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(54) COMPOSITIONS AND METHODS FOR DETECTING PLASMODIUM SPECIES NUCLEIC ACID

ZUSAMMENSETZUNGEN UND VERFAHREN FÜR DEN NACHWEIS VON PLASMODIUMNUKLEINSÄURE

COMPOSITIONS ET PROCÉDÉS DE DÉTECTION D'ACIDE NUCLÉIQUE D'ESPÈCES DE PLASMODIUM

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(56) References cited:
WO-A1-2009/074649
WO-A1-2013/159293
WO-A1-2017/189746
WO-A1-2018/226798
CN-A- 102 220 419
CN-A- 104 673 901

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Description

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/782,945, filed December 20, 2018.

REFERENCE TO SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII Copy, created on October 28, 2019, is named "GDS_0110PC_20191028_Seq_Listing_ST25" and is 56,087 bytes in size.

BACKGROUND

[0003] Malaria is a serious disease caused by intra-erythrocyte parasites of the genus of *Plasmodium.* The parasites are contracted via an infected female *Anopheles* mosquito bite. There are five species of the parasite that cause malaria in humans: *P. falciparum, P. knowlesi, P. malariae, P. ovale* and *P. vivax.* According to the World Health Organization (WHO) there were 216 million cases of Malaria worldwide in 2016, and of those, 445,000 were fatal.

[0004] The United States Food and Drug Administration (U.S. FDA) has implemented strict blood screening guidelines for accepting or deferring donors who have travelled to malaria-endemic regions. Travelers are deferred for one year after traveling to an endemic region, or three years if the donor is a former resident of an endemic region. People who have been diagnosed with malaria are deferred for three years after the completion of treatment and symptom free. There are no approved tests available in the U.S. to screen blood donations for *Plasmodium.* This requires careful screening of prospective donors via a medical questionnaire.

[0005] In endemic countries, WHO recommends testing by thick blood films or using a highly sensitive enzyme immunoassay. In non-endemic countries, WHO recommends donors are deferred for six months from the last potential exposure combined with malaria antibody testing using a highly sensitive enzyme immunoassay. Donors may be reinstated if there is no evidence of malarial antibody. There is a risk that these methods are not effective to detect low levels of parasitemia where transmission may occur. CN102220419B and WO2009074649 relate to detecting Plasmodium using amplification.

[0006] Transfused Transmitted Malaria (TTM) has been documented in the U.S. There have been 46 cases of TTM reported between 1911 and 2015 and the latest case was reported in 2018. There is a need for a specific and sensitive nucleic acid test (NAT) for detecting *Plasmodium* species in a sample to reduce TTM and the number of deferred donors.

SUMMARY

[0007] The present invention is defined by the appended claims. In one aspect, the present invention provides a method for specifically detecting *Plasmodium* species nucleic acid in a sample. The method generally includes (1) contacting a sample, the sample suspected of containing *Plasmodium* species nucleic acid, with at least two oligomers for amplifying a target region of a *Plasmodium* species target nucleic acid, (2) performing an *in vitro* nucleic acid amplification reaction, where any *Plasmodium* target nucleic acid present in said sample is used as a template for generating an amplification product, and (3) detecting the presence or absence of the amplification product, thereby indicating the presence or absence of *Plasmodium* species target nucleic acid in said sample. In some embodiments, the at least two amplification oligomers comprise (a) an amplification oligomer comprising a target-hybridizing sequence (i) that is from about 14 to about 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 162, and includes the sequence of SEQ ID NO:163, or (ii) that is from about 14 to about 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:166, and includes SEQ ID NO: 167 or SEQ ID NO: 168; and (b) an amplification oligomer comprising a target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, is contained in SEQ ID NO: 169 and includes the sequence of SEQ ID NO:171, SEQ ID NO:172, or SEQ ID NO:173. Disclosed, but not part of the claimed invention, is the use of at least two amplification oligomers comprise (a') an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:185 and includes the sequence of SEQ ID NO:37, SEQ ID NO:46, or SEQ ID NO:187; and (b') an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:188 and includes the sequence of SEQ ID NO 83, SEQ ID NO:84, or SEQ ID NO:182.

