika virus is a mosquito-borne Flavivirus that has been associated with human disease ranging from subclinical to mild illnesses. Clinical characteristics of Zika virus infection include fever, headache, malaise, stomach ache, dizziness, anorexia, and maculopapular rash. Zika virus infection has also been associated with serious and sometimes fatal cases of Guillain-Barré syndrome. Additionally, there is mounting evidence indicating that Zika virus infection can cause microcephaly and other birth defects in infants born to infected mothers. Although the primary route of infection is through the bite of a mosquito, sexual transmission and possible transfusion transmission of Zika virus have been reported.

SUMMARY

[0003] The present invention is defined by the appended claims.

[0004] Disclosed herein is a combination of at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence. In some aspects of the combination of at least two amplification oligomers, the first amplification oligomer is contained within SEQ ID NO:24 and contains SEQ ID NO:25. In some aspects, the first amplification oligomer comprises a sequence that is selected from the group consisting of: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53. In some aspects, the target hybridizing region of the second amplification oligomer is contained within SEQ ID NO:150. In some aspects, the target hybridizing region of the second amplification oligomer is 19 contiguous nucleobases in length or is 20 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, and SEQ ID NO:134. In some aspects, the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the T7 promoter sequence consists of SEQ ID NO:179. In some aspects, the second amplification oligomer comprises a sequences selected from the group consisting of: SEQ ID NOs:74 to 89, SEQ ID NO:107, and SEQ ID NO:108.

[0005] In some aspects of the combination of at least two amplification oligomers, the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from one of the following groups (i) SEQ ID NO:16 and SEQ ID NO:78; (ii) SEQ ID NO:16 and SEQ ID NO:80; (iii) SEQ ID NO:16 and SEQ ID NO:75; (iv) SEQ ID NO:16 and SEQ ID NO:89; (v) SEQ ID NO:16 and SEQ ID NO:74; (vi) SEQ ID NO:11 and SEQ ID NO:78; (vii) SEQ ID NO:11 and SEQ ID NO:80; (viii) SEQ ID NO:11 and SEQ ID NO:75; (ix) SEQ ID NO:11 and SEQ ID NO:89; (x) SEQ ID NO:11 and SEQ ID NO:74; (xi) SEQ ID NO:12 and SEQ ID NO:79; (xii) SEQ ID NO:12 and SEQ ID NO:87; (xiii) SEQ ID NO:12 and SEQ ID NO:89; (xiv) SEQ ID NO:12 and SEQ ID NO:74; (xv) SEQ ID NO:17 and SEQ ID NO:79; (xvi) SEQ ID NO:17 and SEQ ID NO:87; (xvii) SEQ ID NO:17 and SEQ ID NO:89; (xviii) SEQ ID NO:17 and SEQ ID NO:74; (xix) SEQ ID NO:53 and SEQ ID NO:78; (xx) SEQ I DNO:53 and SEQ ID NO:80; (xxi) SEQ ID NO:53 and SEQ ID NO:75; (xxii) SEQ ID NO:53 and SEQ ID NO:89; (xxiii) SEQ ID NO:53 and SEQ ID NO:74; (xxiv) SEQ ID NO:18 and SEQ ID NO:78; (xxv) SEQ I DNO:18 and SEQ ID NO:80; (xxvi) SEQ ID NO:18 and SEQ ID NO:75; (xxvii) SEQ ID NO:18 and SEQ ID NO:89; or (xxviii) SEQ ID NO:18 and SEQ ID NO:74.

[0006] In some aspects of the combination of at least two amplification oligomers, the combination comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:11, SEQ ID NO:12,SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53, and wherein the target hybridizing sequence of the first amplification

oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences. In some aspects, the combination of first amplification oligomer, second amplification oligomer and third amplification oligomer is one of: (i) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:16; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 17, 18, & 53; (ii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:11; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:12, 17, 18, & 53; (iii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO: 12; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:17, 18, & 53; (iv) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:17; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:18, & 53; or (v) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:18; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:53. In some aspects of the combination of at least two amplification oligomers, the combination comprises a fourth amplification oligomer for amplifying a target sequence of a Zika nucleic acid, wherein the fourth amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO113, SEQ ID NO: 114, SEQ ID NO:115, SEQ ID NO: 125, SEQ ID NO:127, SEQ ID NO:128, & SEQ ID NO: 134, and wherein the target hybridizing sequence of the fourth amplification oligomer is optionally joined at its 5' end to a promoter sequence, and wherein the target hybridizing sequence of the second amplification oligomer and the target hybridizing sequence of the fourth amplification oligomer are different sequences. In some aspects, the target hybridizing sequence of the fourth amplification oligomer is joined to a promoter sequence is a T7 promoter sequence. In some aspects, the promoter sequence consists of SEQ ID NO:179. In some aspects, the fourth amplification oligomer comprises a sequence that is selected from the group consisting of: SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:87, and SEQ ID NO:89.

[0007] Further disclosed herein is a combination of at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence.

[0008] In some aspects of the combination of at least two amplification oligomers, the target hybridizing sequence of the first amplification oligomer is contained within SEQ ID NO:4. In some aspects, the target hybridizing sequence of the first amplification oligomer contains SEQ ID NO:2. In some aspects, the first amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35. In some aspects, the target hybridizing sequence of the second amplification oligomer is contained within SEQ ID NO: 152. In some aspects, the target hybridizing sequence of the second amplification oligomer contains SEQ ID NO:148. In some aspects, the target hybridizing sequence of the second amplification oligomer contains SEQ ID NO:149. In some aspects, the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the T7 promoter sequence consists of SEQ ID NO:179. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:111, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:122, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, and SEQ ID NO:138. In some aspects, the second amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, and SEQ ID NO:97.

[0009] In some aspects of the combination of at least two amplification oligomers, the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from one of the following groups: (i) SEQ ID NO:30 and SEQ ID NO:92; (ii) SEQ ID NO:30 and SEQ ID NO:94; (iii) SEQ ID NO:30 and SEQ ID NO:95 (iv) SEQ ID NO:35 and SEQ ID NO:92; (v) SEQ ID NO:35 and SEQ ID NO:94; and vi) SEQ ID NO:35 and SEQ ID NO:95. In some aspects, the combination further comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:111, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:122, SEQ ID NO:135,

SEQ ID NO:136, SEQ ID NO:137, & SEQ ID NO:138, wherein, optionally, the target hybridizing sequence of the third amplification oligomer is joined at it 5' end to a promoter sequence, and wherein the target hybridizing sequence of the second amplification oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences. In some aspects, the target hybridizing sequence of the third amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the promoter sequence consists of SEQ ID NO:179. In some aspects, the third amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, and SEQ ID NO:97.

[0010] Further disclosed herein is a combination of at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence.

[0011] In some aspects of the combination of at least two amplification oligomers, the target hybridizing sequence of the first amplification oligomer is contained within SEQ ID NO:6. In some aspects, the target hybridizing sequence of the first amplification oligomer contains SEQ ID NO:7. In some aspects, the target hybridizing sequence of the first amplification oligomer contains SEQ ID NO:8. In some aspects, the first amplification oligomer comprises a target hybridizing sequence that is selected from the group consisting of: SEQ ID NOs:36 to 48. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, & SEQ ID NO:147. In some aspects, the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the T7 promoter sequence consists of SEQ ID NO:179. In some aspects, the second amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:109, and SEQ ID NO:110.

[0012] In some aspects of the combination of at least two amplification oligomers, the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from one of the following groups: (i) SEQ ID NO:36 and SEQ ID NO:103; (ii) SEQ ID NO:36 and SEQ ID NO:104;. (iii) SEQ ID NO:36 and SEQ ID NO:105; (iv) SEQ ID NO:37 and SEQ ID NO:102; (v) SEQ ID NO:37 and SEQ ID NO:103; (vi) SEQ ID NO:37 and SEQ ID NO:105; (vii) SEQ ID NO:38 and SEQ ID NO:105; (viii) SEQ ID NO:39 and SEQ ID NO:104; (ix) SEQ ID NO:39 and SEQ ID NO:105; (x) SEQ ID NO:39 and SEQ ID NO:106; (xi) SEQ ID NO:41 and SEQ ID NO:104; (xii) SEQ ID NO:41 and SEQ ID NO:106; (xiii) SEQ ID NO:43 and SEQ ID NO:106; (xiv) SEQ ID NO:45 and SEQ ID NO:103; (xv) SEQ ID NO:45 and SEQ ID NO:105; (xvi) SEQ ID NO:45 and SEQ ID NO:106; (xvii) SEQ ID NO:46 and SEQ ID NO:106; (xviii) SEQ ID NO:47 and SEQ ID NO:104; and (xix) SEQ ID NO:47 and SEQ ID NO:106.

[0013] Further disclosed herein is a detection probe oligomer for the detection of a Zika virus nucleic acid, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence configured to hybridize under stringent conditions to a target sequence of a Zika virus nucleic acid, and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label, wherein the Zika virus target nucleic acid sequence is selected from the group consisting of: SEQ ID NO:187, SEQ ID NO:188, & SEQ ID NO:189, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the detection probe oligomer comprises a target hybridizing sequence that is configured to selectively hybridize a sequence selected from the group consisting of: SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, & SEQ ID NO:198, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NOs:54 to 63 and 66 to 73. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises from 3 to 7 contiguous nucleobases that are not complementary to the target nucleic acid and that are joined to one of the 3' end or the 5' end of the target hybridizing sequence. In some aspects, the 3 to 7 contiguous nucleobases that are not complementary to the target nucleic acid, are complementary to a portion of the target hybridizing sequence of the detection probe oligomer. In some aspects, the detection probe oligomer comprises a detectable label that is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and the detection probe oligomer further

comprises a quenching compound. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the target hybridizing sequence of the detection probe oligomer does not comprise the one or more nucleobases that are not complementary to the target nucleic acid.

[0014] Further disclosed herein is an amplification reaction mixture comprising a buffered aqueous solution comprising any of the above described combinations of at least two amplification oligomers. Further disclosed herein is an amplification reaction mixture, comprising a dried composition comprising any of the above described combinations of at least two amplification oligomers. In some aspects, the dried composition comprises a bulking agent. In some aspects, the dried composition comprises less than 5% (w/w) of a bulking agent. In some aspects, the bulking agent is a disaccharide form of an amorphous sugar. In some aspects, the bulking agent is one or more of mannitol, trehalose sucrose, lactose, sorbitol, raffinose, and glucose. In some aspects, the dried composition further comprises at least one polymerase enzyme.

[0015] Further disclosed herein is a multiplex amplification reaction mixture, wherein the mixture is a buffered aqueous solution comprising at least two combinations of at least two amplification oligomers selected from the group consisting of: (A) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (B) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (C) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification

oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; and (D) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, and combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence.

[0016] Further disclosed herein is a multiplex amplification reaction mixture, wherein the mixture is a dried composition comprising at least two combinations of at least two amplification oligomers selected from the group consisting of: (A) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (B) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (C) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus

target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; and (D) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, and combined with at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence. In some aspects, the dried composition comprises a bulking agent. In some aspects, the dried composition comprises less than 5% (w/w) of a bulking agent. In some aspects, the bulking agent is a disaccharide form of an amorphous sugar. In some aspects, the bulking agent is one or more of mannitol, trehalose sucrose, lactose, sorbitol, raffinose, and glucose. In some aspects, the dried composition further comprises at least one polymerase enzyme.

[0017] Further disclosed herein is a kit comprising any of the at least two amplification oligomers described herein. In some aspects, the first amplification oligomer is in a first container within the kit and the second amplification oligomer is in a second container within the kit. In one aspect, the kit further comprises a detection probe oligomer. In one aspect, the detection probe oligomer is any detection probe oligomer described herein.

[0018] Further disclosed herein is a detection reaction mixture comprising a buffered aqueous solution and a detection probe oligomer for the detection of a Zika virus nucleic acid, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence configured to hybridize under stringent conditions to a target sequence of a Zika virus nucleic acid, and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label, wherein the Zika virus target nucleic acid sequence is selected from the group consisting of: SEQ ID NO:187, SEQ ID NO:188, & SEQ ID NO:189, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the detection probe oligomer comprises a target hybridizing sequence that is configured to selectively hybridize a sequence selected from the group consisting of: SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, & SEQ ID NO:198, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NOs:54 to 63 and 66 to 73. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the

target hybridizing sequence of the detection probe oligomer comprises from 3 to 7 contiguous nucleobases that are not complementary to the target nucleic acid and that are joined to one of the 3' end or the 5' end of the target hybridizing sequence. In some aspects, the 3 to 7 contiguous nucleobases that are not complementary to the target nucleic acid, are complementary to a portion of the target hybridizing sequence of the detection probe oligomer. In some aspects, the detection probe oligomer comprises a detectable label that is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and the detection probe oligomer further comprises a quenching compound. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the target hybridizing sequence of the detection probe oligomer does not comprise the one or more nucleobases that are not complementary to the target nucleic acid.

[0019] Further disclosed herein is a dried composition comprising a detection probe oligomer for the detection of a Zika virus nucleic acid, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence configured to hybridize under stringent conditions to a target sequence of a Zika virus nucleic acid, and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label, wherein the Zika virus target nucleic acid sequence is selected from the group consisting of: SEQ ID NO:187, SEQ ID NO:188, & SEQ ID NO:189, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the detection probe oligomer comprises a target hybridizing sequence that is configured to selectively hybridize a sequence selected from the group consisting of: SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, & SEQ ID NO:198, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NOs:54 to 63 and 66 to 73. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises from 3 to 7 contiguous nucleobases that are not complementary to the target nucleic acid and that are joined to one of the 3' end or the 5' end of the target hybridizing sequence. In some aspects, the 3 to 7 contiguous nucleobases that are not complementary to the target nucleic acid, are complementary to a portion of the target hybridizing sequence of the detection probe oligomer. In some aspects, the detection probe oligomer comprises a detectable label that is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and the detection probe oligomer further comprises a quenching compound. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the target hybridizing sequence of the detection probe oligomer does not comprise the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the dried composition comprises a bulking agent. In some aspects, the dried composition comprises less than 5% (w/w) of a bulking agent. In some aspects, the bulking agent is a disaccharide form of an amorphous sugar. In some aspects, the bulking agent is one or more of mannitol, trehalose sucrose, lactose, sorbitol, raffinose, and glucose. In some aspects, the dried composition further comprises at least one polymerase enzyme. In some aspects, the the dried composition further comprises one or more of the combinations of at least two amplification oligomers described herein.

[0020] Further disclosed herein are methods for determining the presence or absence of a Zika virus nucleic acid in a sample, the method comprising the steps of: (A) contacting a sample with a combination of at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (B) performing an *in vitro* nucleic acid amplification reaction, wherein any Zika virus target sequence present in the sample is used as a template for generating an amplification product; and (C) detecting the presence or absence of the amplification product, thereby determining the presence or absence of the Zika virus nucleic acid in the sample. In some aspects, the target hybridizing sequence of the first amplification oligomer is contained within SEQ ID NO:24 and contains SEQ ID NO:25. In some aspects, the first amplification oligomer comprises a sequence that is selected from the group consisting of: SEQ ID NO:11, SEQ ID NO:12,SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53. In some aspects, the target hybridizing region of the second amplification oligomer is contained within SEQ ID NO:150. In some aspects, the target hybridizing region of the second amplification oligomer is 19 contiguous

nucleobases in length or is 20 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:113, SEQ ID NO:119, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:130, and SEQ ID NO:131. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, and SEQ ID NO:134. In some aspects, the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the T7 promoter sequence consists of SEQ ID NO:179. In some aspects, the second amplification oligomer comprises a sequences selected from the group consisting of: SEQ ID NOs:74 to 89, SEQ ID NO: 107, and SEQ ID NO:108. In some aspects, the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from the group consisting of: (i) SEQ ID NO:16 and SEQ ID NO:78; (ii) SEQ ID NO:16 and SEQ ID NO:80; (iii) SEQ ID NO:16 and SEQ ID NO:75; (iv) SEQ ID NO:16 and SEQ ID NO:89; (v) SEQ ID NO:16 and SEQ ID NO:74; (vi) SEQ ID NO:11 and SEQ ID NO:78; (vii) SEQ ID NO:11 and SEQ ID NO:80; (viii) SEQ ID NO:11 and SEQ ID NO:75; (ix) SEQ ID NO:11 and SEQ ID NO:89; (x) SEQ ID NO:11 and SEQ ID NO:74; (xi) SEQ ID NO:12 and SEQ ID NO:79; (xii) SEQ ID NO:12 and SEQ ID NO:87; (xiii) SEQ ID NO:12 and SEQ ID NO:89; (xiv) SEQ ID NO:12 and SEQ ID NO:74; (xv) SEQ ID NO:17 and SEQ ID NO:79; (xvi) SEQ ID NO:17 and SEQ ID NO:87; (xvii) SEQ ID NO:17 and SEQ ID NO:89; (xviii) SEQ ID NO:17 and SEQ ID NO:74; (xix) SEQ ID NO:53 and SEQ ID NO:78; (xx) SEQ IDNO:53 and SEQ ID NO:80; (xxi) SEQ ID NO:53 and SEQ ID NO:75; (xxii) SEQ ID NO:53 and SEQ ID NO:89; (xxiii) SEQ ID NO:53 and SEQ ID NO:74; (xxiv) SEQ ID NO:18 and SEQ ID NO:78; (xxv) SEQ IDNO:18 and SEQ ID NO:80; (xxvi) SEQ ID NO:18 and SEQ ID NO:75; (xxvii) SEQ ID NO:18 and SEQ ID NO:89; and (xxviii) SEQ ID NO:18 and SEQ ID NO:74. In one aspect, the combination of at least two amplification oligomers comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53, and wherein the target hybridizing sequence of the first amplification oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences. In one aspect, the combination of first amplification oligomer, second amplification oligomer and third amplification oligomer is: (i) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:16; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 17, 18, & 53; (ii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:11; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:12, 17, 18, & 53; (iii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:12; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:17, 18, & 53; (iv) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:17; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:18, & 53; or (v) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:18; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:53. In some aspects, the combination of at least two amplification oligomers comprises a fourth amplification oligomer for amplifying a target sequence of a Zika nucleic acid, wherein the fourth amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:128, & SEQ ID NO: 134, and wherein the target hybridizing sequence of the fourth amplification oligomer is optionally joined at its 5' end to a promoter sequence, and wherein the target hybridizing sequence of the second amplification oligomer and the target hybridizing sequence of the fourth amplification oligomer are different sequences. In some aspects, the target hybridizing sequence of the fourth amplification oligomer is joined to a promoter sequence is a T7 promoter sequence, wherein preferably the promoter sequence consists of SEQ ID NO:179. In some aspects, the fourth amplification oligomer comprises a sequence that is selected from the group consisting of: SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:87, and SEQ ID NO:89. In some aspects, the detecting step comprises contacting the *in vitro* amplification reaction with at least one detection probe oligomer, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer

is selected from the group consisting of: SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NOs:66 to 73. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises at either its 3' end or its 5' end from 3 to 7 contiguous nucleobases that are not complementary to the target sequence of the Zika virus nucleic acid. In some aspects, the 3 to 7 contiguous nucleobases that are not complementary to the target sequence, are complementary to a portion of the target hybridizing sequence of the detection probe oligomer. In some aspects, the detectable label is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and wherein the detection probe oligomer further comprises a quenching compound. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the target hybridizing sequence does not comprise the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the combination of the first amplification oligomer and the second amplification oligomer is selected from the group consisting of: (i) SEQ ID NO:16 and SEQ ID NO:78; (ii) SEQ ID NO:16 and SEQ ID NO:80; (iii) SEQ ID NO:16 and SEQ ID NO:75; (iv) SEQ ID NO:16 and SEQ ID NO:89; (v) SEQ ID NO:16 and SEQ ID NO:74; (vi) SEQ ID NO:11 and SEQ ID NO:78; (vii) SEQ ID NO:11 and SEQ ID NO:80; (viii) SEQ ID NO:11 and SEQ ID NO:75; (ix) SEQ ID NO:11 and SEQ ID NO:89; (x) SEQ ID NO:11 and SEQ ID NO:74; (xi) SEQ ID NO:12 and SEQ ID NO:79; (xii) SEQ ID NO:12 and SEQ ID NO:87; (xiii) SEQ ID NO:12 and SEQ ID NO:89; (xiv) SEQ ID NO:12 and SEQ ID NO:74; (xv) SEQ ID NO:17 and SEQ ID NO:79; (xvi) SEQ ID NO:17 and SEQ ID NO:87; (xvii) SEQ ID NO:17 and SEQ ID NO:89; (xviii) SEQ ID NO:17 and SEQ ID NO:74; (xix) SEQ ID NO:53 and SEQ ID NO:78; (xx) SEQ I DNO:53 and SEQ ID NO:80; (xxi) SEQ ID NO:53 and SEQ ID NO:75; (xxii) SEQ ID NO:53 and SEQ ID NO:89; (xxiii) SEQ ID NO:53 and SEQ ID NO:74; (xxiv) SEQ ID NO:18 and SEQ ID NO:78; (xxv) SEQ ID NO:18 and SEQ ID NO:80; (xxvi) SEQ ID NO:18 and SEQ ID NO:75; (xxvii) SEQ ID NO:18 and SEQ ID NO:89; and (xxviii) SEQ ID NO:18 and SEQ ID NO:74, and wherein the detection probe oligomer comprises a target hybridizing sequence consisting of SEQ ID NO:69, and the detectable label consists of an acridinium ester label. In some aspects, the combination comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53, and wherein the target hybridizing sequence of the first amplification oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences. In some aspects, the combination of first amplification oligomer, second amplification oligomer and third amplification oligomer is: (i) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:16; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 17, 18, & 53; (ii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:11; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:12, 17, 18, & 53; (iii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:12; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:17, 18, & 53; (iv) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:17; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:18, & 53; or (v) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:18; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, 75, 78, 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:53. In some aspects, the combination comprises a fourth amplification oligomer for amplifying a target sequence of a Zika nucleic acid, wherein the fourth amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:128, & SEQ ID NO: 134, and wherein the target hybridizing sequence of the fourth amplification oligomer is optionally joined at its 5' end to a promoter sequence, and wherein the target hybridizing sequence of the second amplification oligomer and the target hybridizing sequence of the fourth amplification oligomer are different sequences. In some aspects, the target hybridizing sequence of the fourth amplification oligomer is joined to a promoter sequence is a T7 promoter sequence, preferably wherein the promoter sequence consists of SEQ ID NO:179. In some aspects, the fourth amplification oligomer comprises a sequence that is selected from the group consisting of: SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:87, and SEQ ID NO:89. In some aspects, the

amplification step (b) is performed with the detection step (c). In some aspects, before the amplification step (b), the method further comprises purifying the Zika virus target sequence away from one or more components of the sample. In some aspects, the purifying step comprises contacting the sample with at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a moiety that binds to an immobilized probe, wherein the target-hybridizing sequence is selected from the group consisting of SEQ ID NOs:166 to 178. In some aspects, the moiety comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:181 and SEQ ID NO:182. In some aspects, the immobilized probe comprises a nucleic acid sequence consisting of SEQ ID NO:180. In some aspects, the capture probe oligomer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:153 to 165. In some aspects, the *in vitro* amplification reaction is an isothermal amplification reaction. In some aspects, the *in vitro* amplification reaction is a TMA reaction.