[0008] In some embodiments of a method as above where the at least two amplification oligomers comprise the amplification oligomers of (a)(i) and (b), the target-hybridizing sequence of (a)(i) is selected from SEQ ID NOs:21, 23-25, 32, 33, 35, 54, and 55. In other embodiments, the target-hybridizing sequence of (a)(i) is contained in the sequence of SEQ ID NO: 164 and includes the sequence of SEQ ID NO:165; in some such variations, the target-hybridizing sequence

of (a)(i) is selected from SEQ ID NOs:21, 23-25, 32, 33, and 35.

[0009] In some embodiments of a method as above where the at least two amplification oligomers comprise the amplification oligomers of (a)(ii) and (b), the target-hybridizing sequence of (a)(ii) includes the sequence of SEQ ID NO:167; in some such variations, the target-hybridizing sequence of (a)(ii) is selected from the SEQ ID NOs:28-31, 34, 40, 41, and 49-51. In other embodiments, the target-hybridizing sequence of (a)(ii) includes the sequence of SEQ ID NO:168; in some such variations, the target-hybridizing sequence of (a)(ii) is selected from SEQ ID NOs:38, 39, 43, 44, and 53.

[0010] In some embodiments of a method as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the target-hybridizing sequence of (b) is selected from SEQ ID NOs:80-82 and 85-100. In other embodiments, the target-hybridizing sequence of (b) is contained in the sequence of SEQ ID NO: 170 and includes the sequence of SEQ ID NO:171 or SEQ ID NO:172. In some variations where the target-hybridizing sequence of (b) is contained in the sequence of SEQ ID NO: 170 and includes the sequence of SEQ ID NO:171, the target-hybridizing sequence of (b) is selected from SEQ ID NOs:81, 82, 85, 87-90, 94, and 96-98. In some variations where the target-hybridizing sequence of (b) is contained in the sequence of SEQ ID NO: 170 and includes the sequence of SEQ ID NO: 172, the target-hybridizing sequence of (b) is selected from SEQ ID NOs:80, 82, 85, and 87-100.

[0011] Disclosed, but not part of the claimed invention is the use of at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the target-hybridizing sequence of (a') is selected from SEQ ID NOs:37, 46, 183, and 184. In other embodiments, the target-hybridizing sequence of (a') is contained in the sequence of SEQ ID NO: 186; in some such variations, the target-hybridizing sequence of (a') is SEQ ID NO: 183 or SEQ ID NO:184. In certain embodiments where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the target-hybridizing sequence of (b') is selected from SEQ ID NOs:83, 84, and 182. In more particular variations, the target-hybridizing sequence of (a') is SEQ ID NO: 183 or SEQ ID NO: 184 and the target-hybridizing sequence of (b') is SEQ ID NO: 182.

[0012] In some embodiments of a method as above, the amplification oligomer of (b) or (b') is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence of (b) or (b'), respectively. A particularly suitable promoter sequence is a T7 promoter sequence such as, *e.g.,* SEQ ID NO:179. In specific variations where an amplification oligomer of (b) includes a promoter sequence, the amplification oligomer of (b) comprises a sequence selected from SEQ ID NOs:57-59 and 62-77. In specific variations where an amplification oligomer of (b') includes a promoter sequence, the amplification oligomer of (b') comprises a sequence selected from SEQ ID NOs:60, 61, and 181.

[0013] Particularly suitable pairs of amplification oligomer target-hybridizing sequences of (a) and (b), respectively, are (A) SEQ ID NO:30 and SEQ ID NO:82, (B) SEQ ID NO:33 and SEQ ID NO:82, (C) SEQ ID NO:49 and SEQ ID NO:82, (D) SEQ ID NO:21 and SEQ ID NO:89, (E) SEQ ID NO:30 and SEQ ID NO:89, (F) SEQ ID NO:33 and SEQ ID NO:89, (G) SEQ ID NO:49 and SEQ ID NO:89, (H) SEQ ID NO:21 and SEQ ID NO:92, (I) SEQ ID NO:30 and SEQ ID NO:92, (J) SEQ ID NO 21 and SEQ ID NO:94, (K) SEQ ID NO:34 and SEQ ID NO:94, (L) SEQ ID NO:53 and SEQ ID NO:94, (M) SEQ ID NO:21 and SEQ ID NO:95, (N) SEQ ID NO:34 and SEQ ID NO:95, and (O) SEQ ID NO:53 and SEQ ID NO:95. In some such embodiments, the amplification oligomer of (b) is a promoter primer or promoter provider further comprising a promoter sequence (for example, a T7 promoter sequence such as, *e.g.,* SEQ ID NO:179) located 5' to the target-hybridizing sequence of (b).

[0014] In some embodiments of a method as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the at least two amplification oligomers comprise first and second amplification oligomers as in (a). In some such variations, the at least two amplification oligomers comprise first and second amplification oligomers as in (a)(ii). Particularly suitable first and second amplification oligomers of (a)(ii) include a first amplification oligomer comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO:167 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:34) and a second amplification oligomer comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO:168 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:53).

[0015] In other embodiments of a method as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the at least two amplification oligomers comprise an amplification oligomer as in (a)(i) and an amplification oligomer as in (a)(ii). In some such embodiments, the amplification oligomer as in (a)(i) comprises a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:164 and includes the sequence of SEQ ID NO:165 (*e.g.*, the target-hybridizing sequence of SEQ ID NO:21), and the amplification oligomer as in (a)(ii) comprises a target-hybridizing sequence that includes the sequence of SEQ ID NO:167 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:34).

[0016] In some embodiments of a method as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the at least two amplification oligomers comprise first and second amplification oligomers of (b). In some such embodiments, each of the first and second amplification oligomers of (b) comprises a target-hybridizing sequence that is contained in SEQ ID NO:170 and includes the sequence of SEQ ID NO:171 or SEQ ID NO:172 *(e.g.,* a first amplification oligomer comprising the target-hybridizing sequence of SEQ ID NO:94 and a second

amplification oligomer comprising the target-hybridizing sequence of SEQ ID NO:95). In some variations, each of the first and second amplification oligomers of (b) is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence of (b). A particularly suitable promoter sequence is a T7 promoter sequence such as, *e.g.,* SEQ ID NO:179. In specific variations where each of the first and second amplification oligomers of (b) includes a promoter sequence, the first amplification oligomer of (b) comprises the sequence of SEQ ID NO:71 and the second amplification oligomer of (b) comprises the sequence of SEQ ID NO:72.

[0017] In certain embodiments of a method for detecting *Plasmodium* species nucleic acid in a sample as above, the method further includes purifying the target nucleic acid from other components in the sample before step (1). In some such variations, the purifying step comprises contacting the sample with at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a sequence or moiety that binds to an immobilized probe, where the target-hybridizing sequence configured to specifically hybridize to the *Plasmodium* species target nucleic acid. Particularly suitable capture probe oligomer target-hybridizing sequences include sequences that are up to about 30 contiguous nucleotides in length and include a sequence selected from SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof. In more specific variations, the capture probe oligomer target-hybridizing sequence is selected from SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof. In some embodiments, the purifying step comprises contacting the sample with at least two capture probe oligomers (*e.g*., at least two capture probe oligomers as above); in some such variations, the at least two capture probe oligomers include a first capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:19 and a second capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:20.