[0021] Further disclosed herein are methods for determining the presence or absence of a Zika virus nucleic acid in a sample, the method comprising the steps of: (A) contacting a sample with a combination of at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (B) performing an *in vitro* nucleic acid amplification reaction, wherein any Zika virus target sequence present in the sample is used as a template for generating an amplification product; and (C) detecting the presence or absence of the amplification product, thereby determining the presence or absence of the Zika virus nucleic acid in the sample. In some aspects, the target hybridizing sequence of the first amplification oligomer is contained within SEQ ID NO:4. In some aspects, the target hybridizing sequence of the first amplification oligomer contains SEQ ID NO:2. In some aspects, the first amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, and SEQ ID NO:35. In some aspects, the target hybridizing sequence of the second amplification oligomer is contained within SEQ ID NO:152. In some aspects, the target hybridizing sequence of the second amplification oligomer contains SEQ ID NO:148. In some aspects, the target hybridizing sequence of the second amplification oligomer contains SEQ ID NO: 149. In some aspects, the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the T7 promoter sequence consists of SEQ ID NO: 179. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:111, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:122, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, and SEQ ID NO:138. In some aspects, the second amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, and SEQ ID NO:97. In some aspects, the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from the group consisting of: (i) SEQ ID NO:30 and SEQ ID NO:92; (ii) SEQ ID NO:30 and SEQ ID NO:94; (iii) SEQ ID NO:30 and SEQ ID NO:95 (iv) SEQ ID NO:35 and SEQ ID NO:92; (v) SEQ ID NO:35 and SEQ ID NO:94; and (vi) SEQ ID NO:35 and SEQ ID NO:95. In some aspects, the combination further comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:111, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:122, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, & SEQ ID NO:138, wherein, optionally, the target hybridizing sequence of the third amplification oligomer is joined at it 5' end to a promoter sequence, and wherein the target hybridizing sequence of the second amplification oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences. In some aspects, the target hybridizing sequence of the third amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence, preferably wherein the promoter sequence consists of SEQ ID NO:179. In some aspects, the third amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, and SEQ ID NO:97. In some aspects, the detecting step comprises contacting the *in vitro* amplification reaction with at least one detection probe oligomer, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:58. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises at either its 3' end or its 5' end from 3 to 7 contiguous nucleobases that are not complementary to the target sequence of the Zika virus nucleic acid. In some aspects, the 3 to 7 contiguous nucleobases that are not complementary to the target

sequence, are complementary to a portion of the target hybridizing sequence of the detection probe oligomer. In some aspects, the detectable label is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and wherein the detection probe oligomer further comprises a quenching compound. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the detection probe oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:54, SEQ ID NO:55, & SEQ ID NO:58. In some aspects, the combination of the first amplification oligomer and the second amplification oligomer is selected from the group consisting of: (i) SEQ ID NO:30 and SEQ ID NO:92; (ii) SEQ ID NO:30 and SEQ ID NO:94; (iii) SEQ ID NO:30 and SEQ ID NO:95 (iv) SEQ ID NO:35 and SEQ ID NO:92; (v) SEQ ID NO:35 and SEQ ID NO:94; and (vi) SEQ ID NO:35 and SEQ ID NO:95, and wherein the detection probe oligomer comprises a target hybridizing sequence consisting of SEQ ID NO:58, and the detectable label consists of an acridinium ester label. In some aspects, the combination further comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:111, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:122, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, & SEQ ID NO:138, wherein, optionally, the target hybridizing sequence of the third amplification oligomer is joined at it 5' end to a promoter sequence, and wherein the target hybridizing sequence of the second amplification oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences. In some aspects, the target hybridizing sequence of the third amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence, preferably wherein the promoter sequence consists of SEQ ID NO:179. In some aspects, the third amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, and SEQ ID NO:97. In some aspects, the amplification step (b) is performed with the detection step (c). In some aspects, before the amplification step (b), the method further comprises purifying the Zika virus target sequence away from one or more components of the sample. In some aspects, the purifying step comprises contacting the sample with at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a moiety that binds to an immobilized probe, wherein the target-hybridizing sequence is selected from the group consisting of SEQ ID NOs:166 to 178. In some aspects, the moiety comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:181 and SEQ ID NO:182. In some aspects, the immobilized probe comprises a nucleic acid sequence consisting of SEQ ID NO: 180. In some aspects, the capture probe oligomer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:153 to 165. In some aspects, the *in vitro* amplification reaction is an isothermal amplification reaction. In some aspects, the *in vitro* amplification reaction is a TMA reaction.

[0022] Further disclosed herein are methods for determining the presence or absence of a Zika virus nucleic acid in a sample, the method comprising the steps of: (A) contacting a sample with a combination of at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (B) performing an *in vitro* nucleic acid amplification reaction, wherein any Zika virus target sequence present in the sample is used as a template for generating an amplification product; and (C) detecting the presence or absence of the amplification product, thereby determining the presence or absence of the Zika virus nucleic acid in the sample. In some aspects, the target hybridizing sequence of the first amplification oligomer is contained within SEQ ID NO:6. In some aspects, the target hybridizing sequence of the first amplification oligomer contains SEQ ID NO:7. In some aspects, the target hybridizing sequence of the first amplification oligomer contains SEQ ID NO:8. In some aspects, the first amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NOs:36 to 48. In some aspects, the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, & SEQ ID NO:147. In some aspects, the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence. In some aspects, the T7 promoter sequence consists of SEQ ID NO:179. In some aspects, the second amplification oligomer comprises a sequence selected from the group consisting of: SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:109, and SEQ ID NO:110. In some aspects, the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from the group consisting of: (i) SEQ ID NO:36 and SEQ ID NO:103; (ii) SEQ ID NO:36 and SEQ ID NO:104;. (iii) SEQ ID NO:36 and SEQ ID NO:105; (iv) SEQ ID NO:37 and SEQ ID NO:102; (v) SEQ ID NO:37 and SEQ ID NO:103; (vi) SEQ ID NO:37 and SEQ ID NO:105; (vii) SEQ ID NO:38 and SEQ ID NO:105;

(viii) SEQ ID NO:39 and SEQ ID NO:104; (ix) SEQ ID NO:39 and SEQ ID NO:105; (x) SEQ ID NO:39 and SEQ ID NO:106; (xi) SEQ ID NO:41 and SEQ ID NO:104; (xii) SEQ ID NO:41 and SEQ ID NO:106; (xiii) SEQ ID NO:43 and SEQ ID NO:106; (xiv) SEQ ID NO:45 and SEQ ID NO:103; (xv) SEQ ID NO:45 and SEQ ID NO:105; (xvi) SEQ ID NO:45 and SEQ ID NO:106; (xvii) SEQ ID NO:46 and SEQ ID NO:106; (xviii) SEQ ID NO:47 and SEQ ID NO:104; and (xix) SEQ ID NO:47 and SEQ ID NO:106. In some aspects, the detecting step comprises contacting the *in vitro* amplification reaction with at least one detection probe oligomer, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NOs:66 to 73. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the target hybridizing sequence of the detection probe oligomer comprises at either its 3' end or its 5' end from 3 to 7 contiguous nucleobases that are not complementary to the target sequence of the Zika virus nucleic acid. In some aspects, the 3 to 7 contiguous nucleobases that are not complementary to the target sequence, are complementary to a portion of the target hybridizing sequence of the detection probe oligomer. In some aspects, the detectable label is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and wherein the detection probe oligomer further comprises a quenching compound. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the target hybridizing sequence does not comprise the one or more nucleobases that are not complementary to the target nucleic acid. In some aspects, the combination of the first amplification oligomer and the second amplification oligomer is selected from the group consisting of: (i) SEQ ID NO:36 and SEQ ID NO:103; (ii) SEQ ID NO:36 and SEQ ID NO: 104;. (iii) SEQ ID NO:36 and SEQ ID NO: 105; (iv) SEQ ID NO:37 and SEQ ID NO:102; (v) SEQ ID NO:37 and SEQ ID NO:103; (vi) SEQ ID NO:37 and SEQ ID NO:105; (vii) SEQ ID NO:38 and SEQ ID NO:105; (viii) SEQ ID NO:39 and SEQ ID NO:104; (ix) SEQ ID NO:39 and SEQ ID NO:105; (x) SEQ ID NO:39 and SEQ ID NO:106; (xi) SEQ ID NO:41 and SEQ ID NO:104; (xii) SEQ ID NO:41 and SEQ ID NO:106; (xiii) SEQ ID NO:43 and SEQ ID NO:106; (xiv) SEQ ID NO:45 and SEQ ID NO:103; (xv) SEQ ID NO:45 and SEQ ID NO:105; (xvi) SEQ ID NO:45 and SEQ ID NO:106; (xvii) SEQ ID NO:46 and SEQ ID NO:106; (xviii) SEQ ID NO:47 and SEQ ID NO:104; and (xix) SEQ ID NO:47 and SEQ ID NO:106, and wherein the detection probe oligomer comprises a target hybridizing sequence consisting of SEQ ID NO:61, and the detectable label consists of an acridinium ester label. In some aspects, the amplification step (b) is performed with the detection step (c). In some aspects, before the amplification step (b), the method further comprises purifying the Zika virus target sequence away from one or more components of the sample. In some aspects, the purifying step comprises contacting the sample with at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a moiety that binds to an immobilized probe, wherein the target-hybridizing sequence is selected from the group consisting of SEQ ID NOs:166 to 178. In some aspects, the moiety comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:181 and SEQ ID NO:182. In some aspects, the immobilized probe comprises a nucleic acid sequence consisting of SEQ ID NO:180. In some aspects, the capture probe oligomer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:153 to 165. In some aspects, the *in vitro* amplification reaction is an isothermal amplification reaction. In some aspects, the *in vitro* amplification reaction is a TMA reaction.

[0023] Further disclosed herein are multiplex methods for determining the presence or absence of a Zika virus nucleic acid in a sample, the method comprising the steps of: (A) contacting a sample with at least two combinations of at least two amplification oligomers, each for amplifying a separate target sequence of a Zika virus target nucleic acid, wherein comprising at least two combinations of at least two amplification oligomers selected from the group consisting of: (1) (i) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with (ii) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second

amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (2) (i) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with (ii) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (3) (i) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with (ii) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; and (4) (i) at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:189, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 26 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, combined with (ii) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:187, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 19 to 25 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence, and combined with (iii) at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising: at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists essentially of SEQ ID NO:188, accounting for complements thereof, RNA equivalents thereof, or both, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, including complements thereof and/or RNA equivalents thereof, to generate amplification products; wherein a first amplification oligomer comprises a target hybridizing sequence that is from 19 to 23 contiguous nucleobases in length, and wherein a second amplification oligomer comprises a target hybridizing sequence that is from 17 to 23 contiguous nucleobases in length and, optionally, joined at its 5' end to a promoter sequence; (B) performing an *in vitro* nucleic acid amplification reaction, wherein for (A)(1) the at least two amplification oligomer of (i) and/or (ii) hybridize their respective target sequence present in the sample to generate an amplification product(s); or wherein for (A)(2) the at least two amplification oligomer of (i) and/or (ii) hybridize their

respective target sequence present in the sample to generate an amplification product(s); or wherein for (A)(3) the at least two amplification oligomer of (i) and/or (ii) hybridize their respective target sequence present in the sample to generate an amplification product(s); or wherein for (A)(4) the at least two amplification oligomer of (i) and/or (ii) and/or (iii) hybridize their respective target sequence present in the sample to generate an amplification product(s); and (C) detecting the presence or absence of the amplification product(s), thereby determining the presence or absence of the Zika virus nucleic acid in the sample. In some aspects, the detecting step comprises contacting the *in vitro* amplification reaction with at least one detection probe oligomer, wherein the detection probe oligomer comprises: (i) a target hybridizing sequence configured to hybridize under stringent conditions to a target sequence of a Zika virus nucleic acid, and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and (ii) a detectable label, wherein the Zika virus target nucleic acid sequence is selected from the group consisting of: SEQ ID NO:187, SEQ ID NO:188, & SEQ ID NO:189, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the detection probe oligomer comprises a target hybridizing sequence that is configured to selectively hybridize a sequence selected from the group consisting of: SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, & SEQ ID NO:198, including a complement thereof, and/or an RNA equivalent thereof. In some aspects, the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length. In some aspects, the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NOs:54 to 63 and 66 to 73. In some aspects the combinations of amplification oligomers and detection probe oligomers are: for (A)(1)(i), (A)(2)(i) or (A)(4)(i) the detection probe oligomer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:56, 57, & 64 to 73; for (A)(1)(ii), (A)(3)(i), or (A)(4)(ii) the detection probe oligomer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:54, 55, & 58; or for (A)(2)(ii)m (A)(3)(ii), or (A)(4)(iii) the detection probe oligomer is selected from the group consisting of SEQ ID NOs:59 to 63. In some aspects, the detection probe oligomer is selected from the group consisting of TaqMan detection probes, molecular beacons, and molecular torches. In some aspects, the detectable label is a fluorescent label, a luminescent label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label. In some aspects, the detectable label is a chemiluminescent label. In some aspects, the detectable label is an acridinium ester. In some aspects, the detectable label is a fluorescent label and wherein the detection probe oligomer further comprises a quenching compound. In some aspects, the amplification step (b) is performed with the detection step (c). In some aspects, before the amplification step (b), the method further comprises purifying the Zika virus target sequence away from one or more components of the sample. In some aspects, the purifying step comprises contacting the sample with at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a moiety that binds to an immobilized probe, wherein the target-hybridizing sequence is selected from the group consisting of SEQ ID NOs:166 to 178. In some aspects, the moiety comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:181 and SEQ ID NO:182; wherein the immobilized probe comprises a nucleic acid sequence consisting of SEQ ID NO:180; or both. In some aspects, the capture probe oligomer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:153 to 165. In some aspects, the *in vitro* amplification reaction is an isothermal amplification reaction. In some aspects, the *in vitro* amplification reaction is a TMA reaction. These and other aspects will become evident upon reference to the following detailed description and the attached drawings.

DEFINITIONS

[0024] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art pertinent to the methods and compositions described. As used herein, the following terms and phrases have the meanings ascribed to them unless specified otherwise.

[0025] The terms "a," "an," and "the" include plural referents, unless the context clearly indicates otherwise. For example, "a nucleic acid" as used herein is understood to represent one or more nucleic acids. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0026] "Sample" includes any specimen that may contain Zika virus or components thereof, such as nucleic acids or fragments of nucleic acids. For the avoidance of doubt, a medium containing a synthetic *in vitro* transcript comprising a Zika virus nucleic acid is considered a sample. Samples include "biological samples" which include any material derived from a living or dead human that may contain Zika virus or target nucleic acid derived therefrom. Biological samples include, but are not limited to, whole blood, red blood cells, peripheral blood, plasma, serum, lymph node, gastrointestinal tissue (*e.g.*, liver), material collected using a vaginal swab, material collected using a cervical brush, material collected via bronchoscopy, material collected via bronchoaveolar lavage, tissue, sputum, saliva or other body fluids or materials. Also, samples may include processed or purified samples, such as those obtained from passing samples over or through a filtering device, or capturing a nucleic acid of interest in a target capture reaction, or following centrifugation, or by adherence to a medium, matrix, or support.

[0027] "Nucleic acid" refers to a multimeric compound comprising two or more covalently bonded nucleosides and/or nucleoside analogs and/or base analogs, linked together by phosphodiester bonds or other linkages to form a polynu-

cleotide. Sugar moieties of the nucleic acid may be ribose and/or deoxyribose. Sugar moieties may comprise substitutions such as, for example, 2'-methoxy substitutions and 2'-halide substitutions (*e.g.,* 2'-F). Synthetic methods for making nucleic acids *in vitro* are well-known in the art although nucleic acids may be purified from natural sources using routine techniques.

[0028] The term "polynucleotide" as used herein denotes a nucleic acid chain. Throughout this application, nucleic acids are designated by the 5'-terminus to the 3'-terminus. Synthetic nucleic acids, *e.g.,* DNA, RNA, DNA/RNA chimerics, (including when non-natural nucleotides or analogues are included therein), are typically synthesized "3'-to-5'," *i.e.,* by the addition of nucleotides to the 5'-terminus of a growing nucleic acid.

[0029] A "target nucleic acid" as used herein is a nucleic acid comprising a target sequence to be amplified. Target nucleic acids may be DNA or RNA as described herein, and may be either single-stranded or double-stranded. Target nucleic acids may be any of the nucleic acid obtained directly from a sample, a synthetic nucleic acid construct, and a nucleic acid amplification product. The target nucleic acid may include other sequences besides the target sequence, which may not be amplified. For example, a target nucleic acid can be an entire genome, a gene, a region within a genome, an expression product, a chimeric nucleic acid, an amplification product, or a target sequence.

[0030] The term "target sequence" as used herein refers to the particular nucleotide sequence of the target nucleic acid that is to be amplified and/or detected. The "target sequence" includes the complexing sequences to which oligonucleotides (*e.g.*, priming oligonucleotides and/or promoter oligonucleotides) complex during an amplification processes. Where the target nucleic acid is originally single-stranded, the term "target sequence" will also refer to the sequence complementary to the "target sequence" as present in the target nucleic acid. Where the target nucleic acid is originally double-stranded, the term "target sequence" refers to both the sense (+) and antisense (-) strands. A target sequence consisting essentially of a certain SEQ ID NO (*e.g.*, SEQ ID NOs:187, 188 or 189) means that the target sequence may have from 0 to 20 fewer nucleotides on one or both ends of the referenced sequence. Preferably, a target sequence consisting essentially of SEQ ID NO:189 has from 0 to 3 fewer nucleotides on the 5' end and from 0 to 8 fewer nucleotides on the 3' end compared SEQ ID NO:189. Preferably, a target sequence consisting essentially of SEQ ID NO:187 has from 0 to 6 fewer nucleotides on the 5' end compared to SEQ ID NO:187. Preferably, a target sequence consisting essentially of SEQ ID NO:188 has from 0 to 4 fewer nucleotides on the 3' end compared to SEQ ID NO: 188.

[0031] "Target-hybridizing sequence" is used herein to refer to the portion of an oligomer that is configured to hybridize with a target nucleic acid sequence. Preferably, the target-hybridizing sequences are configured to specifically hybridize with a target nucleic acid sequence. Target-hybridizing sequences may be 100% complementary to the portion of the target sequence to which they are configured to hybridize, but not necessarily. Target-hybridizing sequences may also include inserted, deleted and/or substituted nucleotide residues relative to a target sequence. Less than 100% complementarity of a target-hybridizing sequence to a target sequence may arise, for example, when the target nucleic acid is a plurality strains within a species, such as would be the case for an oligomer configured to hybridize to various genotypes of Zika virus species isolates. It is understood that other reasons exist for configuring a target-hybridizing sequence to have less than 100% complementarity to a target nucleic acid.

[0032] The term "targets a sequence" as used herein in reference to a region of Zika virus nucleic acid refers to a process whereby an oligonucleotide hybridizes to the target sequence in a manner that allows for target capture, amplification and/or detection as described herein. In one preferred embodiment, the oligonucleotide is complementary with the targeted Zika virus nucleic acid sequence and contains no mismatches. In another preferred embodiment, the oligonucleotide is complementary but contains 1, 2, 3, 4, or 5 mismatches with the targeted Zika virus nucleic acid sequence. Preferably, the oligonucleotide that hybridizes to the Zika virus nucleic acid sequence includes at least 10 to as many as 50 nucleotides complementary to the target sequence. It is understood that at least 10 and as many as 50 is an inclusive range such that 10, 50 and each whole number there between are included. Preferably, the oligomer specifically hybridizes to the target sequence.

[0033] The term "configured to" denotes an actual arrangement of the polynucleotide sequence configuration of a referenced oligonucleotide target-hybridizing sequence. For example, amplification oligomers that are configured to generate a specified amplicon from a target sequence have polynucleotide sequences that hybridize to the target sequence and can be used in an amplification reaction to generate the amplicon. Also as an example, oligonucleotides that are configured to specifically hybridize to a target sequence have a polynucleotide sequence that specifically hybridizes to the referenced sequence under stringent hybridization conditions.

[0034] The term "configured to specifically hybridize to" as used herein means that the target-hybridizing sequence of an amplification oligonucleotide, detection probe, or other oligonucleotide is designed to have a polynucleotide sequence that could target a sequence of the referenced Zika virus target nucleic acid. Such an oligonucleotide is not limited to targeting that sequence only, but is rather useful as a composition, in a kit, or in a method for targeting a Zika virus target nucleic acid. The oligonucleotide is designed to function as a component of an assay for amplification and detection of Zika virus from a sample, and therefore is designed to target Zika virus in the presence of other nucleic acids commonly found in testing samples. "Specifically hybridize to" does not mean exclusively hybridize to, as some small level of hybridization to non-target nucleic acids may occur, as is understood in the art. Rather, "specifically hybridize

to" means that the oligonucleotide is configured to function in an assay to primarily hybridize the target so that an accurate detection of target nucleic acid in a sample can be determined.

[0035] The interchangeable terms "oligomer," "oligo," and "oligonucleotide" refer to a nucleic acid having generally less than 1,000 nucleotide (nt) residues, including polymers in a range having a lower limit of about 5 nt residues and an upper limit of about 900 nt residues, including all whole numbers therein. Oligonucleotides may be purified from naturally occurring sources or may be synthesized using any of a variety of well-known enzymatic or chemical methods. The term oligonucleotide does not denote any particular function to the reagent; rather, it is used generically to cover all such reagents described herein. An oligonucleotide may serve various different functions. For example, it may function as a primer if it is specific for and capable of hybridizing to a complementary strand and can further be extended in the presence of a nucleic acid polymerase; it may function as a primer and provide a promoter if it contains a sequence recognized by an RNA polymerase and allows for transcription (*e.g.*, a T7 Primer); and it may function to detect a target nucleic acid if it is capable of hybridizing to the target nucleic acid, or an amplicon thereof, and further provides a detectible moiety (*e.g.,* an acridinium-ester compound).

[0036] As used herein, an oligonucleotide "substantially corresponding to" a specified reference nucleic acid sequence means that the oligonucleotide is sufficiently similar to the reference nucleic acid sequence such that the oligonucleotide has similar hybridization properties to the reference nucleic acid sequence in that it would hybridize with the same target nucleic acid sequence under stringent hybridization conditions. One skilled in the art will understand that "substantially corresponding oligonucleotides" can vary from a reference sequence and still hybridize to the same reference sequence (e.g., a primer sequence hybridizing to a target nucleic acid sequence). It is also understood that a first nucleic acid corresponding to a second nucleic acid includes the RNA or DNA equivalent thereof as well as DNA/RNA chimerics thereof, and includes the complements thereof, unless the context clearly dictates otherwise. This variation from the nucleic acid may be stated in terms of a percentage of identical bases within the sequence or the percentage of perfectly complementary bases between the probe or primer and its target sequence. Thus, in certain embodiments, an oligonucleotide "substantially corresponds" to a reference nucleic acid sequence if these percentages of base identity or complementarity are from 100% to about 80%. In preferred embodiments, the percentage is from 100% to about 85%, including all whole and partial numbers therein. Similarly, a region of a nucleic acid or amplified nucleic acid can be referred to herein as corresponding to a reference nucleic acid sequence. One skilled in the art will understand the various modifications to the hybridization conditions that might be required at various percentages of complementarity to allow hybridization to a specific target sequence without causing an unacceptable level of non-specific hybridization.

[0037] As used herein, the phrase "or its complement, or an RNA equivalent, or DNA/RNA chimeric thereof," with reference to a DNA sequence, includes (in addition to the referenced DNA sequence) the complement of the DNA sequence, an RNA equivalent of the referenced DNA sequence, an RNA equivalent of the complement of the referenced DNA sequence, a DNA/RNA chimeric of the referenced DNA sequence, and a DNA/RNA chimeric of the complement of the referenced DNA sequence. Similarly, the phrase "or its complement, or a DNA equivalent or DNA/RNA chimeric thereof," with reference to an RNA sequence, includes (in addition to the referenced RNA sequence) the complement of the RNA sequence, a DNA equivalent of the referenced RNA sequence, a DNA equivalent of the complement of the referenced RNA sequence, a DNA/RNA chimeric of the referenced RNA sequence, and a DNA/RNA chimeric of the complement of the referenced RNA sequence.

[0038] As used herein, a "blocking moiety" is a substance used to "block" the 3'-terminus of an oligonucleotide or other nucleic acid so that it cannot be efficiently extended by a nucleic acid polymerase. Oligomers not intended for extension by a nucleic acid polymerase may include a blocker group that replaces the 3' OH to prevent enzyme-mediated extension of the oligomer in an amplification reaction. For example, blocked amplification oligomers and/or detection probes present during amplification may not have functional 3' OH and instead include one or more blocking groups located at or near the 3' end. In some embodiments a blocking group near the 3' end and may be within five residues of the 3' end and is sufficiently large to limit binding of a polymerase to the oligomer. In other embodiments a blocking group is covalently attached to the 3' terminus. Many different chemical groups may be used to block the 3' end, *e.g.,* alkyl groups, non-nucleotide linkers, alkane-diol dideoxynucleotide residues, and cordycepin.

[0039] An "amplification oligomer" is an oligomer, at least the 3'-end of which is complementary to a target nucleic acid, and which hybridizes to a target nucleic acid, or its complement, and participates in a nucleic acid amplification reaction. The nucleic acid amplification reaction can be through an extension of the 3' end of the amplification oligomer. The amplification can also be through a promoter sequence attached to the amplification oligomer. Examples of amplification oligomers include, but are not limited to, primers and promoter-based amplification oligomers. Promoter-based amplification oligomers are referred to as promoter primers, promoter providers, T7 amplification oligomers, T7 providers and T7 primers.

[0040] As used herein, a "primer" is an amplification oligomer that hybridizes to a target nucleic acid and contains a 3' OH end that is extended by a polymerase in an amplification process.

[0041] As used herein, a "promoter-based amplification oligomer," referred to also as "promoter primers," "promoter providers," "T7 amplification oligomers," "T7 providers" and "T7 primers," refers to an oligonucleotide comprising at least

first and second regions. The "first region" of a promoter-based amplification oligonucleotide comprises a base sequence that hybridizes to a target sequence (e.g., a target-hybridizing sequence), where the first region is situated 3', but not necessarily adjacent to, a second region that is a promoter sequence. In some instances, the 3' end of the first region is not extended by a polymerase, and the promoter-based amplification oligomer participates in or facilitates amplification by the promoter sequence. In certain instances, there is an intervening sequence or sequences between the target-hybridizing sequence at the 3' end of the amplification oligomer and the promoter sequence at the 5' end of the amplification oligomer. One example of an intervening sequence is a tag sequence that is useful as an added sequence in an amplification product.

[0042] As used herein, a "promoter" is a specific nucleic acid sequence that is recognized by a DNA-dependent RNA polymerase ("transcriptase") as a signal to bind to the nucleic acid and begin the transcription of RNA at a specific site.

[0043] "Amplification" refers to any known procedure for obtaining multiple copies of a target nucleic acid sequence or its complement or fragments thereof. The multiple copies may be referred to as amplicons or amplification products. Known amplification methods include, for example, replicase-mediated amplification (*see, e.g.,* US Patent No. 4,786,600), polymerase chain reaction (PCR; *see, e.g.,* US Patent Nos. 4,683,195; 4,683,202; and 4,800,159), ligase chain reaction (LCR; *see, e.g.,* US Patent Nos. 5,427,930 and 5,516,663), strand-displacement amplification (SDA; *see, e.g.,* US Patent Nos. 5,422,252; 5,547,861; and 5,648,211), and transcription-mediated or transcription-associated amplification.

[0044] "Transcription-associated amplification" or "transcription-mediated amplification" (TMA) refer to nucleic acid amplification that uses an RNA polymerase to produce multiple RNA transcripts from a nucleic acid template. These methods generally employ an RNA polymerase, a DNA polymerase, deoxyribonucleoside triphosphates, ribonucleoside triphosphates, and a template complementary oligonucleotide that includes a promoter sequence, and optionally may include one or more other oligonucleotides. TMA methods and single-primer transcription-associated amplification methods are embodiments of amplification methods used for detection of Zika virus target sequences as described herein. Variations of transcription-associated amplification are well-known in the art as previously disclosed in detail (*see, e.g.,* US Patent Nos. 4,868,105; 5,124,246; 5,130,238; 5,399,491; 5,437,990; 5,554,516; and 7,374,885; and International Patent Application Pub. Nos. WO 88/01302; WO 88/10315; and WO 95/03430). The person of ordinary skill in the art will appreciate that the disclosed compositions may be used in amplification methods based on extension of oligomer sequences by a polymerase.

[0045] As used herein, the term "real-time TMA" refers to single-primer transcription-mediated amplification ("TMA") of target nucleic acid that is monitored by real-time detection means.

[0046] The term "amplicon" or "amplification product" as used herein refers to the nucleic acid molecule generated during an amplification procedure that is complementary or homologous to a sequence contained within the target sequence. The complementary or homologous sequence of an amplicon is sometimes referred to herein as a "target-specific sequence." Amplicons generated using the amplification oligomers described herein may comprise non-target specific sequences, such as those added by a tag sequence on an amplification oligomer, and/or modified nucleotides included in the amplification oligomers or in the dNTP mixture. Amplicons can be double-stranded or single-stranded and can include DNA, RNA, or both. For example, DNA-dependent RNA polymerase transcribes single-stranded amplicons from double-stranded DNA during transcription-mediated amplification procedures. These single-stranded amplicons are RNA amplicons and can be either strand of a double-stranded complex, depending on how the amplification oligomers are configured. Thus, amplicons can be single-stranded RNA. RNA-dependent DNA polymerases synthesize a DNA strand that is complementary to an RNA template. Thus, amplicons can be double-stranded DNA and RNA hybrids. RNA-dependent DNA polymerases often include RNase activity, or are used in conjunction with an RNase, which degrades the RNA strand. Thus, amplicons can be single stranded DNA. RNA-dependent DNA polymerases and DNA-dependent DNA polymerases synthesize complementary DNA strands from DNA templates. Thus, amplicons can be double-stranded DNA. RNA-dependent RNA polymerases synthesize RNA from an RNA template. Thus, amplicons can be double-stranded RNA. DNA-dependent RNA polymerases synthesize RNA from double-stranded DNA templates, also referred to as transcription. Thus, amplicons can be single stranded RNA. Amplicons and methods for generating amplicons are known to those skilled in the art. For convenience herein, a single strand of RNA or a single strand of DNA may represent an amplicon generated by an amplification oligomer combination described herein. Such representation is not meant to limit the amplicon to the representation shown. Skilled artisans in possession of the instant disclosure will use amplification oligomers and polymerase enzymes to generate any of the numerous types of amplicons.

[0047] A "non-target-specific sequence," as is used herein refers to a region of an oligomer sequence, wherein said region does not stably hybridize with a target sequence under standard hybridization conditions. Oligomers with non-target-specific sequences include, but are not limited to, promoter primers (*e.g.,* the 5' promoter sequence of the amplification oligomer) and molecular beacons and torches (*e.g.,* one or both of the stem members).

[0048] "Detection probe," "detection oligonucleotide," and "detection probe oligomer" are used interchangeably to refer to a nucleic acid oligomer that hybridizes specifically to a target sequence in a nucleic acid, or in an amplified nucleic acid, under conditions that promote hybridization to allow detection of the target sequence or amplified nucleic acid. Detection may either be direct (*e.g.,* a probe hybridized directly to its target sequence) or indirect (*e.g.,* a probe

linked to its target via an intermediate molecular structure). Detection probes may be DNA, RNA, analogs thereof or combinations thereof (*e.g.*, DNA/RNA chimerics) and they may be labeled or unlabeled. Detection probes may further include modifications such as, *e.g.,* 2'-O-methyl linkages. A detection probe may comprise target-specific sequences and other sequences that contribute to the three-dimensional conformation of the probe (*see, e.g.,* US Patent Nos. 5,118,801; 5,312,728; 6,849,412; 6,835,542; 6,534,274; and 6,361,945; and US Patent Application Pub. No. 20060068417).

[0049] As used herein, a "label" refers to a moiety or compound joined directly or indirectly to a probe that is detected or leads to a detectable signal. Direct labeling can occur through bonds or interactions that link the label to the probe. Indirect labeling can occur through use of a bridging moiety or "linker" such as a binding pair member, an antibody or additional oligomer, which is either directly or indirectly labeled, and which may amplify the detectable signal. Labels include any detectable moiety, such as a radionuclide, ligand (*e.g.,* biotin, avidin), enzyme or enzyme substrate, reactive group, or chromophore (*e.g.*, dye, particle, or bead that imparts detectable color), luminescent compound (*e.g.*, bioluminescent, phosphorescent, or chemiluminescent labels), or fluorophore. Labels may be detectable in a homogeneous assay *see, e.g.,* US Patent Nos. 5,283,174; 5,656,207; and 5,658,737. Labels include chemiluminescent compounds, *e.g.*, acridinium ester ("AE") compounds that include standard AE and derivatives (*see, e.g.,* US Patent Nos. 5,656,207; 5,658,737; and 5,639,604). Synthesis and methods of attaching labels to nucleic acids and detecting labels are well known. (*See, e.g.,* Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Habor, NY, 1989, Chapter 10. *See also* US Patent Nos. 5,658,737; 5,656,207; 5,547,842; 5,283,174; and 4,581,333). More than one label, and more than one type of label, may be present on a particular probe, or detection may use a mixture of probes in which each probe is labeled with a compound that produces a detectable signal *(see, e.g.,* US Patent Nos. 6,180,340 and 6,350,579).

[0050] "Capture probe," "capture oligonucleotide," "target capture oligonucleotide," and "capture probe oligomer" are used interchangeably to refer to a nucleic acid oligomer that specifically hybridizes to a target sequence in a target nucleic acid by standard base pairing and joins to a binding partner on an immobilized probe to capture the target nucleic acid to a support. One example of a capture oligomer includes two binding regions: a target sequence-binding region (*e.g.,* target-hybridizing sequence) and an immobilized probe-binding region, usually on the same oligomer, although the two regions may be present on two different oligomers joined together by one or more linkers. Another embodiment of a capture oligomer uses a target-sequence binding region that includes random or non-random poly-GU, poly-GT, or poly U sequences to bind non-specifically to a target nucleic acid and link it to an immobilized probe on a support.

[0051] As used herein, an "immobilized oligonucleotide," "immobilized probe," or "immobilized nucleic acid" refers to a nucleic acid binding partner that directly or indirectly joins a capture oligomer to a solid support. An immobilized probe joined to a solid support facilitates separation of a capture probe bound target from unbound material in a sample. Immobilized probes, solid supports and capture oligomers are described in the literature (*e.g.,* U.S. Patent No. 6,110,678).

[0052] By "complementary" is meant that the nucleotide sequences of similar regions of two single-stranded nucleic acids, or two different regions of the same single-stranded nucleic acid, have a nucleotide base composition that allow the single-stranded regions to hybridize together in a stable double-stranded hydrogen-bonded region under stringent hybridization or amplification conditions. Sequences that hybridize to each other may be completely complementary or partially complementary to the intended target sequence by standard nucleic acid base pairing (*e.g.,* G:C, A:T, or A:U pairing). By "sufficiently complementary" is meant a contiguous sequence that is capable of hybridizing to another sequence by hydrogen bonding between a series of complementary bases, which may be complementary at each position in the sequence by standard base pairing or may contain one or more residues, including abasic residues that are not complementary. Sufficiently complementary contiguous sequences typically are at least 80% (including all whole and partial numbers from 80% to just less than 100%) complementary to a sequence to which an oligomer is intended to specifically hybridize and that stably hybridize with its target sequence under appropriate hybridization conditions,. (*see, e.g.,* Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 at §§ 1.90-1.91, 7.37-7.57, 9.47-9.51 and 11.47-11.57, particularly §§ 9.50-9.51, 11.12-11.13, 11.45-11.47 and 11.55-11.57).

[0053] By "preferentially hybridize" or "specifically hybridize" is meant that under stringent hybridization assay conditions, probes hybridize to their target sequences, or replicates thereof, to form stable probe:target hybrids, while at the same time formation of stable probe:non-target hybrids is minimized. Thus, a probe hybridizes to a target sequence or replicate thereof to a sufficiently greater extent than to a non-target sequence, to enable one having ordinary skill in the art to accurately detect or quantitate RNA replicates or complementary DNA (cDNA) of the target sequence formed during the amplification. Appropriate hybridization conditions are well-known in the art, may be predicted based on sequence composition, or can be determined by using routine testing methods (*see, e.g.,* Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 at §§ 1.90-1.91, 7.37-7.57, 9.47-9.51 and 11.47-11.57, particularly §§ 9.50-9.51, 11.12-11.13, 11.45-11.47 and 11.55-11.57).

[0054] "Sample preparation" refers to any steps or method that treats a sample for subsequent amplification and/or detection of Zika virus nucleic acids present in the sample. Sample preparation may include physical/mechanical dis-

ruption and/or chemical lysis of cellular components to release intracellular components into a substantially aqueous or organic phase and removal of debris, such as by using filtration, centrifugation or adsorption. Sample preparation may include use of a targe capture oligonucleotide that selectively or non-specifically capture a target nucleic acid and separate it from other sample components (*e.g.*, as described in US Patent No. 6,110,678 and International Patent Application Pub. No. WO 2008/016988). Sample preparation can include pooling a plurality of samples into a single pooled batch. Preferably for pooling, an aliquot of each sample is pooled into the larger batch. More preferably for pooling, an aliquot of each sample is first lysed and then pooled into the larger batch. The larger batch of pooled samples can be from a plurality of samples wherein the plurality is from 2 to about 200 individual samples.