[0018] In some embodiments of a method for detecting *Plasmodium* species nucleic acid in a sample as above, the detecting step (3) comprises contacting the *in vitro* nucleic acid amplification reaction with at least one detection probe oligomer comprising a target-hybridizing sequence configured to specifically hybridize to the amplification product under conditions whereby the presence or absence of the amplification product is determined, thereby indicating the presence or absence of *Plasmodium* species in the sample. In some such embodiments where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the detection probe oligomer target-hybridizing sequence is from about 13 to about 40 nucleotides in length and is (i) contained in the sequence of SEQ ID NO: 196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO: 175, SEQ ID NO:176, SEQ ID NO: 177, and SEQ ID NO:178, including complements, DNA equivalents, and DNA/RNA equivalents thereof. Suitable detection probe oligomer target-hybridizing sequences include SEQ ID NOs: 131, 132, 135, 140, 145, 147-157, and 159-161, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In certain embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO:174 or SEQ ID NO:175, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in some such variations comprising the sequence of SEQ ID NO:174 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 148-155 and 159, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in other such variations comprising SEQ ID NO:175 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 147, 156, 157, 160, and 161, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In certain embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:177 and SEQ ID NO:178, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in some such variations, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 131, 132, 135, 140, 147, 156, 157, 160, and 161, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0019] In other embodiments of a method as above where the detecting step (3) comprises contacting the *in vitro* nucleic acid amplification reaction with at least one detection probe oligomer and where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the detection probe oligomer target-hybridizing sequence is at least about 13 nucleotides in length and is (i) contained in the sequence of SEQ ID NO: 189 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:190 and SEQ ID NO:191, including complements, DNA equivalents, and DNA/RNA equivalents thereof. In some such variations, the detection probe oligomer target-hybridizing sequence is selected from the SEQ ID NOs:125-130 and 143, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0020] In particular variations of a method for detecting *Plasmodium* species nucleic acid in a sample as above, where the detecting step (3) comprises contacting the *in vitro* nucleic acid amplification reaction with at least one detection probe oligomer and where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the amplification oligomer target-hybridizing sequence of (a), the amplification oligomer target-hybridizing sequence of (b), and the detection probe oligomer target-hybridizing sequence, respectively, are (A) SEQ ID NO:30, SEQ ID NO:82, and SEQ ID NO:151 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 151 or its complement;

(B) SEQ ID NO:30, SEQ ID NO:82, and SEQ ID NO:157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 157 or its complement; (C) SEQ ID NO:33, SEQ ID NO:82, and SEQ ID NO: 155 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 155 or its complement; (D) SEQ ID NO:49, SEQ ID NO:82, and SEQ ID NO:150 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:150 or its complement; (E) SEQ ID NO:49, SEQ ID NO:82, and SEQ ID NO: 155 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 155 or its complement; (F) SEQ ID NO:21, SEQ ID NO:89, and SEQ ID NO:148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (G) SEQ ID NO:21, SEQ ID NO:89, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (H) SEQ ID NO:30, SEQ ID NO:89; and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (I) SEQ ID NO:30, SEQ ID NO:89; and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (I) SEQ ID NO:33, SEQ ID NO:89; and SEQ ID NO:158 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:158 or its complement; (K) SEQ ID NO:49, SEQ ID NO:89, and SEQ ID NO: 150 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:150 or its complement; (L) SEQ ID NO:21, SEQ ID NO:92, and SEQ ID NO:148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (M) SEQ ID NO:21, SEQ ID NO:92, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (N) SEQ ID NO:30, SEQ ID NO:92; and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (O) SEQ ID NO:30, SEQ ID NO:92; and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (P) SEQ ID NO:21, SEQ ID NO:94, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (Q) SEQ ID NO:21, SEQ ID NO:94, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (R) SEQ ID NO:34, SEQ ID NO:94, and SEQ ID NO:148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (S) SEQ ID NO:34, SEQ ID NO:94, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (T) SEQ ID NO:34, SEQ ID NO:94, and SEQ ID NO:157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:157 or its complement; (U) SEQ ID NO:53, SEQ ID NO:94, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (V) SEQ ID NO:53, SEQ ID NO:94, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (W) SEQ ID NO:53, SEQ ID NO:94, and SEQ ID NO:157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:157 or its complement; (X) SEQ ID NO:21, SEQ ID NO:95, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (Y) SEQ ID NO:21, SEQ ID NO:95, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (Z) SEQ ID NO:34, SEQ ID NO:95, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (AA) SEQ ID NO:34, SEQ ID NO:95, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (AB) SEQ ID NO:34, SEQ ID NO:95, and SEQ ID NO:157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 157 or its complement; (AC) SEQ ID NO:53, SEQ ID NO:95, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (AD) SEQ ID NO:53, SEQ ID NO:95, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; or (AE) SEQ ID NO:53, SEQ ID NO:95, and SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 157 or its complement.