[0055] "Separating" or "purifying" means that one or more components of a sample are removed or separated from other sample components. Sample components include target nucleic acids usually in a generally aqueous solution phase, which may also include cellular fragments, proteins, carbohydrates, lipids, and other nucleic acids. "Separating" or "purifying" does not connote any degree of purification. Typically, separating or purifying removes at least 70%, or at least 80%, or at least 95% of the target nucleic acid from other sample components.

[0056] As used herein, a "DNA-dependent DNA polymerase" is an enzyme that synthesizes a complementary DNA copy from a DNA template. All known DNA-dependent DNA polymerases require a complementary primer to initiate synthesis. It is known that under suitable conditions a DNA-dependent DNA polymerase may synthesize a complementary DNA copy from an RNA template. RNA-dependent DNA polymerases typically also have DNA-dependent DNA polymerase activity.

[0057] As used herein, a "DNA-dependent RNA polymerase" or "transcriptase" is an enzyme that synthesizes multiple RNA copies from a double-stranded or partially double-stranded DNA molecule having a promoter sequence that is usually double-stranded. The RNA molecules ("transcripts") are synthesized in the 5'-to-3' direction beginning at a specific position just downstream of the promoter.

[0058] As used herein, an "RNA-dependent DNA polymerase" or "reverse transcriptase" ("RT") is an enzyme that synthesizes a complementary DNA copy from an RNA template. All known reverse transcriptases also have the ability to make a complementary DNA copy from a DNA template; thus, they are both RNA- and DNA-dependent DNA polymerases. RTs may also have an RNAse H activity. A primer is required to initiate synthesis with both RNA and DNA templates.

[0059] As used herein, a "selective RNAse" is an enzyme that degrades the RNA portion of an RNA:DNA duplex but not single-stranded RNA, double-stranded RNA or DNA. An exemplary selective RNAse is RNAse H. Enzymes possessing the same or similar activity as RNAse H may also be used. Selective RNAses may be endonucleases or exonucleases. Most reverse transcriptase enzymes contain an RNAse H activity in addition to their polymerase activities.

[0060] The term "specificity," in the context of an amplification and/or detection system, is used herein to refer to the characteristic of the system which describes its ability to distinguish between target and non-target sequences dependent on sequence and assay conditions. In terms of nucleic acid amplification, specificity generally refers to the ratio of the number of specific amplicons produced to the number of side-products (*e.g.*, the signal-to-noise ratio). In terms of detection, specificity generally refers to the ratio of signal produced from target nucleic acids to signal produced from non-target nucleic acids.

[0061] The term "sensitivity" is used herein to refer to the precision with which a nucleic acid amplification reaction can be detected or quantitated. The sensitivity of an amplification reaction is generally a measure of the smallest copy number of the target nucleic acid that can be reliably detected in the amplification system, and will depend, for example, on the detection assay being employed, and the specificity of the amplification reaction, *e.g.,* the ratio of specific amplicons to side-products.

[0062] As used herein, the term "relative light unit" ("RLU") is an arbitrary unit of measurement indicating the relative number of photons emitted by the sample at a given wavelength or band of wavelengths. RLU varies with the characteristics of the detection means used for the measurement.

[0063] "Sample Transport Solution" generally refers to a solution containing 15 mM sodium phosphate monobasic, 15 mM sodium phosphate dibasic, 1 mM EDTA, 1 mM EGTA, and 110 mM lithium lauryl sulfate (LLS), at pH 6.7.

[0064] "Target Capture Reagent" generally refers to a solution containing 250 mM HEPES, 310 mM lithium hydroxide, 1.88 M lithium chloride, 100 mM EDTA, at pH 6.4, and 250 μg/ml of magnetic particles (1 micron SERA-MAG^TM MG-CM particles, GE Healthcare Lifesciences) with dT_{14} oligomers covalently bound thereto.

[0065] "Wash Solution" generally refers to a solution containing 10 mM HEPES, 150 mM sodium chloride, 6.5 mM sodium hydroxide, 1 mM EDTA, 0.3% (v/v) ethanol, 0.02% (w/v) methyl paraben, 0.01% (w/v) propyl paraben, and 0.1% (w/v) sodium lauryl sulfate, at pH 7.5.

[0066] "Probe Reagent" generally refers to a solution containing one or more labeled detection probes in a solution made up of 100 mM lithium succinate, 2% (w/v) LLS, 15 mM mercaptoethanesulfonate, 1.2 M lithium chloride, 20 mM EDTA, and 3% (v/v) ethanol, at pH 4.7.

[0067] "Amplification Reagent" generally refers to a concentrated mixture mixed with other reaction components to produce a mixture containing 47.6 mM Na-HEPES, 12.5 mM N-acetyl-L-cysteine, 2.5% TRITON™ X-100, 54.8 mM KCl, 23 mM MgCl2, 3 mM NaOH, 0.35 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 7.06 mM rATP, 1.35 mM rCTP, 1.35

mM UTP, 8.85 mM rGTP, 0.26 mM Na2EDTA, 5% v/v glycerol, 2.9% trehalose, 0.225% ethanol, 0.075% methylparaben, 0.015% propylparaben, and 0.002% Phenol Red, at pH 7.5-7.6, although other formulations of amplification reagent may function equally well. Primers may be added to the amplification reagent or added to amplification reactions separate from the amplification reagent. Enzymes in an amplification reagent can include one or more of Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) and bacteriophage T7 RNA polymerase for which units are functionally defined as: 1 U of MMLV-RT incorporates 1 nmol of dTTP in 10 min at 37°C using 200-400 micromolar oligo dT-primed poly(A) as template, and 1 U of T7 RNA polymerase incorporates 1 nmol of ATP into RNA in 1 hr at 37°C using a DNA template containing a T7 promoter.

[0068] "Hybridization Reagent" generally refers to a solution made up of 100 mM succinic acid, 2% (w/v) LLS, 100 mM lithium hydroxide, 15 mM aldrithiol-2, 1.2 M lithium chloride, 20 mM EDTA, and 3.0% (v/v) ethanol, at pH 4.7.

[0069] "Selection Reagent" generally refers to a solution containing 600 mM boric acid, 182.5 mM sodium hydroxide, 1% (v/v) octoxynol (TRITON® X-100), at pH 8.5.

[0070] "Detection Reagents" include "Detect Reagent I," which generally refers to a solution containing 1 mM nitric acid and 32 mM hydrogen peroxide, and "Detect Reagent II," which generally refers to a solution of 1.5 M sodium hydroxide.

DETAILED DESCRIPTION

[0071] Described herein are compositions, kits, and methods for amplifying and detecting Zika virus nucleic acid from a sample. Preferably, the samples are biological samples. The compositions, kits, and methods provide oligonucleotide sequences that recognize target sequences of the Zika virus genome. Such oligonucleotides may be used as amplification oligonucleotides. Other oligonucleotides may be used as probes for detecting amplified sequences of Zika virus, or for capture of Zika virus target nucleic acid.

[0072] The methods provide for the sensitive and specific detection of Zika virus nucleic acids. The methods include performing a nucleic acid amplification of a Zika virus target nucleic acid and detecting the amplified product by, for example, specifically hybridizing the amplified product with a nucleic acid detection probe that provides a signal to indicate the presence of Zika virus in the sample. The amplification step includes contacting the sample with one or more amplification oligomers specific for a target sequence in a Zika virus target nucleic acid to produce an amplified product if Zika virus nucleic acid is present in the sample. Amplification synthesizes additional copies of the target sequence or its complement by using at least one nucleic acid polymerase and an amplification oligomer to produce the copies from a template strand (*e.g.,* by extending the sequence from a primer using the template strand). One embodiment for detecting the amplified product uses a hybridizing step that includes contacting the amplified product with at least one probe specific for a sequence amplified by the selected amplification oligomers, *e.g.,* a sequence contained in the target sequence flanked by a pair of selected amplification oligomers.

[0073] The detection step may be performed using any of a variety of known techniques to detect a signal specifically associated with the amplified target sequence, such as, *e.g.,* by hybridizing the amplification product with a labeled detection probe and detecting a signal resulting from the labeled probe. The detection step may also provide additional information on the amplified sequence, such as, *e.g.,* all or a portion of its nucleic acid base sequence. Detection may be performed after the amplification reaction is completed, or may be performed simultaneously with amplifying the target sequence, *e.g.,* in real time. In one embodiment, the detection step allows homogeneous detection, *e.g.,* detection of the hybridized probe without removal of unhybridized probe from the mixture (*see, e.g.,* US Patent Nos. 5,639,604 and 5,283,174).

[0074] In embodiments that detect the amplified product near or at the end of the amplification step, a linear detection probe may be used to provide a signal to indicate hybridization of the probe to the amplified product. One example of such detection uses a luminescentally labeled probe that hybridizes to target nucleic acid. Luminescent label is then hydrolyzed from non-hybridized probe. Detection is performed by chemiluminescence using a luminometer. (*see, e.g.,* International Patent Application Pub. No. WO 89/002476). In other embodiments that use real-time detection, the detection probe may be a hairpin probe such as, for example, a molecular beacon, molecular torch, or hybridization switch probe that is labeled with a reporter moiety that is detected when the probe binds to amplified product. Such probes may comprise target-hybridizing sequences and non-target-hybridizing sequences. Various forms of such probes have been described previously (*see, e.g.,* US Patent Nos. 5,118,801; 5,312,728; 5,925,517; 6,150,097; 6,849,412; 6,835,542; 6,534,274; and 6,361,945; and US Patent Application Pub. Nos. 20060068417A1 and 20060194240A1).

[0075] Preferred compositions described herein are configured to specifically hybridize to Zika virus nucleic acids from one or more Zika virus strains and, preferably with minimal cross-reactivity to other, non-Zika virus nucleic acids suspected of being in a sample (*e.g.,* other blood borne pathogens). In some aspects, the disclosed compositions are configured to specifically hybridize to Zika virus nucleic acid with minimal cross-reactivity to one or more of hepatitis C virus (HCV), human immunodeficiency virus 1 (HIV 1), hepatitis B virus (HBV), Dengue virus plasmodium, babesia, and West Nile virus. In one aspect, the compositions are part of a multiplex system that further includes components and methods for

detecting one of more of these organisms.

[0076] SEQ ID NO:1. (5' to 3') Gen Bank Accession Number/Version Number/GI Number AY632535.2 GI:226374362.

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[0077] In certain aspects of the disclosures herein, a combination of at least two oligomers is provided for determining the presence or absence of Zika virus in a sample. Typically, the oligomer combination includes at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid corresponding to the sequence within SEQ ID NO:1. In such embodiments, at least one amplification oligomer comprises a target-hybridizing sequence in the sense

orientation and at least one amplification oligomer comprises a target-hybridizing sequence in the antisense orientation, where the amplification oligomers are each configured to specifically hybridize to a Zika virus target sequence corresponding to a sequence contained within SEQ ID NO:1 and where the target-hybridizing sequences are selected so that the amplification oligomers are situated to hybridize the ends of the target sequence to be amplified. In some variations, the at least two amplification oligomers are configured to specifically hybridize to a target sequence in one or more of the following nucleic acid sequences SEQ ID NO:183, SEQ ID NO: 184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO:194, the reverse-complement of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO: 194, an RNA equivalents of any one of SEQ ID NO: 183, SEQ ID NO:184, SEQ ID NO: 185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO:194, and reverse-complements of an RNA equivalent of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO: 194. In some variations, the at least two amplification oligomers are configured to specifically hybridize to a target sequence consisting essentially of: SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194, the reverse-complement of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO: 186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO: 194, an RNA equivalents of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO: 194, or reverse-complements of an RNA equivalent of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO: 185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO: 190, SEQ ID NO: 194.

Table 1: Exemplary Amplification Oligomer Target-hybridizing Sequences for Amplification of Zika virus Target Sequences

SEQ ID NO:	Sequence (5' to 3')
2	TCYCTTGGAGTGCTTGTGA
3	TCYCTTGGAGTGCTTGTGATT
4	TCYCTTGGAGTGCTTGTGATTYT
5	TCYCTTGGAGTGCTTGTGATTY
6	YCCYAAYAAACCTGGAGATGAGTA
7	AAYAAACCTGGAGATG
8	YAAYAAACCTGGAGATG
9	TGGCTTGAAGCAAGAATGCT
10	GCTTGAAGCAAGAAT
11	AGGACAGCAGCTGGCATCAT
12	AGGACGGCAGCTGGCATCAT
13	AGGACAGCAGCTGGCATCATG
14	AGGACGGCAGCTGGCATCATG
15	AGGACAGCAGCTGGCATCATGA
16	AGGACGGCAGCTGGCATCATGA
17	AGGACAGCAGCTGGCATCATGAA
18	AGGACGGCAGCTGGCATCATGAA
19	ACGGCAGCTGGCATCATGAA
20	GACGGCAGCTGGCATCATGAA
21	ACAGCAGCTGGCATCATGAAGAA
22	AGGACAGCAGCTGGCATCATGAAGAA
23	AGAACAGCAGCTGGCATCATGAAGAA
24	AGRACRGCAGCTGGCATCATGAAGAA

(continued)

SEQ ID NO:	Sequence (5' to 3')
25	ACRGCAGCTGGCATCAT
26	RACRGCAGCTGGCATCATGAA
27	GTTGTGGATGGAATAGTGGT
28	TCTCTTGGAGTGCTTGTGATTC
29	TCTCTTGGAGTGCTTGTGATT
30	TCTCTTGGAGTGCTTGTGA
31	TCTCTTGGAGTGCTTGTGATTCT
32	TCCCTTGGAGTGCTTGTGATTCT
33	TCCCTTGGAGTGCTTGTGATTC
34	TCCCTTGGAGTGCTTGTGATT
35	TCCCTTGGAGTGCTTGTGA
36	AACAAACCTGGAGATGAGTA
37	CAACAAACCTGGAGATGAGTA
38	AACAAACCTGGAGATGAGT
39	CAACAAACCTGGAGATGAGT
40	CAATAAACCTGGAGATGAGT
41	CCCAATAAACCTGGAGATGAGT
42	CCYAAYAAACCTGGAGATGAGTA
43	CCCAACAAACCTGGAGATGAGTA
44	YCCYAAYAAACCTGGAGATG
45	TCCTAACAAACCTGGAGATG
46	CCCAACAAACCTGGAGATGAGT
47	CCYAAYAAACCTGGAGATGAG
48	CCCAACAAACCTGGAGATGAG
49	AGRACRGCAGCTGGCATCATGAAGA
50	AGRACRGCAGCTGGCATCATGAAGAA
51	AGRACRGCAGCTGGCATCATGA
52	AGRACRGCAGCTGGCATCAT
53	AGRACRGCAGCTGGCATCATGAA
111	TRRCTACCAGCACTGCCAT
112	GTCWATWGTCATTGTGT
113	GTCATTGTGTCAATGTCAG
114	GTCTATTGTCATTGTGT
115	CTGCTATGAGTAACACTTGTCCCATCTT
116	TAGCTACCAGCACTGCCAT
117	CTACCAGCACTGCCATTGATGTGC
118	CTGCYATGAGTARCACYTGYCCCATCTT
119	GTCWATWGTCATTGTGTCA

(continued)

SEQ ID NO:	Sequence (5' to 3')
120	YTGYCCCATCTTYTTYT
121	GTCWATWGTCATTGTGTCAATGTCAG
122	TRRCTACCAGCACTGCCATTG
123	GRAGCATTCTTGCTTCAAGCCA
124	GTCAAGRAGCATTCTTGCTTCA
125	GTCATTGTGTCAATGTCAGT
126	TCATTGTGTCAATGTCAGT
127	GTCTATTGTCATTGTGTCA
128	ATTGTCATTGTGTCAATGTCAGT
129	ATTGTCATTGTGTCAATGTCA
130	ATTGTCATTGTGTCAATGTC
131	ATTGTCATTGTGTCAATGT
132	TTGTCCCATCTTCTTCT
133	GTAACACTTGTCCCATCTT
134	GTCTATTGTCATTGTGTCAATGTCAG
135	CTACCAGCACTGCCATTGATGTGCT
136	CCAGCACTGCCATTGATGTGCT
137	CTACCAGCACTGCCATTGATGT
138	TAGCTACCAGCACTGCCATTG
139	AGCATTCTTGCTTCAAGCCA
140	GCATTCTTGCTTCAAGCCA
141	GGAGCATTCTTGCTTCAAGCCA
142	GTCAAGAAGCATTCTTGCTTCA
143	TAGAGCGAGGCTATGAGGCCATC
144	TAGAGCGAGGCTATGAGGCCAT
145	TAGAGCGAGGCTATGAGGCCA
146	TAGAGCGAGGCTATGAGG
147	TAGAGCGAGGCTATGAG

[0078] In certain embodiments, an amplification oligomer as described herein is a promoter-based amplification oligomer comprising a target-hybridizing sequence and further comprising a promoter sequence located 5' to the target-hybridizing sequence wherein the promoter sequence is non-complementary to the Zika virus target nucleic acid. For example, in some embodiments of an oligomer combination as described herein for amplification of a Zika virus target sequence, an amplification oligomer as described above in Table 1 is a promoter-based amplification oligomer, wherein a target-hybridizing sequence further comprises a 5' promoter sequence. In particular embodiments, the promoter sequence is a T7 RNA polymerase promoter sequence such as, for example, a T7 promoter sequence having the sequence shown in SEQ ID NO:179. In specific variations, the amplification oligomer has the nucleotide sequence shown in SEQ ID NOs:74-110.

[0079] In some embodiments, an oligomer combination as described herein further comprises at least one capture probe oligomer comprising a target-hybridizing sequence substantially corresponding to a sequence contained in the complement of SEQ ID NO:1, wherein the target-hybridizing sequence is covalently attached to a sequence or moiety that binds to an immobilized probe. In specific variations, the target-hybridizing sequence comprises or consists of a

sequence substantially corresponding to, or identical to, a sequence selected from SEQ ID NOs: 166-178, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In more specific variations, the capture probe oligomer has a sequence selected from SEQ ID NOs: 153-165. An oligomer combination may include at least two capture probe oligomers as above. In more specific embodiments, the at least one capture probe oligomer includes providing the at least one capture probe oligomer in a target capture reaction mixture. In one aspect, each of the at least one capture probe oligomers is provided in the target capture reaction mixture at a concentration from about 3 pmoles/reaction to about 6 pmoles/reaction (inclusive of all whole and partial numbers of the range (*e.g.,* 4, 4.75, 5.12, 5.98, 6)). When a plurality of at least one capture probe oligomer is used in a target capture reaction the concentration of each capture probe oligomer may be equal to the concentration of the others or there may be varied concentrations, as described herein.

[0080] In certain variations, an oligomer combination as described herein further comprises at least one detection probe oligomer configured to specifically hybridize to a Zika virus target sequence that is amplifiable using the first and second amplification oligomers (*e.g.,* a Zika virus target sequence that is flanked by the target-hybridizing sequences of the first and second amplification oligomers). Particularly suitable detection probe oligomers include, for example, oligomers comprising a target-hybridizing sequence substantially corresponding to, or identical to, a sequence selected from SEQ ID NOs:54-63 & 66-73, including complements, DNA equivalents, and DNA/RNA chimerics thereof. A detection probe oligomer may contain a 2'-methoxy backbone at one or more linkages in the nucleic acid backbone. In some variations, an oligomer combination includes at least two detection probe oligomers. In more specific embodiments, the at least one detection probe oligomer includes providing the at least one detection probe oligomer in an amplicon detection reaction mixture. In one aspect, each of the at least one detection probe oligomers is provided in the detection reaction mixture at about 2.0 E+06 RLU/reaction to about 6.0 E+06 RLU/reaction (inclusive of all whole and partial numbers of the range (e.g., 2.0 E+06, 2.138 E+06, 3.385 E +06 RLU)). When a plurality of at least one detection probe oligomer is used in a detection reaction the concentration of each detection oligomer may be equal to the concentration of the others or there may be varied concentrations, as described herein.