[0021] In particular variations of a method for detecting *Plasmodium* species nucleic acid in a sample as above, where the detecting step (3) comprises contacting the *in vitro* nucleic acid amplification reaction with at least one detection probe oligomer and where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the amplification oligomer target-hybridizing sequence of (a'), the amplification oligomer target-hybridizing sequence of (b'), and the detection probe oligomer target-hybridizing sequence, respectively, are (A) SEQ ID NO:183, SEQ ID NO:182, and SEQ ID NO: 126 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 126 or its complement; (B) SEQ ID NO: 183, SEQ ID NO: 182, and SEQ ID NO: 127 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 127 or its complement; (C) SEQ ID NO: 183, SEQ ID NO: 182, and SEQ ID NO: 128 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 128 or its complement; (D) SEQ ID NO: 183, SEQ ID NO: 182, and SEQ ID NO: 143 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 143 or its complement; (E) SEQ ID NO: 183, SEQ ID NO: 182, and SEQ ID NO: 129 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 129 or its complement; or (F) SEQ ID NO: 184, SEQ ID NO: 182, and SEQ ID NO: 126 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 126 or its complement.

[0022] In some embodiments of a method as above utilizing at least one detection probe oligomer, the detection probe oligomer comprises a 2' methoxy modification on at least one of a nucleotide residue member of the detection probe oligomer nucleotide sequence.

[0023] In some embodiments of a method as above utilizing at least one detection probe oligomer, the detection probe oligomer further includes a detectable label such as, for example, a fluorescent or chemiluminescent label. A particularly

suitable chemiluminescent label is a chemiluminescent acridinium ester (AE) compound linked between two nucleobases of the detection probe oligomer. In some embodiments comprising a detectably labeled probe oligomer, the detectable label is a fluorescent label and the detection probe oligomer further includes a non-fluorescent quencher.

[0024] In some embodiments of a method as above, the detecting step (3) occurs during the amplifying step (2). In some such embodiments, the method utilizes a detection probe oligomer comprising a fluorescent label and a quencher (e.g., a molecular torch, a molecular beacon, or a TaqMan detection probe).

[0025] In some embodiments of a method as above utilizing at least one detection probe oligomer, the detection probe further includes a non-target-hybridizing sequence. In particular variations, a detection probe oligomer comprising a non-target-hybridizing sequence is a hairpin detection probe such as, *e.g.,* a molecular beacon or a molecular torch.

[0026] In certain embodiments, a method for detecting *Plasmodium* species nucleic acid in a sample as above utilizes at least two detection probe oligomers. In some such embodiments, the at least two detection probe oligomers comprise first and second detection probe oligomers, where (A) the first detection probe oligomer comprises a target-hybridizing sequence that is (i) contained in the sequence of SEQ ID NO: 197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO: 175 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (B) the second detection probe oligomer comprises a target-hybridizing sequence that is (i) contained in the sequence of SEQ ID NO: 197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO: 176 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof. In a more specific variations, the first detection probe oligomer comprises the target-hybridizing sequence of SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and the second detection probe oligomer comprises a target-hybridizing sequence selected from SEQ ID NO: 148 and SEQ ID NO:152, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0027] In certain variations of a method for detecting *Plasmodium* species nucleic acid in a sample as above, the *in vitro* nucleic acid amplification reaction at step (2) is an isothermal amplification reaction *(e.g.,* a transcription-mediated amplification (TMA) reaction).

[0028] In certain variations of a method for detecting *Plasmodium* species nucleic acid in a sample as above, the amplification reaction is a real-time amplification reaction.

[0029] In some embodiments of a method for detecting *Plasmodium* species nucleic acid in a sample as above, the sample is a clinical sample. In some embodiments, the sample is a blood sample such as, for example, a red blood cell sample (*e.g*., a lysed blood cell sample or lysed red blood cell sample).

[0030] In another aspect, the present invention provides a combination of at least two oligomers for determining the presence or absence of *Plasmodium* species in a sample. The oligomer combination generally includes at least two oligomers for amplifying a target region *of Plasmodium* species target nucleic acid. In some embodiments, the at least two amplification oligomers comprise (a) an amplification oligomer comprising a target-hybridizing sequence (i) that is from about 14 to about 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 162, and includes the sequence of SEQ ID NO: 163, or (ii) that is from about 14 to about 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 166, and includes SEQ ID NO: 167 or SEQ ID NO: 168; and (b) an amplification oligomer comprising a target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, is contained in SEQ ID NO: 169 and includes the sequence of SEQ ID NO: 171, SEQ ID NO: 172, or SEQ ID NO: 173. Disclosed, but not part of the claimed invention is the use of at least two amplification oligomers comprise (a') an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO: 185 and includes the sequence of SEQ ID NO:37, SEQ ID NO:46, or SEQ ID NO: 187; and (b') an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO: 188 and includes the sequence of SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO: 182.