[0081] Typically, a detection probe oligomer further includes a label. Particularly suitable labels include compounds that emit a detectable light signal, *e.g.,* fluorophores or luminescent (*e.g.,* chemiluminescent) compounds that can be detected in a homogeneous mixture. More than one label, and more than one type of label, may be present on a particular probe, or detection may rely on using a mixture of probes in which each probe is labeled with a compound that produces a detectable signal (*see, e.g.,* US Pat. Nos. 6,180,340 and 6,350,579). Labels may be attached to a probe by various means including covalent linkages, chelation, and ionic interactions, but preferably the label is covalently attached. For example, in some embodiments, a detection probe has an attached chemiluminescent label such as, *e.g.,* an acridinium ester (AE) compound (*see, e.g.,* US Patent Nos. 5,185,439; 5,639,604; 5,585,481; and 5,656,744), which in typical variations is attached to the probe by a non-nucleotide linker (*see, e.g.,* US Patent Nos. 5,585,481; 5,656,744; and 5,639,604, particularly at column 10, line 6 to column 11, line 3, and Example 8). In other embodiments, a detection probe comprises both a fluorescent label and a quencher, a combination that is particularly useful in fluorescence resonance energy transfer (FRET) assays. Specific variations of such detection probes include, *e.g.,* a TaqMan detection probe (Roche Molecular Diagnostics), a molecular torch (*see, e.g.,* US Patent Nos. 6,849,412; 6,835,542; 6,534,274; and 6,361,945), and a "molecular beacon" (*see, e.g.,* Tyagi et al., Nature Biotechnol. 16:49-53, 1998; US Patent Nos. 5,118,801 and 5,312,728). In some embodiments, the detections probe oligomers are molecular torch oligomers comprising a target-hybridizing sequence selected from SEQ ID NOs:54-63 & 66-73, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0082] In another aspect, the present invention provides methods for determining the presence or absence of Zika virus in a sample using an oligomer combination as described herein. Such a method generally includes (1) contacting the sample with at least two oligomers for amplifying a Zika virus nucleic acid target sequence corresponding to a Zika virus target nucleic acid, where the oligomers include at least two amplification oligomers as described herein; (2) performing an *in vitro* nucleic acid amplification reaction, where any Zika virus target nucleic acid present in the sample is used as a template for generating an amplification product; and (3) detecting the presence or absence of the amplification product, thereby determining the presence or absence of Zika virus in the sample. A detection method in accordance with the present invention typically further includes the step of obtaining the sample to be contacted with the at least two oligomers. In certain embodiments, "obtaining" a sample to be used in steps (1)-(3) includes, for example, receiving the sample at a testing facility or other location where one or more steps of the method are performed, and/or retrieving the sample from a location (*e.g.,* from storage or other depository) within a facility where one or more steps of the method are performed. In certain embodiments, the sample is contacted with at least two amplification oligomers for amplifying a Zika virus nucleic acid target sequence corresponding to a Zika virus target nucleic acid, where the oligomers include at least two amplification oligomers as described above; (2) performing an *in vitro* nucleic acid amplification reaction, where any Zika virus target nucleic acid present in the sample is used as a template for generating an amplification product and the amplification product sequence substantially corresponds to, or consists essentially of, or is identical to one or more of the following sequences SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO:195, SEQ

ID NO:196, SEQ ID NO:197, SEQ ID NO:198, the reverse-complement of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO:195, SEQ ID NO: 196, SEQ ID NO:197, SEQ ID NO:198, an RNA equivalents of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO: 185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO: 194 SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, and reverse-complements of an RNA equivalent of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO: 186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO: 195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198. In certain embodiments, an amplification product is contacted with a detection probe oligomer that is configured to specifically hybridize to a target sequence in one or more of the following sequences SEQ ID NO:183, SEQ ID NO: 184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO: 195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, the reverse-complement of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, an RNA equivalents of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO: 195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:198, and reverse-complements of an RNA equivalent of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO: 186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194 SEQ ID NO:195, SEQ ID NO: 196, SEQ ID NO:197, SEQ ID NO:198.

[0083] In certain embodiments, the method further includes purifying the Zika virus target nucleic acid from other components in the sample before the contacting step. Such purification may include methods of separating and/or concentrating organisms contained in a sample from other sample components. In particular embodiments, purifying the target nucleic acid includes capturing the target nucleic acid to specifically or non-specifically separate the target nucleic acid from other sample components. Non-specific target capture methods may involve selective precipitation of nucleic acids from a substantially aqueous mixture, adherence of nucleic acids to a support that is washed to remove other sample components, or other means of physically separating nucleic acids from a mixture that contains Zika virus nucleic acid and other sample components.

[0084] In some embodiments, a Zika virus target nucleic is selectively separated or purified away from other sample components by specifically hybridizing the Zika virus target nucleic acid to a capture probe oligomer. The capture probe oligomer comprises a target-hybridizing sequence configured to specifically hybridize to a Zika virus target sequence so as to form a target-sequence:capture-probe complex that is separated from sample components. Suitable capture probe target-hybridizing sequences include sequences substantially corresponding to, or identical to, a sequence selected from SEQ ID NOs: 166-178, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In a preferred variation, the specific target capture binds the Zika virus target:capture-probe complex to an immobilized probe to form a target:capture-probe:immobilized-probe complex that is separated from the sample and, optionally, washed to remove non-target sample components (*see, e.g.,* US Patent Nos. 6,110,678; 6,280,952; and 6,534,273). In such variations, the capture probe oligomer further comprises a moiety that binds attaches the capture probe, with its bound target sequence, to an immobilized probe attached to a solid support, thereby permitting the hybridized target nucleic acid to be separated from other sample components. In a preferred embodiment, the immobilized probe is a substantially homopolymeric sequence, in a more preferred embodiment, the immobilized probe is a poly-T sequence. By way of example only, in a preferred embodiment, the immobilized probe is a dT_{14} sequence (SEQ ID NO: 180). By way of example only, in a preferred embodiment, the capture probe comprises a moiety that is a nucleic acid sequence, preferably SEQ ID NO:181 or SEQ ID NO:182.

[0085] In more specific embodiments, the capture probe oligomer includes a tail portion (*e.g.,* a 3' tail) that is a nucleic acid sequence, wherein the nucleic acid sequence is not complementary to the Zika virus target sequence but that specifically hybridizes to a sequence on the immobilized probe, thereby serving as the moiety allowing the target nucleic acid to be separated from other sample components, such as previously described in, *e.g.,* U.S. Patent No. 6,110,678. Any sequence may be used in a tail region, which is generally about 5 to 50 nt long, and preferred embodiments include a substantially homopolymeric tail of about 10 to 40 nt (*e.g.,* A_{10} to A_{40}), more preferably about 14 to 33 nt (*e.g.,* A_{14} to A_{30} or T_3A_{14} to T_3A_{30}), that bind to a complementary immobilized sequence (*e.g.,* poly-T) attached to a solid support, *e.g.,* a matrix or particle. By way of example only, in a preferred embodiment the substantially homopolymeric tail is SEQ ID NO181 or 182 and the immobilized probe is SEQ ID NO:180. For example, in specific embodiments of a capture probe comprising a 3' tail, the capture probe has a sequence selected from SEQ ID NOs: 153-165.

[0086] Target capture typically occurs in a solution phase mixture that contains one or more capture probe oligomers that hybridize specifically to the Zika virus target sequence under hybridizing conditions, usually at a temperature higher than the T_m of the tail-sequence:immobilized-probe-sequence duplex. For embodiments comprising a capture probe tail, the Zika virus-target: capture-probe complex is captured by adjusting the hybridization conditions so that the capture probe tail hybridizes to the immobilized probe, and the entire complex on the solid support is then separated from other sample components. The support with the attached immobilized-probe:capture-probe:Zika virus-target-sequence may

be washed one or more times to further remove other sample components. Preferred embodiments use a particulate solid support, such as paramagnetic beads, so that particles with the attached Zika virus-target:capture-probe:immobilized-probe complex may be suspended in a washing solution and retrieved from the washing solution, preferably by using magnetic attraction. To limit the number of handling steps, the Zika virus target nucleic acid may be amplified by simply mixing the Zika virus target sequence in the complex on the support with amplification oligomers and proceeding with amplification steps.

[0087] Amplifying a Zika virus target sequence utilizes an *in vitro* amplification reaction using at least two amplification oligomers that hybridize a target sequence to be amplified. In particular embodiments, the target sequence to be amplified substantially corresponds to all or a portion of SEQ ID NO:1 from about nucleotide position 3340 to about nucleotide position 3862, or from about nucleotide position 5806 to about nucleotide position 6380, or from about nucleotide position 7132 to about nucleotide position 7641, or a combination thereof. In particular embodiments, the target sequence is contained within a sequence from about nucleotide position 3340 to about nucleotide position 3862 of SEQ ID NO:1, or from about nucleotide position 5806 to about nucleotide position 6380 or SEQ ID NO:1, or from about nucleotide position 7132 to about nucleotide position 7641 of SEQ ID NO:1. In particular embodiments, a target sequence is contained within SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194, the reverse-complement of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194, an RNA equivalents of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194, or reverse-complements of an RNA equivalent of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194. In particular embodiments, a target sequence is contained within a sequence consisting essentially of SEQ ID NO:183, SEQ ID NO: 184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO: 189, SEQ ID NO:190, SEQ ID NO:194, the reverse-complement of any one of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO: 189, SEQ ID NO:190, SEQ ID NO:194, an RNA equivalents of any one of SEQ ID NO:183, SEQ ID NO: 184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194, or reverse-complements of an RNA equivalent of SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:194. Particularly suitable amplification oligomer combinations for amplification of these Zika virus regions are described herein. Suitable amplification methods include, for example, replicase-mediated amplification, polymerase chain reaction (PCR), ligase chain reaction (LCR), strand-displacement amplification (SDA), and transcription-mediated or transcription-associated amplification (TMA). Such amplification methods are well-known in the art and are readily used in accordance with the methods of the present invention.

[0088] For example, some amplification methods that use TMA amplification include the following steps. Briefly, the target nucleic acid that contains the sequence to be amplified is provided as single-stranded nucleic acid (*e.g.,* ssRNA or ssDNA). Those skilled in the art will appreciate that conventional melting of double stranded nucleic acid (*e.g.,* dsDNA) may be used to provide single-stranded target nucleic acids. A promoter-based amplification oligomer binds specifically to the target nucleic acid at its target sequence and a reverse transcriptase (RT) extends the 3' end of the promoter primer using the target strand as a template to create a cDNA copy of the target sequence strand, resulting in an RNA:DNA duplex. An RNase digests the RNA strand of the RNA:DNA duplex and a second primer binds specifically to its target sequence, which is located on the cDNA strand downstream from the promoter primer end. RT synthesizes a new DNA strand by extending the 3' end of the second primer using the first cDNA template to create a dsDNA that contains a functional promoter sequence. An RNA polymerase specific for the promoter sequence then initiates transcription to produce RNA transcripts that are about 100 to 1000 amplified copies ("amplicons") of the initial target strand in the reaction. Amplification continues when the second primer binds specifically to its target sequence in each of the amplicons and RT creates a DNA copy from the amplicon RNA template to produce an RNA:DNA duplex. RNase in the reaction mixture digests the amplicon RNA from the RNA:DNA duplex and the promoter primer binds specifically to its complementary sequence in the newly synthesized DNA. RT extends the 3' end of the promoter primer to create a dsDNA that contains a functional promoter to which the RNA polymerase binds to transcribe additional amplicons that are complementary to the target strand. The autocatalytic cycles of making more amplicon copies repeat during the course of the reaction resulting in about a billion-fold amplification of the target nucleic acid present in the sample. The amplified products may be detected in real-time during amplification, or at the end of the amplification reaction by using a probe that binds specifically to a target sequence contained in the amplified products. Detection of a signal resulting from the bound probes indicates the presence of the target nucleic acid in the sample.

[0089] In some embodiments, TMA methods utilize a "reverse" TMA reaction. In such variations, the initial or "forward" amplification oligomer is a priming oligonucleotide that hybridizes to the target nucleic acid in the vicinity of the 3'-end of the target sequence. A reverse transcriptase (RT) synthesizes a cDNA strand by extending the 3'-end of the primer using the target nucleic acid as a template. The second or "reverse" amplification oligomer is a promoter primer or promoter provider having a target-hybridizing sequence configured to hybridize to a target-sequence contained within

the synthesized cDNA strand. Where the second amplification oligomer is a promoter primer, RT extends the 3' end of the promoter primer using the cDNA strand as a template to create a second, cDNA copy of the target sequence strand, thereby creating a dsDNA that contains a functional promoter sequence. Amplification then continues essentially as described above for initiation of transcription from the promoter sequence utilizing an RNA polymerase. Alternatively, where the second amplification oligomer is a promoter provider, a terminating oligonucleotide, which hybridizes to a target sequence that is in the vicinity to the 5'-end of the target sequence, is typically utilized to terminate extension of the priming oligomer at the 3'-end of the terminating oligonucleotide, thereby providing a defined 3'-end for the initial cDNA strand synthesized by extension from the priming oligomer. The target-hybridizing sequence of the promoter provider then hybridizes to the defined 3'-end of the initial cDNA strand, and the 3'-end of the cDNA strand is extended to add sequence complementary to the promoter sequence of the promoter provider, resulting in the formation of a double-stranded promoter sequence. The initial cDNA strand is then used a template to transcribe multiple RNA transcripts complementary to the initial cDNA strand, not including the promoter portion, using an RNA polymerase that recognizes the double-stranded promoter and initiates transcription therefrom. Each of these RNA transcripts is then available to serve as a template for further amplification from the first priming amplification oligomer.

[0090] Detection of the amplified products may be accomplished by a variety of methods. The nucleic acids may be associated with a surface that results in a physical change, such as a detectable electrical change. Amplified nucleic acids may be detected by concentrating them in or on a matrix and detecting the nucleic acids or dyes associated with them *(e.g.,* an intercalating agent such as ethidium bromide or cyber green), or detecting an increase in dye associated with nucleic acid in solution phase. Other methods of detection may use nucleic acid detection probes that are configured to specifically hybridize to a sequence in the amplified product and detecting the presence of the probe:product complex, or by using a complex of probes that may amplify the detectable signal associated with the amplified products *(e.g.,* US Patent Nos. 5,424,413; 5,451,503; and 5,849,481). Directly or indirectly labeled probes that specifically associate with the amplified product provide a detectable signal that indicates the presence of the target nucleic acid in the sample. In particular, the amplified product will contain a target sequence in or complementary to a sequence in the Zika virus genomic RNA, and a probe will bind directly or indirectly to a sequence contained in the amplified product to indicate the presence of Zika virus nucleic acid in the tested sample.

[0091] Preferred embodiments of detection probes that hybridize to the complementary amplified sequences may be DNA or RNA oligomers, or oligomers that contain a combination of DNA and RNA nucleotides, or oligomers synthesized with a modified backbone, *e.g.,* an oligomer that includes one or more 2'-methoxy substituted ribonucleotides. Probes used for detection of the amplified Zika virus sequences may be unlabeled and detected indirectly *(e.g.,* by binding of another binding partner to a moiety on the probe) or may be labeled with a variety of detectable labels. Particular embodiments of detection probes suitable for use in accordance with methods of the present invention are further described herein. In some preferred embodiments of the method for detecting Zika virus sequences, such as in certain embodiments using transcription-mediated amplification (TMA), the detection probe is a linear chemiluminescently labeled probe, more preferably, a linear acridinium ester (AE) labeled probe.

[0092] Other embodiments using transcription-mediated amplification utilize a promoter-based amplification oligomer, which comprises a first target-hybridizing sequence and, situated 5' to the first region, a second region comprising a promoter sequence for an RNA polymerase, but which is not modified to prevent the initiation of DNA synthesis from its 3'-terminus. In some embodiments, a promoter primer for use in accordance with the detection method comprises a target-hybridizing sequence having a sequence substantially corresponding to, or identical to, a sequence selected from SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, and SEQ ID NO:111.

[0093] Assays for detection of the Zika virus nucleic acid may optionally include a non-Zika virus internal control (IC) nucleic acid that is amplified and detected in the same assay reaction mixtures by using amplification and detection oligomers specific for the IC sequence. IC nucleic acid sequences can be RNA template sequences (e.g., and *in vitro* transcript), synthetic nucleic acid sequences that are spiked into a sample or the IC nucleic acid sequences may be a cellular component. IC nucleic acid sequences that are cellular components can be from exogenous cellular sources or endogenous cellular sources relative to the specimen. In these instances, an internal control nucleic acid is co-amplified with the Zika virus nucleic acid in the amplification reaction mixtures. The internal control amplification product and the Zika virus target sequence amplification product can be detected independently. Two different internal control systems were employed in the procedures described below.

[0094] A first arrangement for internal control systems was useful for monitoring the integrity of amplification and detection reactions that employ paired sets of primers and an oligonucleotide probe that hybridized amplification product at a position between the primer binding sites, or the complements thereof. In a simple application, the internal control

template nucleic acid can be distinguished from the analyte template nucleic acid at the sequence of bases serving as the probe binding site. These bases may be scrambled, replaced by an unrelated base sequence, or simply contain a sufficient number of point mutations to result in differential probe binding. In this way, nucleic acid products resulting from amplification of analyte nucleic acid can be detected by an analyte-specific probe, and not by an internal control-specific probe. Likewise, amplicons resulting from amplification of internal control nucleic acid can be detected by an internal control-specific probe, and not by an analyte-specific probe. This configuration allows that both analyte and internal control nucleic acid templates may be amplified using identical primers, or primer sets.

[0095] In certain embodiments, amplification and detection of a signal from the amplified IC sequence demonstrates that the assay reagents, conditions, and performance of assay steps were properly used in the assay if no signal is obtained for the intended target Zika virus nucleic acid *(e.g.,* samples that test negative for Zika virus). An IC may also be used as an internal calibrator for the assay when a quantitative result is desired, *i.e.,* the signal obtained from the IC amplification and detection is used to set a parameter used in an algorithm for quantitating the amount of Zika virus nucleic acid in a sample based on the signal obtained for an amplified Zika virus target sequence. ICs are also useful for monitoring the integrity of one or more steps in an assay. A preferred embodiment of a synthetic IC nucleic acid sequence is a randomized sequence that has been derived from a naturally occurring source *(e.g.,* an HIV sequence that has been rearranged in a random manner). Another preferred IC nucleic acid sequence may be an RNA transcript isolated from a naturally occurring source or synthesized *in vitro,* such as by making transcripts from a cloned randomized sequence such that the number of copies of IC included in an assay may be accurately determined. The primers and probe for the IC target sequence are configured and synthesized by using any well-known method provided that the primers and probe function for amplification of the IC target sequence and detection of the amplified IC sequence using substantially the same assay conditions used to amplify and detect the Zika virus target sequence. In preferred embodiments that include a target capture-based purification step, it is preferred that a target capture probe specific for the IC target be included in the assay in the target capture step so that the IC is treated in the assay in a manner analogous to that for the intended Zika virus analyte in all of the assay steps.

[0096] Also provided by the subject invention is a reaction mixture for determining the presence or absence of a Zika virus target nucleic acid in a sample. A reaction mixture in accordance with the present invention at least comprises one or more of the following: an oligomer combination as described herein for amplification of a Zika virus target nucleic acid; a capture probe oligomer as described herein for purifying the Zika virus target nucleic acid; and a detection probe oligomer as described herein for determining the presence or absence of a Zika virus amplification product. The reaction mixture may further include a number of optional components such as, for example, arrays of capture probe nucleic acids. For an amplification reaction mixture, the reaction mixture will typically include other reagents suitable for performing *in vitro* amplification such as, *e.g.,* buffers, salt solutions, appropriate nucleotide triphosphates *(e.g.,* dATP, dCTP, dGTP, dTTP, ATP, CTP, GTP and UTP), and/or enzymes *(e.g.,* reverse transcriptase, and/or RNA polymerase), and will typically include test sample components, in which a Zika virus target nucleic acid may or may not be present. In addition, for a reaction mixture that includes a detection probe together with an amplification oligomer combination, selection of amplification oligomers and detection probe oligomers for a reaction mixture are linked by a common target sequence *(i.e.,* the reaction mixture will include a probe that binds to a sequence amplifiable by an amplification oligomer combination of the reaction mixture). Reaction mixtures may be aqueous solutions or dried compositions (e.g., lyophilized compositions, spray dried compositions, etc.). Dried compositions may comprise buffers, bulking agents, and stabilizers. In one embodiment, a bulking agent is present in the dried composition, preferably at a concentration of less than about 5% (w/w). The bulking agent may be a disaccharide form of an amorphous sugar. Preferably, the bulking agent is one or more of mannitol, trehalose sucrose, lactose, sorbitol, raffinose, and glucose.

[0097] Also provided by the subject disclosure are kits for practicing the methods as described herein. A kit in accordance with the present disclosure at least comprises one or more of the following: an amplification oligomer combination as described herein for amplification of a Zika virus target nucleic acid; a capture probe oligomer as described herein for purifying the Zika virus target nucleic acid; a detection probe oligomer as described herein for determining the presence or absence of a Zika virus amplification product; and a probe protection oligomer as described herein for detuning sensitivity of an assay for detecting the Zika virus target nucleic acid. The kits may further include a number of optional components such as, for example, arrays of capture probe nucleic acids. Other reagents that may be present in the kits include reagents suitable for performing *in vitro* amplification such as, *e.g.,* buffers, salt solutions, appropriate nucleotide triphosphates *(e.g.,* dATP, dCTP, dGTP, dTTP, ATP, CTP, GTP and UTP), and/or enzymes *(e.g.,* reverse transcriptase, and/or RNA polymerase). Oligomers as described herein may be packaged in a variety of different embodiments, and those skilled in the art will appreciate that the disclosure embraces many different kit configurations. For example, a kit may include amplification oligomers for only one target sequence of a Zika virus genome, or it may include amplification oligomers for multiple Zika virus target sequences. In addition, for a kit that includes a detection probe together with an amplification oligomer combination, selection of amplification oligomers and detection probe oligomers for a kit are linked by a common target sequence *(i.e.,* the kit will include a probe that binds to a sequence amplifiable by an amplification oligomer combination of the kit). In certain embodiments, the kit further includes a set of instructions for practicing

methods in accordance with the present invention, where the instructions may be associated with a package insert and/or the packaging of the kit or the components thereof.

Order of Steps

[0098] Target Capture: Nucleic acids underwent specimen processing and target capture prior to amplification essentially according to the procedures disclosed in published International Patent Application No. PCT/US2000/18685, except that templates were captured using Zika virus target capture oligonucleotides having the target capture oligonucleotide sequences given herein. Notably, capture oligonucleotides do not participate in the amplification or detection reactions of the assay. IVT or Virus-containing samples were combined with a target capture reagent to facilitate nucleic acid release and hybridization to capture oligonucleotides disposed on magnetic beads. Incubation was performed to capture Zika virus nucleic acids from the sample. Following the incubation, the magnetic beads and any capture target nucleic acids were transferred to a magnetic wash station for 10-20 min. for a wash step. Captured target nucleic acids were then assayed in an amplification reaction.

[0099] Transcription mediated amplification (TMA) reactions were carried out essentially as described in U.S. Patent No. 5,399,491 (Kacian et al.). Isolated target nucleic acids were combined with primers in amplification reagent heated to 60°C for 10 minutes and then cooled to 42°C to facilitate primer annealing. Enzyme reagent was then added to the mixtures and the amplification reactions were carried out, as will be familiar to those having an ordinary level of skill in the art.

[0100] Detection: After a one hour incubation at 42°C, the amplification reaction volumes were subjected to hybridization assays employing probes internally labeled with a chemiluminescent compound using techniques familiar to those having an ordinary level of skill in the art, and then used in amounts equivalent to about 2 E+06 to about 6 E+06 RLU for each probe in the hybridization reaction. *(See e.g.,* U.S. Patent Nos. 5,585,481 and 5,639,604). Hybridization reactions were followed by addition of an aliquot of 0.15 M sodium tetraborate (pH 8.5), and 1% TRITON X-100 (Union Carbide Corporation; Danbury, CT). These mixtures were first incubated at 60°C for 10 minutes to inactivate the chemiluminescent label linked to unhybridized probe, and cooled briefly to room temperature (i.e., 15-30°C) prior to reading the hybridization signal. Chemiluminescence due to hybridized probe in each sample was assayed using commercially available instrumentation (Gen-Probe Incorporated; San Diego, CA) configured for injection of 1 mM nitric acid and 0.1 % (v/v) hydrogen peroxide, followed by injection of a solution containing 1 N sodium hydroxide. Results for the chemiluminescent reactions were measured in relative light units (RLU). In this procedure, the signal/noise value corresponded to the chemiluminescent signal (measured in RLU) generated by label associated with specifically hybridized probe divided by a background signal measured in the absence of a target nucleic acid.

Table 2: Zika virus Assay Reagents

Reagent Name	Description
Internal Control Reagent	A HEPES buffered solution containing detergent and an RNA transcript.
Target Capture Reagent	A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles.
Amplification Reagent	Primers, dNTPs, NTPs and co-factors in TRIS buffered solution containing ProClin 300 as preservative.
Enzyme Reagent	MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.
Probe Reagent	Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.
IC Buffer	A HEPES buffered solution containing detergent.

[0101] The following are non-limiting examples of Zika virus amplification and detection assays.

EXAMPLE 1

[0102] Numerous sets of amplification and detection oligonucleotides were configured to discriminately amplify and detect a zika virus. In this example, the transcription mediated amplification (TMA) reactions were carried out essentially as described by Kacian et al., in U.S. Patent No. 5,399,491. Amplification reactions were conducted for various combinations of amplification oligonucleotides using about 5 pmoles per reaction of each T7 primer and nonT7 primer in a 75

uL amplification reaction mixture. Amplification products were detected by hybridization protection assay (HPA) using about 2 10^6 reactive light units (RLU) per reaction of each of an acridinium ester (AE)-labeled detection probe.

[0103] A first set of amplification and detection oligonucleotides were configured to amplify a target sequence within a zika virus region, wherein the zika virus region corresponds to nucleobases 7132 to 7641 of SEQ ID NO:1 (zika07k region). The amplification oligomers were: for the nonT7 primers, SEQ ID NOs:11-23 & 27; and for the promoter primers, a target-hybridizing sequence from SEQ ID NOs:113-115, & 125-134 joined at their 3' ends to the promoter primer sequence SEQ ID NO:179 (SEQ ID NOs:74-76, 78-83, 85-87, & 89). The detection probes were esterase-labeled detection probes, SEQ ID NOs:69-73.

[0104] A second set of amplification and detection oligonucleotides were configured to amplify a target sequence within a zika virus region, wherein the zika virus region corresponds to nucleobases 3340 to 3862 of SEQ ID NO:1 (zika03k region). The amplification oligomers were: for the nonT7 primers, SEQ ID NOs:28-35; and for the promoter primers, a target-hybridizing sequence from SEQ ID NOs:116, 117, & 135-138 joined at their 3' ends to the promoter primer sequence SEQ ID NO:179 (SEQ ID NOs:90-92, 94, 95, & 97). The detection probe was an esterase-labeled detection probe, SEQ ID NO:58.

[0105] A third set of amplification and detection oligonucleotides were configured to amplify a target sequence within a zika virus region, wherein the zika virus region corresponds to nucleobases 5806 to 6380 of SEQ ID NO:1 (zika06k region). The amplification oligomers were: for the nonT7 primers, SEQ ID NOs:36-41, 43, 45, 46, & 48; and for the promoter primers, a target-hybridizing sequence from SEQ ID NOs:98-106 joined at their 3' ends to the promoter primer sequence SEQ ID NO:179 (SEQ ID NOs:139-147). The detection probes were esterase-labeled detection probes, SEQ ID NOs:59 & 61-63.

[0106] *In vitro* transcripts (IVTs) were made for testing each set of amplification and detection oligonucleotides (SEQ ID NOs:191-193, respectively). The stock concentration of each IVT was determined and then the stock IVTs were each diluted to about 10 copies per mL. Separate target capture reactions were prepared, as described above, and using the following target capture oligonucleotides: for capture of SEQ ID NO:191, SEQ ID NOs:160-163; for capture of SEQ ID NO:192, SEQ ID NOs:153-156; and for capture of SEQ ID NO:193, SEQ ID NOs: 157-159. Captured IVT were then combined with an amplification reaction mixture and an isothermal amplification reaction was performed. Amplification oligomer pairs used for these tests were combinations of the non-T7 primers and the promoter primers listed in each of the above amplification and detection oligomer sets. Amplification product was detected using an esterase-labeled detection probe. The detection probes SEQ ID NOs:69, 58, and 61 were used to detect amplification products generated from the amplification oligomers of set one, set two, and set three, respectively. Detection probe signal (RLU) was read using a luminometer. The RLU data for each reaction was then calculated against a cutoff value to provide a signal to cutoff value (SCO). Tables 3-5, below, report the SCO data for combinations of amplification and detection oligonucleotides from each of sets one to three.

Table 3: Set One Amplification and Detection Oligonucleotide Combinations Results.

SEQ ID NO: 27	SEQ ID NO:23	SEQ ID NO:22	SEQ ID NO:21	SEQ ID NO:20	SEQ ID NO:19	SEQ ID NO:18	SEQ ID NO:17	SEQ ID NO:16	SEQ ID NO:15	SEQ ID NO:14	SEQ ID NO:13	SEQ ID NO:12	SEQ ID NO:11	SCO
88.8	2941.0	531.0	1687.1	1289.2	446.1	4304.3	374.3	725.5	337.1	978.8	469.8	408.9	296.0	SEQ ID NO: 74
366.3	1000.6	349.4	3458.6	2993.7	174.2	0.4	380.7	692.1	0.1	1929.4	195.6	4467.9	3328.0	SEQ ID NO: 75
439.5	824.3	257.4	452.4	88.3	292.8	88.5	139.4	344.4	119.2	203.9	722.2	173.1	832.0	SEQ ID NO: 76
	1426.6	550.8	520.0	2965.9	396.6	233.9	617.0	239.5	1002.6	206.1	0.3	302.2	536.5	SEQ ID NO: 78
	997.5	910.2	1269.0	121.6	179.7	196.2	1082.9	1279.5	868.0	2519.8	2.7	3452.4	714.1	SEQ ID NO: 79
	711.9	3103.7	342.4	714.8	424.9	1262.0	714.2	1072.9	2047.3	740.9	578.7	604.2	378.2	SEQ ID NO: 80
	8.0	11.9	12.0	1.0	4.6	2.4	1.2	5.0	1.1	0.4	0.1	0.3	3.3	SEQ ID NO: 81

(continued)

SEQ ID NO: 27	SEQ ID NO:23	SEQ ID NO:22	SEQ ID NO:21	SEQ ID NO:20	SEQ ID NO:19	SEQ ID NO:18	SEQ ID NO:17	SEQ ID NO:16	SEQ ID NO:15	SEQ ID NO:14	SEQ ID NO:13	SEQ ID NO:12	SEQ ID NO:11	SCO
	3.9	46.7	17.6	6.9	8.3	1.2	33.8	1.0	3.7	6.7	2.2	11.2	4.7	SEQ ID NO: 82
	77.3	2.2	619.6	166.1	1150.8	632.8	46.5	181.1	53.4	70.1	86.2	660.1	5.4	SEQ ID NO: 83
	3.7	0.4	1.7	3.5	11.8	20.1	12.5	14.8	19.9	26.2	2.0	0.2	12.4	SEQ ID NO: 85
	1.0	437.7	131.5	191.8	1694.7	370.5	15.3	387.1	387.7	192.4	785.5	424.6	3032.3	SEQ ID NO: 86
	363.1	433.1	1192.5	714.9	447.0	296.6	1447.8	1034.7	1307.1	597.1	2176.1	1073.1	333.6	SEQ ID NO: 87

Table 4: Set Two Amplification and Detection Oligonucleotide Combinations Results.

SCO	SEQ ID N0:90	SEQ ID NO:91	SEQ ID NO:92	SEQ ID NO:94	SEQ ID NO:95	SEQ ID NO:97
SEQ ID NO:28	1.9	5.0	369.4	5.6	241.7	28.3
SEQ ID NO:29	0.6	0.5	412.6	197.6	684.8	15.9
SEQ ID N0:30	1.3	21.4	182.9	869.5	364.0	0.9
SEQ ID NO:31	0.1	1.0	2930.5	934.7	1449.6	1239.5
SEQ ID NO:32	2.1	1.9	724.4	7.9	675.3	174.9
SEQ ID NO:33	1.0	1.4	179.9	0.2	549.4	394.4
SEQ ID NO:34	1.4	1.5	183.8	1.1	256.4	238.5
SEQ ID NO:35	0.6	0.1	546.8	1.1	278.9	188.5

Table 5: Set Three Amplification and Detection Oligonucleotide Combinations Results.

SCO	SEQ ID NO:102	SEQ ID NO:103	SEQ ID NO: 104	SEQ ID NO:105	SEQ ID NO:106
SEQ ID NO:36	0.5	1.6	2.6	2.4	1.2
SEQ ID NO:37	1.7	3.7	0.8	0.2	4.0
SEQ ID NO:38	0.1	0.2	1.5	3.2	0.9
SEQ ID NO:39	0.7	0.5	4.2	3.0	1.7
SEQ ID NO:40	0.3	0.1	0.9	1.1	0.4
SEQ ID NO:41	0.5	0.6	2.2	0.1	2.0
SEQ ID NO:43	0.8	0.1	0.6	0.2	8.1
SEQ ID NO:45	1.4	8.6	0.2	3.0	6.0
SEQ ID NO:46	0.2	0.7	0.1	0.3	7.8
SEQ ID NO:48	0.3	0.2	1.8	0.3	1.6

[0107] An SCO greater than 1 indicates that the amplification and detection oligonucleotide combination provided a robust detection signal. A more preferable SCO is greater than 10, and an even more preferable SCO is greater than 100. These results indicate that various combinations of oligonucleotides were effective in providing detectable zika virus IVT amplification product above a cutoff value. A number of these amplification and detection oligonucleotide combinations were used in further testing.

EXAMPLE 2

[0108] This example describes multiplex amplification reactions using various primer sets for amplification of two Zika virus target sequences. The Zika virus assay involved three main steps, which take place in a single tube: sample preparation; Zika virus RNA target amplification by Transcription-Mediated Amplification (TMA); and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA), as described above. Table 6 below lists the amplification oligonucleotide combinations used in this assay.

Table 6: Zika virus Amplification Oligomers

Condition	Non T7	T7
1	SEQ ID NO:16; SEQ ID NO:11; & SEQ ID NO:30	SEQ ID NO:78; SEQ ID NO:80; & SEQ ID NO:94

(continued)

Condition	Non T7	T7
2	SEQ ID NO:16; SEQ ID NO:11; & SEQ ID NO:35	SEQ ID NO:78; SEQ ID NO:80; & SEQ ID NO:92
3	SEQ ID NO:16; SEQ ID NO:11; & SEQ ID NO:30	SEQ ID NO:78; SEQ ID NO:75; & SEQ ID NO:94
4	SEQ ID NO:16; SEQ ID NO:11; & SEQ ID NO:35	SEQ ID NO:78; SEQ ID NO:75; & SEQ ID NO:92
5	SEQ ID NO:12; SEQ ID NO:17; & SEQ ID NO:30	SEQ ID NO:79; SEQ ID NO:87; SEQ ID NO:94; & SEQ ID NO:95

[0109] Each of the conditions listed in Table 6 were tested in a transcription-mediated amplification (TMA) reaction using (1) Zika virus *in vitro* transcripts (IVT represented by SEQ ID NOs:191 & 192) each at 10 and 0 copies per mL of internal control buffer (see example 3, below), and (2) a stock virus P6-740 (available from the Center for Disease Control, Atlanta, GA) at 0, 1 e-2 and 3 e-3 plaque forming units per mL of BI0052 (Zika virus negative human serum). Transcription mediated amplification (TMA) reactions were carried out essentially as described by Kacian et al., in U.S. Patent No. 5,399,491. Amplification reactions were conducted for various primer combinations using about 5 pmoles per reaction of each T7 primer and nonT7 primer in a 75 uL amplification reaction mixture. Amplification products were detected by hybridization protection assay (HPA) using about 2 10^6 reactive light units (RLU) per reaction of each of an AE-labeled detection probe having the nucleobase sequences shown in SEQ ID NOs:58 & 69. Each condition was tested in replicates (5 replicates) for each of the IVT dilutions and each of the stock virus dilutions. Signal-to-noise ratios were calculated for each primer pair by dividing the RLU value observed at 10 copies of Zika virus IVT by the background RLU value observed at 0 copies of Zika virus IVT. Total RLU values for the conditions and dilutions are shown in Table 7 below.

Table 7: RLU Values of Zika virus T7/nonT7 Amplification Oligo Combinations

Target/Dilution	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5
Stock Virus 0 pfu/mL	963	1,178	1,081	934	1,813
	814	955	1,328	2,386	1,473
	736	878	1,019	963	1,484
	734	853	1,300	918	1,402
	1,415	759	861	826	1,429
Stock Virus 1 e-2 pfu/mL	2,000,485	1,870,648	994,460	1,355,632	1,929,586
	1,941,247	1,927,834	1,713,798	1,818,677	1,957,834
	1,135,584	1,864,776	1,696,065	1,588,766	1,993,748
	1,930,067	1,821,843	1,786,136	1,802,995	1,943,361
	1,814,498	1,732,891	1,789,571	1,729,585	1,838,143
Stock Virus 3 e-3 pfu/mL	1,529,585	1,820,238	1,597,110	1,233,004	2,038,343
	1,048,528	1,920,906	1,225,351	1,851,168	2,026,636
	1,027,851	1,875,564	1,091,207	1,080,080	2,043,965
	1,854,326	1,887,128	1,870,477	1,711,219	2,056,585
	998,130	1,905,529	981,560	1,253,642	2,029,214
IVT 0 copies/mL	926	1,095	1,163	1,139	2,577
	884	2,375	1,075	994	1,350
	806	1,014	1,112	972	1,527
	850	1,398	1,061	926	6,352
	825	846	1,007	1,052	1,741
IVT #1 (SEQ ID NO:191) 10 copies/mL	984,644	958,137	963,564	441,459	2,079,278
	1,034,198	924,170	952,185	586,993	410,577
	968,755	1,812,164	927,418	1,606,937	643,748
	945,882	907,020	896,725	133,370	1,053,188
	1,504	1,648,693	899,610	835,627	1,093,569
IVT #2 (SEQ ID NO:192) 10 copies/mL	1,076	746,827	28,652	359,399	2,075
	978	943,480	666,687	609,472	994,794
	1,022	30,263	1,128	947,261	972,426
	1,133	961,979	1,120	1,198	971,269
	1,228	1,174	1,163	849,962	966,070

[0110] Primer pairs that demonstrated an RLU above 100,000 were considered to be successful for amplification of Zika virus target nucleic acid to at least as low as 10 copies of target nucleic acid per mL. These data show that all of the amplification oligonucleotide combinations performed well to amplify as low as 3 e-3 pfu per mL of stock zika virus and as low as 10 copies per mL of IVT#1 (SEQ ID NO: 191). Condition 1 failed to amplify and/or detect 10 copies of IVT#2 (SEQ ID NO: 192), and condition 3 successfully amplified and/or detected this target in only 1 out of 5 replicates. Conditions 2, 4, & 5 combinations improved the number of successfully amplified and/or detected replicates, but still none showed 5 of 5 successful amplification and detection reactions. Thus, the combinations of just amplification oligomers SEQ ID NOs:30 and 94 performed poorly in this set of tests (conditions 1 and 3), however, adding SEQ ID NO:95 to the combination resulted in a very good performing assay (condition 5). Similarly, the combination of amplification oligonucleotides SEQ ID NOs:35 & 92 performed well in these tests 3 and 4 out of 5 (conditions 2 and 4). Further combinations were tested.

EXAMPLE 3

[0111] This example describes Zika virus amplification and detection assays performed using different oligomer combinations. Sample and oligonucleotide combinations are listed in Tables 8-9 below. Amplification and detection reactions were performed using the Hologic Panther System (Hologic, Inc., Marlborough, MA).