[0031] In some embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a)(i) and (b), the target-hybridizing sequence of (a)(i) is selected from SEQ ID NOs:21, 23-25, 32, 33, 35, 54, and 55. In other embodiments, the target-hybridizing sequence of (a)(i) is contained in the sequence of SEQ ID NO: 164 and includes the sequence of SEQ ID NO: 165; in some such variations, the target-hybridizing sequence of (a)(i) is selected from SEQ ID NOs:21, 23-25, 32, 33, and 35.

[0032] In some embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a)(ii) and (b), the target-hybridizing sequence of (a)(ii) includes the sequence of SEQ ID NO: 167; in some such variations, the target-hybridizing sequence of (a)(ii) is selected from the SEQ ID NOs:28-31, 34, 40, 41, and 49-51. In other embodiments, the target-hybridizing sequence of (a)(ii) includes the sequence of SEQ ID NO: 168; in some such variations, the target-hybridizing sequence of (a)(ii) is selected from SEQ ID NOs:38, 39, 43, 44, and 53.

[0033] In some embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the target-hybridizing sequence of (b) is selected from SEQ ID NOs:80-82 and 85-100 In other embodiments, the target-hybridizing sequence of (b) is contained in the sequence of SEQ ID NO: 170 and includes the sequence of SEQ ID NO:171 or SEQ ID NO:172. In some variations where the target-

hybridizing sequence of (b) is contained in the sequence of SEQ ID NO: 170 and includes the sequence of SEQ ID NO: 171, the target-hybridizing sequence of (b) is selected from SEQ ID NOs:81, 82, 85, 87-90, 94, and 96-98. In some variations where the target-hybridizing sequence of (b) is contained in the sequence of SEQ ID NO: 170 and includes the sequence of SEQ ID NO:172, the target-hybridizing sequence of (b) is selected from SEQ ID NOs:80, 82, 85, and 87-100.

[0034] In some embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the target-hybridizing sequence of (a') is selected from SEQ ID NOs:37, 46, 183, and 184. In other embodiments, the target-hybridizing sequence of (a') is contained in the sequence of SEQ ID NO: 186; in some such variations, the target-hybridizing sequence of (a') is SEQ ID NO:183 or SEQ ID NO:184. In certain embodiments where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the target-hybridizing sequence of (b') is selected from SEQ ID NOs:83, 84, and 182. In more particular variations, the target-hybridizing sequence of (a') is SEQ ID NO:183 or SEQ ID NO:184 and the target-hybridizing sequence of (b') is SEQ ID NO:182.

[0035] In some embodiments of an oligomer combination as above, the amplification oligomer of (b) or (b') is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence of (b) or (b'), respectively. A particularly suitable promoter sequence is a T7 promoter sequence such as, *e.g.,* SEQ ID NO: 179. In specific variations where an amplification oligomer of (b) includes a promoter sequence, the amplification oligomer of (b) comprises a sequence selected from SEQ ID NOs:57-59 and 62-77. In specific variations where an amplification oligomer of (b') includes a promoter sequence, the amplification oligomer of (b') comprises a sequence selected from SEQ ID NOs:60, 61, and 181.

[0036] Particularly suitable pairs of amplification oligomer target-hybridizing sequences of (a) and (b), respectively, are (A) SEQ ID NO:30 and SEQ ID NO:82, (B) SEQ ID NO:33 and SEQ ID NO:82, (C) SEQ ID NO:49 and SEQ ID NO:82, (D) SEQ ID NO:21 and SEQ ID NO:89, (E) SEQ ID NO:30 and SEQ ID NO:89, (F) SEQ ID NO:33 and SEQ ID NO:89, (G) SEQ ID NO:49 and SEQ ID NO:89, (H) SEQ ID NO:21 and SEQ ID NO:92, (I) SEQ ID NO:30 and SEQ ID NO:92, (J) SEQ ID NO 21 and SEQ ID NO:94, (K) SEQ ID NO:34 and SEQ ID NO:94, (L) SEQ ID NO:53 and SEQ ID NO:94, (M) SEQ ID NO:21 and SEQ ID NO:95, (N) SEQ ID NO:34 and SEQ ID NO:95, and (O) SEQ ID NO:53 and SEQ ID NO:95. In some such embodiments, the amplification oligomer of (b) is a promoter primer or promoter provider further comprising a promoter sequence (for example, a T7 promoter sequence such as, *e.g.,* SEQ ID NO: 179) located 5' to the target-hybridizing sequence of (b).