Table 8: Zika virus-specific Oligonucleotides

Condition	Non T7	T7
1	SEQ ID NO:12; SEQ ID NO:17; & SEQ ID NO:30	SEQ ID NO:79; SEQ ID NO:87; SEQ ID NO:94; & SEQ ID NO:95
2	SEQ ID NO:12; SEQ ID NO:17; & SEQ ID NO:30	SEQ ID NO:87; SEQ ID NO:89 SEQ ID NO:94; & SEQ ID NO:95
3	SEQ ID NO:12; SEQ ID NO:17; & SEQ ID NO:30	SEQ ID NO:79; SEQ ID NO:89 SEQ ID NO:94; & SEQ ID NO:95
4	SEQ ID NO:16; SEQ ID NO:11; & SEQ ID NO:35	SEQ ID NO:78; SEQ ID NO:80; & SEQ ID NO:92
5	SEQ ID NO:16; SEQ ID NO:11; & SEQ ID NO:35	SEQ ID NO:78; SEQ ID NO:89; & SEQ ID NO:92
6	SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:30; & SEQ ID NO:35	SEQ ID NO:74; & SEQ ID NO:95

Table 9: Samples Tested

Sample Identifier	Sample	Description
A	IVT#1 (SEQ ID NO:191)	Zika virus In Vitro Transcript (IVT) in IC buffer (Table 2)
B	IVT#2 (SEQ ID NO:192)	Zika virus In Vitro Transcript (IVT) in IC buffer (Table 2)
C	Virus P6-740	Stock virus in BI0052 serum
D	Donated blood	Zika virus spiked blood samples diluted in human plasma
E	Clinical Samples	Plasma samples from Zika virus positive donors

[0112] A multiplex TMA amplification and detection reaction was performed to determine the analytical sensitivity of a combination of amplification and detection oligomers. This Zika virus assay involved three main steps, which take place in a single tube: sample preparation; Zika virus RNA target amplification by Transcription-Mediated Amplification (TMA); and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA), as described above. Tested samples included *in vitro* transcripts (IVTs) serially diluted in IC buffer, donated human blood spiked with Zika virus RNA and serially diluted in human plasma, cadaveric plasma or cadaveric serum spiked with Zika virus RNA, and clinical samples determined to be positive for Zika virus. Transcription mediated amplification (TMA) reactions were carried out essentially as described by Kacian et al., in U.S. Patent No. 5,399,491. Amplification reactions were conducted for various combinations of amplification oligonucleotides using about 5 pmoles per reaction of each T7 primer and nonT7 primer in a 75 uL amplification reaction mixture. Amplification products were detected by hybridization protection assay (HPA) using about 2 10^6 reactive light units (RLU) per reaction of each of an acridinium ester (AE)-labeled detection probe. Each amplification oligonucleotide condition was tested in replicates of 5 on each of the sample dilutions. Amplification oligomer combinations are shown in Table 8, above. Detection reactions were performed using SEQ ID NOs:58 & 69 as AE-labeled detection probe oligomers. Tables 10-12 show the results of these reactions. Table 10 shows the RLU values for conditions 1-5 of amplification oligonucleotides. Table 11 and Table 12 show the analytical sensitivity and clinical sensitivity for condition 6 of amplification oligonucleotides. In Table 11 average means average of the reactive samples unless all samples were non-reactive in which case average means average of all non-reactive

samples.

Table 10: RLU Values of Zika virus T7/nonT7 Amplification Oligo Combinations

Sample Type Copies / mL	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5
A 10 copies /mL	48,757	757,559	**762,192**	9,523	**781,594**
	375,358	8,702	**418,044**	63,009	**267,402**
	1,078	776,747	**798,691**	354,815	**807,916**
	758,423	800,552	**758,118**	27,867	**791,362**
	746,033	304,704	**763,195**	308,653	**761,598**
B 10 copies/mL	906	1,930	**705,223**	126,982	**455,773**
	25,945	**716,734**	1,219	4,289	2,859
	1,002	4,320	**713,626**	82,863	3,843
	373,889	6,343	15,160	2,831	2,106
	1,246	3,818	1,186	2,303	775
C 1 e-2 pfu/mL	4,491	1,287	3,077	6,013	1,459
	3,012	1,102	2,844	6,269	11,925
	6,754	957	2,294	1,772	4,123
	7,372	1,538	1,230	682	1,237
	5,139	6,444	**676,451**	1,352	783
C 3 e-3 pfu/mL	4,058	10,823	17,014	4,723	1,134
	987	16,253	2,328	1,416	2,029
	1,527	10,552	3,119	3,592	2,732
	4,586	24,009	**922,487**	3,562	795
	1,170	6,060	3,090	744	9,447
C 0 pfu/mL	944	1,074	1,046	4,074	5,482
	11,680	880	3,649	4,061	2,660
	6,761	2,365	3,059	18,396	3,426
	7,895	1,186	9,854	2,312	1,513
	3,503	5,757	12,162	16,504	11,853

Table 11: Analytical Sensitivity of a Multiplexed Amplification and Detection Reaction

Sample Type(s) Copies/mL	#Reactive / Tested	% Reactive (95% CI)	SCO	
			Average	%CV
A&B 90 Copies/mL	20/20	100 (84-100)	33.5	4.3%
A&B 30 Copies/mL	72/72	100 (95-100)	33.2	4.7%
A&B 10 Copies/mL	66/72	92 (83-96)	32.0	13.6%
A&B 3 Copies/mL	39/72	54 (43-65)	28.7	23.3%
A&B 1 Copy/mL	16/72	22 (14-33)	27.7	26.4%
A&B 0.3 Copies/mL	2/72	3 (1-10)	24.5	50.7%
D 90 Copies/mL	20/20	100 (84-100)	32.5	3.3%
D 30 Copies/mL	72/72	100 (95-100)	32.6	4.1%
D 10 Copies/mL	72/72	100 (95-100)	31.5	9.7%
D 3 Copies/mL	62/72	86 (76-92)	25.8	29.3%
D 1 Copy/mL	27/72	38 (27-49)	24.1	37.1%
D 0.3 Copies/mL	14/72	19 (12-30)	17.7	32.8%
D 0.1 Copies/mL	1/72	1 (0-8)	15.7	N/A
No Template 0 Copies/mL	0/144	0 (0-5)	0.0	N/A

Table 12: Clinical Sensitivity of a Multiplexed Amplification and Detection Reaction

Sample Origin Country	Neat			Pooled		
	# Reactive / Tested	% Reactive	SCO	# Reactive / Tested	% Reactive	SCO
Colombia	1/1	100%	30.5	4/4	100%	30.7
Colombia	1/1	100%	31.3	4/4	100%	31.8
Colombia	1/1	100%	31.3	4/4	100%	31.6
Colombia	1/1	100%	32.5	4/4	100%	19.7
Colombia	1/1	100%	32.8	4/4	100%	28.1
Colombia	1/1	100%	32.5	4/4	100%	31.4
Colombia	1/1	100%	31.2	4/4	100%	30.8
Colombia	1/1	100%	29.8	4/4	100%	31.1

(continued)

Sample Origin Country	Neat			Pooled		
	# Reactive / Tested	% Reactive	SCO	# Reactive / Tested	% Reactive	SCO
Colombia	1/1	100%	32.1	3/4	75%	25.9
Dominican Republic	1/1	100%	30.9	4/4	100%	31.5
Dominican Republic	1/1	100%	31.8	4/4	100%	27.0
Dominican Republic	1/1	100%	32.3	4/4	100%	31.7
Dominican Republic	1/1	100%	32.1	4/4	100%	30.5
Dominican Republic	1/1	100%	30.6	4/4	100%	31.2
Dominican Republic	1/1	100%	31.8	1/4	25%	16.2
Dominican Republic	1/1	100%	33.4	4/4	100%	27.5
Dominican Republic	1/1	100%	30.3	4/4	100%	31.3
Dominican Republic	1/1	100%	31.0	4/4	100%	22.8
Dominican Republic	1/1	100%	32.1	4/4	100%	30.9
Dominican Republic	1/1	100%	31.7	4/4	100%	31.9
Dominican Republic	1/1	100%	32.0	4/4	100%	32.5
Dominican Republic	1/1	100%	29.6	4/4	100%	31.6
Dominican Republic	1/1	100%	29.8	4/4	100%	31.6
Colombia	1/1	100%	30.3	4/4	100%	31.7
Colombia	1/1	100%	31.7	4/4	100%	32.2
Colombia	1/1	100%	29.6	4/4	100%	30.2

[0113] These data show that conditions 3 and 5 performed well at amplifying as low as 10 copies per mL of IVT#1 (SEQ ID NO: 191). Conditions 1, 2 and 4 amplified 2 to 3 of 5 replicates containing 10 copies per mL of SEQ ID NO;191. Conditions 1, 2, 3 & 5 amplifies 1 to 2 out of 5 replicates containing 10 copies per mL of IVT#2 (SEQ ID NO: 192). In this experiment, none of the conditions performed well at amplifying target nucleic acid from various pfu/mL of P6-740 virus, with only conditions 3 amplifying 1 of 5 replicates at each concentration. Probit analysis of the multiplexed assay showed detection probabilities in copies / mL (95% fiducial limits) for the IVTA&B and spiked blood donor samples to be (i) 204 (2.0-2.9) for 50% probability and 13.4 (9.9-20.3) for 95% probability, and (ii) 1.0 (0.8-1.2) for 50% probability, and 5.9 (4.3-8.9) for 95% probability. Clinical samples showed 100% (95% CI 87-100%) sensitivity (26 of 26) in neat samples and 96% (95% CI 91-98%) sensitivity (100 of 104) in 1:16 pooled samples. Thus, these amplification oligonucleotide combinations demonstrate very sensitive detection of Zika virus RNA down to about 6 to about 13 copies of Zika virus nucleic acid per mL for an assay specificity of greater than 99.90%.

SEQUENCES

[0114]

Table 13: Exemplary Oligomer Sequences, Reference Sequences, and Regions

SEQ ID NO:	SEQUENCE (5' TO 3')
1	AY632535.2 GI:226374362[1]
2	TCYCTTGGAGTGCTTGTGA
3	TCYCTTGGAGTGCTTGTGATT
4	TCYCTTGGAGTGCTTGTGATTYT
5	TCYCTTGGAGTGCTTGTGATTY

(continued)

SEQ ID NO:	SEQUENCE (5' TO 3')
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7	AAYAAACCTGGAGATG
8	YAAYAAACCTGGAGATG
9	TGGCTTGAAGCAAGAATGCT
10	GCTTGAAGCAAGAAT
11	AGGACAGCAGCTGGCATCAT
12	AGGACGGCAGCTGGCATCAT
13	AGGACAGCAGCTGGCATCATG
14	AGGACGGCAGCTGGCATCATG
15	AGGACAGCAGCTGGCATCATGA
16	AGGACGGCAGCTGGCATCATGA
17	AGGACAGCAGCTGGCATCATGAA
18	AGGACGGCAGCTGGCATCATGAA
19	ACGGCAGCTGGCATCATGAA
20	GACGGCAGCTGGCATCATGAA
21	ACAGCAGCTGGCATCATGAAGAA
22	AGGACAGCAGCTGGCATCATGAAGAA
23	AGAACAGCAGCTGGCATCATGAAGAA
24	AGRACRGCAGCTGGCATCATGAAGAA
25	ACRGCAGCTGGCATCAT
26	RACRGCAGCTGGCATCATGAA
27	GTTGTGGATGGAATAGTGGT
28	TCTCTTGGAGTGCTTGTGATTC
29	TCTCTTGGAGTGCTTGTGATT
30	TCTCTTGGAGTGCTTGTGA
31	TCTCTTGGAGTGCTTGTGATTCT
32	TCCCTTGGAGTGCTTGTGATTCT
33	TCCCTTGGAGTGCTTGTGATTC
34	TCCCTTGGAGTGCTTGTGATT
35	TCCCTTGGAGTGCTTGTGA
36	AACAAACCTGGAGATGAGTA
37	CAACAAACCTGGAGATGAGTA
38	AACAAACCTGGAGATGAGT
39	CAACAAACCTGGAGATGAGT
40	CAATAAACCTGGAGATGAGT
41	CCCAATAAACCTGGAGATGAGT
42	CCYAAYAAACCTGGAGATGAGTA
43	CCCAACAAACCTGGAGATGAGTA

(continued)

SEQ ID NO:	SEQUENCE (5' TO 3')
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45	TCCTAACAAACCTGGAGATG
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48	CCCAACAAACCTGGAGATGAG
49	AGRACRGCAGCTGGCATCATGAAGA
50	AGRACRGCAGCTGGCATCATGAAGAA
51	AGRACRGCAGCTGGCATCATGA
52	AGRACRGCAGCTGGCATCAT
53	AGRACRGCAGCTGGCATCATGAA
54	TRAAGAAGAGAATGACCAC
55	URAAGAAGAGAAUGACCAC
56	GTTGTGGAKGGAATAGTGGT
57	GUUGUGGAKGGAAUAGUGGT
58	UGAAGAAGAGAAUGACCAC
59	UGUAUGGAGGUGGGUGUGC
60	CUGGCUUGAAGCAAGAAUGCT
61	UGGCUUGAAGCAAGAAUGCT
62	UGGCUUGAAGCAAGAAT
63	GCUUGAAGCAAGAAUGCT
64	CATTGACACAATG
65	ACTGACATTGACACAATGACWAT
66	ACUGACAUUGACACAAUGACWAT
67	GACAUUGACACAAUGACWAT
68	CAUUGACACAAUGACWAT
69	GUUGUGGAUGGAAUAGUGGT
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71	ACUGACAUUGACACAAUG
72	GACAUUGACACAAUGACAAT
73	CAUUGACACAAUGACAAT
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75	AATTTAATACGACTCACTATAGGGAGAGTCATTGTGTCAATGTCAGT
76	AATTTAATACGACTCACTATAGGGAGATCATTGTGTCAATGTCAGT
77	AATTTAATACGACTCACTATAGGGAGAGTCWATWGTCATTGTGTCA
78	AATTTAATACGACTCACTATAGGGAGAGTCTATTGTCATTGTGTCA
79	AATTTAATACGACTCACTATAGGGAGAGTCTATTGTCATTGTGT
80	AATTTAATACGACTCACTATAGGGAGAATTGTCATTGTGTCAATGTCAGT
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94	AATTTAATACGACTCACTATAGGGAGATAGCTACCAGCACTGCCAT
95	AATTTAATACGACTCACTATAGGGAGACTACCAGCACTGCCATTGATGTGC
96	AATTTAATACGACTCACTATAGGGAGATRRCTACCAGCACTGCCATTG
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108	AATTTAATACGACTCACTATAGGGAGACTGCYATGAGTARCACYTGYCCCATCTT
109	AATTTAATACGACTCACTATAGGGAGAGRAGCATTCTTGCTTCAAGCCA
110	AATTTAATACGACTCACTATAGGGAGAGTCAAGRAGCATTCTTGCTTCA
111	TRRCTACCAGCACTGCCAT
112	GTCW A TWGTCA TTGTGT
113	GTCATTGTGTCAATGTCAG
114	GTCTATTGTCATTGTGT
115	CTGCTATGAGTAACACTTGTCCCATCTT
116	TAGCTACCAGCACTGCCAT
117	CTACCAGCACTGCCATTGATGTGC
118	CTGCYATGAGTARCACYTGYCCCATCTT
119	GTCW A TWGTCA TTGTGTCA

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SEQ ID NO:	SEQUENCE (5' TO 3')
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124	GTCAAGRAGCATTCTTGCTTCA
125	GTCA TTGTGTCAA TGTCAGT
126	TCATTGTGTCAATGTCAGT
127	GTCTATTGTCATTGTGTCA
128	A TTGTCA TTGTGTCAA TGTCAGT
129	ATTGTCATTGTGTCAATGTCA
130	A TTGTCA TTGTGTCAA TGTC
131	ATTGTCATTGTGTCAATGT
132	TTGTCCCATCTTCTTCT
133	GTAACACTTGTCCCATCTT
134	GTCTA TTGTCA TTGTGTCAA TGTCAG
135	CTACCAGCACTGCCATTGATGTGCT
136	CCAGCACTGCCATTGATGTGCT
137	CTACCAGCACTGCCATTGATGT
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140	GCATTCTTGCTTCAAGCCA
141	GGAGCATTCTTGCTTCAAGCCA
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146	TAGAGCGAGGCTATGAGG
147	TAGAGCGAGGCTATGAG
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152	TRRCTACCAGCACTGCCATTGATGTGC
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154	GGCCUUAUCUCCAUUCCAUACCATTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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156	AGCCACAUCUCCUCCAGUGUUCATTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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171	GCCUUAUCUCCAUUCCAUACCA
172	AGCCACAUCUCCUCCAGUGUUCA
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174	AGCUUGAACUCUCCCUCAAUGGCGG
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197	YCCYGTTGTGGAKGGAA TAGTGGTR
198	YCCYGTTGTGGAKGGAATAGTGGTRACTGACATTGACACAATGACWATWGACCCCCA RGTGGA

[0115] From the foregoing, it will be appreciated that, although specific embodiments of the disclosure have been

described herein for purposes of illustration, various modifications may be made without deviating from the scope of the disclosure.

SEQUENCE LISTING

[0116]

<110> Gen-Probe Incorporated Kui, GAO Jeffrey, LINNEN

<120> COMPOSITIONS AND METHODS FOR DETECTING ZIKA VIRUS NUCLEIC ACID

<130> DIA.0025.02

<140> TBA
<141> 2017-06-09

<150> 62/348,563
<151> 2016-06-10

<160> 198

<170> PatentIn version 3.5

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acaaagatca tcatgagcac atcaatggca gtgctggtag tca 103

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uggcgcacua cauguacuug aucccaggcc uacaagcggc agcagcgcgu gcugcccaga 180

aaaggacagc agcuggcauc augaagaauc ccguugugga uggaauagug guaacugaca 240

uugacacaau gacaauagac ccccaggugg agaagaagau gggacaagug uuacucauag 300

caguagccau cuccagugcu gugcugcugc ggaccgccug gggaugggggg gaggcuggag 360

cucugaucac agcagcgacc uccaccuugu gggaaggcuc uccaaacaaa uacuggaacu 420

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auggagauaa ggcccaggaa agaaccagag agcaacuuag ugaggucaau ggugacagcg 180

gggucaaccg aucauaugga ccacuucucu cuuggagugc uugugauucu acucauggug 240

caggaggggu ugaagaagag aaugaccaca aagaucauca ugagcacauc aauggcagug 300

cugguaguca ugaucuuggg aggauuuuca augagugacc uggccaagcu ugugauccug 360

augggugcua cuuucgcaga aaugaacacu ggaggagaug uagcucacuu ggcauuggua 420

gcggcauuua aagucagacc agccuugcug gucuccuuca uuuucagagc caauuggaca 480

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caugccuguc acgcaugcua gugcugcuca gaggagagga cguauaggca ggaacccuaa 180

caaaccugga gaugaguaca uguauggagg uggggugugca gagacugaug aaggccaugc 240

acacuggcuu gaagcaagaa ugcuucuuga caacaucuac cuccaggaug gccucauagc 300

cucgcucuau cggccugagg ccgauaaggu agccgccauu gagggagagu uuaagcugag 360

gacagagcaa aggaagaccu ucguggaacu caugaagaga ggagaccuuc ccgucuggcu 420

agccuaucag guugcaucug ccggaauaac uuacacagac agaagauggu gcuuugaugg 480

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gga 63

Claims

1. A combination of at least two amplification oligomers for amplifying a Zika virus nucleic acid in a sample, the oligomer combination comprising:

   at least two amplification oligomers for amplifying a target sequence of a Zika virus target nucleic acid, wherein the target sequence consists of SEQ ID NO:189 or SEQ ID NO: 190, the RNA equivalent of SEQ ID NO: 189 or SEQ ID NO: 190, wherein the first amplification oligomer and the second amplification oligomer are configured to hybridize to opposite ends of the target sequence, to generate amplification products;
   wherein a first amplification oligomer comprises: (i)a target hybridizing sequence that is from 17 to 26 contiguous nucleobases of SEQ ID NO: 24, wherein said contiguous nucleobases of the target-hybridizing sequence include SEQ ID NO: 25, or (ii) a target-hybridizing sequence consisting of SEQ ID NO: 27,
   wherein a second amplification oligomer comprises a target hybridizing sequence that is selected from the group consisting of SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 126 and SEQ ID NO: 128 and, optionally, joined at its 5' end to a promoter sequence.

2. The combination of claim 1, wherein the target hybridizing sequence of the second amplification oligomer is selected from the group consisting of: SEQ ID NO:113 and SEQ ID NO:126.

3. The combination of claim 1 or claim 2, wherein the second amplification oligomer is joined at its 5' end to a promoter sequence that is a T7 promoter sequence; preferably,
   wherein the T7 promoter sequence consists of SEQ ID NO:179.

4. The combination of claim 2, wherein the second amplification oligomer comprises a sequences selected from the group consisting of: SEQ ID NO:74 and SEQ ID NO:76.

5. The combination of any one of claims 1 to 4, wherein the first amplification oligomer comprises a sequence that is selected from the group consisting of: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID

NO:22 and SEQ ID NO:23.

6. The combination of any one of claims 1 to 5, wherein the combination of first amplification oligomer and second amplification oligomer comprise nucleic acid sequences selected from the group consisting of:

(v) SEQ ID NO:16 and SEQ ID NO:74;
(x) SEQ ID NO:11 and SEQ ID NO:74;
(xii) SEQ ID NO:12 and SEQ ID NO:87;
(xvi) SEQ ID NO:17 and SEQ ID NO:87;
(xviii) SEQ ID NO:17 and SEQ ID NO:74;
(xxv) SEQ ID NO:18 and SEQ ID NO:80;
and
(xxviii) SEQ ID NO:18 and SEQ ID NO:74.

7. The combination of any one of claims 1 to 6, wherein the combination further comprises a third amplification oligomer for amplifying a target sequence of a Zika virus nucleic acid, wherein the third amplification oligomer comprises a target hybridizing sequence selected from the group consisting of: SEQ ID NO:11, SEQ ID NO: 12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53, and wherein the target hybridizing sequence of the first amplification oligomer and the target hybridizing sequence of the third amplification oligomer are different sequences; preferably,
wherein the combination of first amplification oligomer, second amplification oligomer and third amplification oligomer is:

(i) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:16; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74, and 80, & 89; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 17, 18, & 53;
(ii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:11; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74 and 80; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:12, 17, 18, & 53;
(iii) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:12; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74 and 87; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:17, 18, & 53;
(iv) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:17; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74 and 87; and a third amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:18, & 53; or
(v) a first amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:18; a second amplification oligomer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:74 and 80; and a third amplification oligomer comprising a nucleic acid sequence consisting of SEQ ID NO:53.

8. An amplification reaction mixture, wherein the mixture is a buffered aqueous solution comprising the combination of at least two amplification oligomers of any one of claims 1 to 7; or
wherein the mixture is a dried composition comprising the at least two amplification oligomers of any one of claims 1 to 7, preferably,
wherein the dried composition further comprises at least one polymerase enzyme.

9. A kit comprising the at least two amplification oligomers of any one of claims 1 to 7; preferably,
wherein the first amplification oligomer is in a first container within the kit and the second amplification oligomer is in a second container within the kit.

10. The kit of claim 9, wherein the kit further comprises a detection probe oligomer.

11. A method for determining the presence or absence of a Zika virus nucleic acid in a sample, the method comprising the steps of:

(A) contacting a sample with the combination of at least two amplification oligomers according to any of claims 1 to 7;
(B) performing an *in vitro* nucleic acid amplification reaction, wherein any Zika virus target sequence present in the sample is used as a template for generating an amplification product; and
(C) detecting the presence or absence of the amplification product,

thereby determining the presence or absence of the Zika virus nucleic acid in the sample.

12. The method of claim 11, wherein the detecting step comprises contacting the *in vitro* amplification reaction with at least one detection probe oligomer, wherein the detection probe oligomer comprises:

(i) a target hybridizing sequence and, optionally, one or more nucleobases that are not complementary to the Zika virus target nucleic acid, and
(ii) a detectable label; preferably,

wherein the target hybridizing sequence of the detection probe oligomer is from 17 to 23 contiguous nucleobases in length; and/or
wherein the target hybridizing sequence of the detection probe oligomer is selected from the group consisting of: SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NOs:66 to 73; and/or
wherein the detectable label is a fluorescent label, a luminescent label, a chemiluminescent label, an acridium ester label, a chromophore label, a radionuclide label, a ligand label, an enzyme label, or a reactive group label.

Patentansprüche

1. Kombination aus mindestens zwei Vervielfältigungsoligomeren zum Vervielfältigen einer Zikavirus-Nukleinsäure in einer Probe, wobei die Oligomerkombination Folgendes umfasst:

mindestens zwei Vervielfältigungsoligomere zum Vervielfältigen einer Zielsequenz einer Zikavirus-Zielnukleinsäure, wobei die Zielsequenz aus SEQ ID NO:189 oder SEQ ID NO: 190, dem RNA-Äquivalent von SEQ ID NO: 189 oder SEQ ID NO: 190, besteht, wobei das erste Vervielfältigungsoligomer und das zweite Vervielfältigungsoligomer konfiguriert sind, um an entgegengesetzte Enden der Zielsequenz zu hybridisieren, um Vervielfältigungsprodukte zu erzeugen;
wobei ein erstes Vervielfältigungsoligomer Folgendes umfasst: (i) eine Zielhybridisierungssequenz, die zusammenhängende Nukleinbasen von SEQ ID NO: 24 mit einer Länge von 17 bis 26 aufweist, wobei die zusammenhängende Nukleinbasen der Zielhybridisierungssequenz SEQ ID NO: 25 beinhalten, oder (ii) eine Zielhybridisierungssequenz, die aus SEQ ID NO: 27 besteht,
wobei ein zweites Vervielfältigungsoligomer eine Zielhybridisierungssequenz umfasst, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 126 und SEQ ID NO: 128 und optional an seinem 5'-Ende an eine Promotorsequenz gebunden ist.

2. Kombination nach Anspruch 1, wobei die Zielhybridisierungssequenz des zweiten Vervielfältigungsoligomers ausgewählt ist aus der Gruppe, bestehend aus: SEQ ID NO:113 und SEQ ID NO:126.

3. Kombination nach Anspruch 1 oder 2, wobei das zweite Vervielfältigungsoligomer an seinem 5'-Ende an eine Promotorsequenz gebunden ist, die eine T7-Promotorsequenz ist,
wobei die T7-Promotorsequenz bevorzugt aus SEQ ID NO:179 besteht.

4. Kombination nach Anspruch 2, wobei das zweite Vervielfältigungsoligomer eine Sequenz umfasst, die ausgewählt ist aus der Gruppe, bestehend aus: SEQ ID NO:74 und SEQ ID NO:76.

5. Kombination nach einem der Ansprüche 1 bis 4, wobei das erste Vervielfältigungsoligomer eine Sequenz umfasst, die ausgewählt ist aus der Gruppe, bestehend aus: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 und SEQ ID NO:23.

6. Kombination nach einem der Ansprüche 1 bis 5, wobei die Kombination aus dem ersten Vervielfältigungsoligomer und dem zweiten Vervielfältigungsoligomer Nukleinsäuresequenzen umfasst, die ausgewählt sind aus der Gruppe,

bestehend aus:

(v) SEQ ID NO:16 und SEQ ID NO:74;
(x) SEQ ID NO:11 und SEQ ID NO:74;
(xii) SEQ ID NO:12 und SEQ ID NO:87;
(xvi) SEQ ID NO:17 und SEQ ID NO:87;
(xviii) SEQ ID NO:17 und SEQ ID NO:74;
(xxv) SEQ ID NO:18 und SEQ ID NO:80;
und
(xxviii) SEQ ID NO:18 und SEQ ID NO:74.

7. Kombination nach einem der Ansprüche 1 bis 6, wobei die Kombination weiter ein drittes Vervielfältigungsoligomer zum Vervielfältigen einer Zielsequenz einer Zikavirus-Nukleinsäure umfasst, wobei das dritte Vervielfältigungsoligomer eine Zielhybridisierungssequenz umfasst, die ausgewählt ist aus der Gruppe, bestehend aus: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52 und SEQ ID NO:53, und wobei die Zielhybridisierungssequenz des ersten Vervielfältigungsoligomers und die Zielhybridisierungssequenz des dritten Vervielfältigungsoligomers unterschiedliche Sequenzen sind,
wobei die Kombination aus dem ersten Vervielfältigungsoligomer, dem zweiten Vervielfältigungsoligomer und dem dritten Vervielfältigungsoligomer bevorzugt die Folgende ist:

(i) ein erstes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, bestehend aus SEQ ID NO:16; ein zweites Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO:74 und 80 & 89; und ein drittes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 11, 12, 17, 18 & 53;
(ii) ein erstes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, bestehend aus SEQ ID NO:11; ein zweites Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 74 und 80; und ein drittes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 12, 17, 18 & 53;
(iii) ein erstes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, bestehend aus SEQ ID NO:12, ein zweites Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 74 und 87; und ein drittes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO: 17, 18 & 53;
(iv) ein erstes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, bestehend aus SEQ ID NO:17; ein zweites Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO:74 und 87; und ein drittes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO:18 & 53; oder
(v) ein erstes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, bestehend aus SEQ ID NO:18; ein zweites Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO:74 und 80; und ein drittes Vervielfältigungsoligomer, umfassend eine Nukleinsäuresequenz, die ausgewählt ist aus der Gruppe, bestehend aus SEQ ID NO:53.

8. Vervielfältigungs-Reaktionsmischung, wobei die Mischung eine gepufferte wässrige Lösung ist, umfassend die Kombination aus mindestens zwei Vervielfältigungsoligomeren nach einem der Ansprüche 1 bis 7; oder
wobei die Mischung eine getrocknete Zusammensetzung ist, umfassend die mindestens zwei Vervielfältigungsoligomere nach einem der Ansprüche 1 bis 7,
wobei bevorzugt die getrocknete Mischung weiter mindestens ein Polymeraseenzym umfasst.

9. Kit, umfassend die mindestens zwei Vervielfältigungsoligomere nach einem der Ansprüche 1 bis 7;
wobei bevorzugt sich das erste Vervielfältigungsoligomer in einem ersten Behälter innerhalb des Kits befindet und sich das zweite Vervielfältigungsoligomer in einem zweiten Behälter innerhalb des Kits befindet.

10. Kit nach Anspruch 9, wobei das Kit weiter ein Nachweistester-Oligomer umfasst.

11. Verfahren zum Bestimmen der Anwesenheit oder Abwesenheit einer Zikavirus-Nukleinsäure in einer Probe, wobei das Verfahren die folgenden Schritte umfasst:

(A) Kontaktieren einer Probe mit der Kombination aus mindestens zwei Vervielfältigungsoligomeren nach einem der Ansprüche 1 bis 7;
(B) Durchführen einer *in vitro* Nukleinsäure-Vervielfältigungsreaktion, wobei eine beliebige, in der Probe vorhandene Zikavirus-Zielsequenz als eine Vorlage zum Erzeugen eines Vervielfältigungsprodukts verwendet wird; und
(C) Nachweisen der Anwesenheit oder Abwesenheit des Vervielfältigungsprodukts,

wodurch die Anwesenheit oder Abwesenheit der Zikavirus-Nukleinsäure in der Probe nachgewiesen wird.

12. Verfahren nach Anspruch 11, wobei der Nachweisschritt das Kontaktieren der *in vitro* Vervielfältigungsreaktion mit mindestens einem Nachweistester-Oligomer umfasst, wobei das Nachweistester-Oligomer Folgendes umfasst:

(i) eine Zielhybridisierungssequenz und optional eine oder mehrere Nukleinbasen, die der Zikavirus-Zielnukleinsäure nicht komplementär sind, und
(ii) eine nachweisbare Markierung;

wobei die Zielhybridisierungssequenz des Nachweistester-Oligomers bevorzugt zusammenhängende Nukleinbasen mit einer Länge von 17 bis 23 beträgt; und/oder
wobei die Zielhybridisierungssequenz des Nachweistester-Oligomers ausgewählt ist aus der Gruppe, bestehend aus: SEQ ID NO:56, SEQ ID NO:57 und SEQ ID NO:66 bis 73; und/oder
wobei die nachweisbare Markierung eine fluoreszente Markierung, eine lumineszente Markierung, eine chemilumineszente Markierung, eine Acridiniumester-Markierung, eine Chromophor-Markierung, eine Radionuklid-Markierung, eine Ligand-Markierung, eine Enzym-Markierung oder eine reaktive Gruppen-Markierung ist.

Revendications

1. Combinaison d'au moins deux oligomères d'amplification pour amplifier un acide nucléique de virus Zika dans un échantillon, la combinaison d'oligomères comprenant :

au moins deux oligomères d'amplification pour amplifier une séquence cible d'un acide nucléique cible de virus Zika, dans lequel la séquence cible consiste en SEQ ID NO : 189 ou SEQ ID NO : 190, l'équivalent ARN de SEQ ID NO : 189 ou SEQ ID NO : 190, dans laquelle le premier oligomère d'amplification et le deuxième oligomère d'amplification sont configurés pour s'hybrider aux extrémités opposées de la séquence cible, pour générer des produits d'amplification ;
dans laquelle un premier oligomère d'amplification comprend : (i) une séquence d'hybridation cible qui contient 17 à 26 bases nucléiques contiguës de SEQ ID NO : 24, dans laquelle lesdites bases nucléiques contiguës de la séquence d'hybridation cible incluent SEQ ID NO : 25, ou (ii) une séquence d'hybridation cible consistant en SEQ ID NO : 27,
dans laquelle un deuxième oligomère d'amplification comprend une séquence d'hybridation cible qui est sélectionnée dans le groupe consistant en SEQ ID NO : 113, SEQ ID NO : 115, SEQ ID NO : 126 et SEQ ID NO : 128 et, facultativement, jointe à son extrémité 5' à une séquence de promoteur.

2. Combinaison selon la revendication 1, dans laquelle la séquence d'hybridation cible du deuxième oligomère d'amplification est sélectionnée dans le groupe consistant en SEQ ID NO : 113 et SEQ ID NO : 126.

3. Combinaison selon la revendication 1 ou la revendication 2, dans laquelle le deuxième oligomère d'amplification est joint à son extrémité 5' à une séquence de promoteur qui est une séquence de promoteur de T7 ; de préférence, dans laquelle la séquence de promoteur de T7 consiste en SEQ ID NO : 179.

4. Combinaison selon la revendication 2, dans laquelle le deuxième oligomère d'amplification comprend une séquence sélectionnée dans le groupe consistant en : SEQ ID NO : 74 et SEQ ID NO : 76.

5. Combinaison selon l'une quelconque des revendications 1 à 4, dans laquelle le premier oligomère d'amplification comprend une séquence qui est sélectionnée dans le groupe consistant en SEQ ID NO : 11, SEQ ID NO : 12, SEQ ID NO : 13, SEQ ID NO : 14, SEQ ID NO : 15, SEQ ID NO : 16, SEQ ID NO : 17, SEQ ID NO : 18, SEQ ID NO : 19, SEQ ID NO : 20, SEQ ID NO : 21, SEQ ID NO : 22 et SEQ ID NO : 23.

6. Combinaison selon l'une quelconque des revendications 1 à 5, dans laquelle la combinaison du premier oligomère d'amplification et du deuxième oligomère d'amplification comprend des séquences d'acide nucléique sélectionnées dans le groupe consistant en :

(v) SEQ ID NO : 16 et SEQ ID NO : 74;
(x) SEQ ID NO : 11 et SEQ ID NO : 74;
(xii) SEQ ID NO : 12 et SEQ ID NO : 87 ;
(xvi) SEQ ID NO : 17 et SEQ ID NO : 87 ;
(xviii) SEQ ID NO : 17 et SEQ ID NO : 74 ;
(xxv)SEQ ID NO : 18 et SEQ ID NO : 80 ;
et
(xxviii) SEQ ID NO : 18 et SEQ ID NO : 74.

7. Combinaison selon l'une quelconque des revendications 1 à 6, dans laquelle la combinaison comprend en outre un troisième oligomère d'amplification pour amplifier une séquence cible d'un acide nucléique de virus Zika, dans laquelle le troisième oligomère d'amplification comprend une séquence d'hybridation cible sélectionnée dans le groupe consistant en : SEQ ID NO : 11, SEQ ID NO : 12, SEQ ID NO : 13, SEQ ID NO : 14, SEQ ID NO : 15, SEQ ID NO : 16, SEQ ID NO : 17, SEQ ID NO : 18, SEQ ID NO : 19, SEQ ID NO : 20, SEQ ID NO : 21, SEQ ID NO : 22, SEQ ID NO : 23, SEQ ID NO : 26, SEQ ID NO : 49, SEQ ID NO : 50, SEQ ID NO : 51, SEQ ID NO : 52, et SEQ ID NO : 53, et dans laquelle la séquence d'hybridation cible du premier oligomère d'amplification et la séquence d'hybridation cible du troisième oligomère d'amplification sont des séquences différentes ; de préférence,
dans laquelle la combinaison du premier oligomère d'amplification, du deuxième oligomère d'amplification et du troisième oligomère d'amplification est :

(i) un premier oligomère d'amplification comprenant une séquence d'acide nucléique consistant en SEQ ID NO : 16; un deuxième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 74, et 80, & 89 ; et un troisième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 11, 12, 17, 18, & 53 ;
(ii) un premier oligomère d'amplification comprenant une séquence d'acide nucléique consistant en SEQ ID NO : 11; un deuxième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 74 et 80; et un troisième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 12, 17, 18, & 53 ;
(iii) un premier oligomère d'amplification comprenant une séquence d'acide nucléique consistant en SEQ ID NO : 12; un deuxième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 74 et 87 ; et un troisième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 17, 18, & 53 ;
(iv) un premier oligomère d'amplification comprenant une séquence d'acide nucléique consistant en SEQ ID NO : 17; un deuxième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 74 et 87 ; et un troisième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 18 & 53 ; ou
(v) un premier oligomère d'amplification comprenant une séquence d'acide nucléique consistant en SEQ ID NO : 18; un deuxième oligomère d'amplification comprenant une séquence d'acide nucléique sélectionnée dans le groupe consistant en les SEQ ID NO : 74 et 80; et un troisième oligomère d'amplification comprenant une séquence d'acide nucléique consistant en SEQ ID NO : 53.

8. Mélange réactionnel d'amplification, dans lequel le mélange est une solution aqueuse tamponnée comprenant la combinaison d'au moins deux oligomères d'amplification selon l'une quelconque des revendications 1 à 7 ; ou
dans lequel le mélange est une composition séchée comprenant les au moins deux oligomères d'amplification selon l'une quelconque des revendications 1 à 7, de préférence,
dans lequel la composition séchée comprend en outre au moins une enzyme polymérase.

9. Kit comprenant les au moins deux oligomères d'amplification selon l'une quelconque des revendications 1 à 7 ; de préférence,
dans lequel le premier oligomère d'amplification est dans un premier récipient à l'intérieur du kit et le deuxième oligomère d'amplification est dans un deuxième récipient à l'intérieur du kit.

10. Kit selon la revendication 9, dans lequel le kit comprend en outre un oligomère sonde de détection.

11. Procédé de détermination de la présence ou de l'absence d'un acide nucléique de virus Zika dans un échantillon, le procédé comprenant les étapes consistant à :

(A) mettre en contact un échantillon avec la combinaison d'au moins deux oligomères d'amplification selon l'une quelconque des revendications 1 à 7 ;
(B) mettre en œuvre une réaction d'amplification d'acide nucléique *in vitro,* dans lequel toute séquence cible de virus Zika présente dans l'échantillon est utilisée comme matrice pour générer un produit d'amplification ; et
(C) détecter la présence ou l'absence du produit d'amplification, en déterminant ainsi la présence ou l'absence de l'acide nucléique de virus Zika dans l'échantillon.

12. Procédé selon la revendication 11, dans lequel l'étape de détection comprend la mise en contact de la réaction d'amplification *in vitro* avec au moins un oligomère sonde de détection, dans lequel l'oligomère sonde de détection comprend :

(i) une séquence d'hybridation cible et, facultativement, une ou plusieurs bases nucléiques qui ne sont pas complémentaires de l'acide nucléique cible de virus Zika, et
(ii) un marqueur détectable ; de préférence,

dans lequel la séquence d'hybridation cible de l'oligomère sonde de détection présente une longueur de 17 à 23 bases nucléiques contiguës ; et/ou
dans lequel la séquence d'hybridation cible de l'oligomère sonde de détection est sélectionnée dans le groupe consistant en : SEQ ID NO : 56, SEQ ID NO : 57, et SEQ ID NO : 66 à 73 ; et/ou
dans lequel le marqueur détectable est un marqueur fluorescent, un marqueur luminescent, un marqueur chimio-luminescent, un marqueur de type ester d'acridium, un marqueur chromophore, un marqueur de type radionucléide, un marqueur de type ligand, un marqueur enzymatique, ou un marqueur à groupe réactif.

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