[0037] In some embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the at least two amplification oligomers comprise first and second amplification oligomers as in (a). In some such variations, the at least two amplification oligomers comprise first and second amplification oligomers as in (a)(ii). Particularly suitable first and second amplification oligomers of (a)(ii) include a first amplification oligomer comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO: 167 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:34) and a second amplification oligomer comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO: 168 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:53).

[0038] In other embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the at least two amplification oligomers comprise an amplification oligomer as in (a)(i) and an amplification oligomer as in (a)(ii). In some such embodiments, the amplification oligomer as in (a)(i) comprises a target-hybridizing sequence that is contained in the sequence of SEQ ID NO: 164 and includes the sequence of SEQ ID NO: 165 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:21), and the amplification oligomer as in (a)(ii) comprises a target-hybridizing sequence that includes the sequence of SEQ ID NO: 167 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:34).

[0039] In some embodiments of an oligomer combination as above where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the at least two amplification oligomers comprise first and second amplification oligomers of (b). In some such embodiments, each of the first and second amplification oligomers of (b) comprises a target-hybridizing sequence that is contained in SEQ ID NO: 170 and includes the sequence of SEQ ID NO:171 or SEQ ID NO:172 *(e.g.,* a first amplification oligomer comprising the target-hybridizing sequence of SEQ ID NO:94 and a second amplification oligomer comprising the target-hybridizing sequence of SEQ ID NO:95). In some variations, each of the first and second amplification oligomers of (b) is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence of (b). A particularly suitable promoter sequence is a T7 promoter sequence such as, *e.g.,* SEQ ID NO:179. In specific variations where each of the first and second amplification oligomers of (b) includes a promoter sequence, the first amplification oligomer of (b) comprises the sequence of SEQ ID NO:71 and the second amplification oligomer of (b) comprises the sequence of SEQ ID NO:72.

[0040] In certain embodiments of an oligomer combination for detecting *Plasmodium* species nucleic acid in a sample as above, the oligomer combination further includes at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a sequence or moiety that binds to an immobilized probe, where the target-hybridizing

sequence configured to specifically hybridize to the *Plasmodium* species target nucleic acid. Particularly suitable capture probe oligomer target-hybridizing sequences include sequences that are up to about 30 contiguous nucleotides in length and include a sequence selected from SEQ ID NOs: 11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof. In more specific variations, the capture probe oligomer target-hybridizing sequence is selected from SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof. In some embodiments, the oligomer combination includes at least two capture probe oligomers *(e.g.,* at least two capture probe oligomers as above); in some such variations, the at least two capture probe oligomers include a first capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:19 and a second capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:20.

[0041] In some embodiments of an oligomer combination for detecting *Plasmodium* species nucleic acid in a sample as above, the oligomer combination further includes at least one detection probe oligomer comprising a target-hybridizing sequence configured to specifically hybridize to a *Plasmodium* species amplicon amplifiable by the at least two amplification oligomers. In some such embodiments where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the detection probe oligomer target-hybridizing sequence is from about 13 to about 40 nucleotides in length and is (i) contained in the sequence of SEQ ID NO:196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, and SEQ ID NO:178, including complements, DNA equivalents, and DNA/RNA equivalents thereof. Suitable detection probe oligomer target-hybridizing sequences include SEQ ID NOs:131, 132, 135, 140, 145, 147-157, and 159-161, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In certain embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO:174 or SEQ ID NO:175, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in some such variations comprising the sequence of SEQ ID NO: 174 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 148-155 and 159, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in other such variations comprising SEQ ID NO:175 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs:147, 156, 157, 160, and 161, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In certain embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:177 and SEQ ID NO:178, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in some such variations, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs:131, 132, 135, 140, 147, 156, 157, 160, and 161, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0042] In other embodiments of an oligomer combination as above further including at least one detection probe oligomer and where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the detection probe oligomer target-hybridizing sequence is at least about 13 nucleotides in length and is (i) contained in the sequence of SEQ ID NO:189 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:190 and SEQ ID NO:191, including complements, DNA equivalents, and DNA/RNA equivalents thereof. In some such variations, the detection probe oligomer target-hybridizing sequence is selected from the SEQ ID NOs: 125-130 and 143, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0043] In particular variations of an oligomer combination as above further including at least one detection probe oligomer and where the at least two amplification oligomers comprise the amplification oligomers of (a) and (b), the amplification oligomer target-hybridizing sequence of (a), the amplification oligomer target-hybridizing sequence of (b), and the detection probe oligomer target-hybridizing sequence, respectively, are (A) SEQ ID NO:30, SEQ ID NO:82, and SEQ ID NO:151 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 151 or its complement; (B) SEQ ID NO:30, SEQ ID NO:82, and SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 157 or its complement; (C) SEQ ID NO:33, SEQ ID NO:82, and SEQ ID NO:155 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:155 or its complement; (D) SEQ ID NO:49, SEQ ID NO:82, and SEQ ID NO: 150 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 150 or its complement; (E) SEQ ID NO:49, SEQ ID NO:82, and SEQ ID NO:155 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:155 or its complement; (F) SEQ ID NO:21, SEQ ID NO:89, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (G) SEQ ID NO:21, SEQ ID NO:89, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (H) SEQ ID NO:30, SEQ ID NO:89; and SEQ ID NO:148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (I) SEQ ID NO:30, SEQ ID NO:89; and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (I) SEQ ID NO:33, SEQ ID NO:89; and SEQ ID NO:158 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:158 or its complement; (K) SEQ ID NO:49, SEQ ID NO:89, and SEQ ID NO: 150 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:150 or its complement; (L) SEQ ID NO:21, SEQ ID NO:92, and SEQ ID NO: 148 or its complement, or

a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (M) SEQ ID NO:21, SEQ ID NO:92, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (N) SEQ ID NO:30, SEQ ID NO:92; and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (O) SEQ ID NO:30, SEQ ID NO:92; and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (P) SEQ ID NO:21, SEQ ID NO:94, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (Q) SEQ ID NO:21, SEQ ID NO:94, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (R) SEQ ID NO:34, SEQ ID NO:94, and SEQ ID NO:148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (S) SEQ ID NO:34, SEQ ID NO:94, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (T) SEQ ID NO:34, SEQ ID NO:94, and SEQ ID NO:157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:157 or its complement; (U) SEQ ID NO:53, SEQ ID NO:94, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (V) SEQ ID NO:53, SEQ ID NO:94, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (W) SEQ ID NO:53, SEQ ID NO:94, and SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:157 or its complement; (X) SEQ ID NO:21, SEQ ID NO:95, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 148 or its complement; (Y) SEQ ID NO:21, SEQ ID NO:95, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; (Z) SEQ ID NO:34, SEQ ID NO:95, and SEQ ID NO: 148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (AA) SEQ ID NO:34, SEQ ID NO:95, and SEQ ID NO: 152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 152 or its complement; (AB) SEQ ID NO:34, SEQ ID NO:95, and SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:157 or its complement; (AC) SEQ ID NO:53, SEQ ID NO:95, and SEQ ID NO:148 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:148 or its complement; (AD) SEQ ID NO:53, SEQ ID NO:95, and SEQ ID NO:152 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:152 or its complement; or (AE) SEQ ID NO:53, SEQ ID NO:95, and SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO:157 or its complement.

[0044] In particular variations of an oligomer combination as above further including at least one detection probe oligomer and where the at least two amplification oligomers comprise the amplification oligomers of (a') and (b'), the amplification oligomer target-hybridizing sequence of (a'), the amplification oligomer target-hybridizing sequence of (b'), and the detection probe oligomer target-hybridizing sequence, respectively, are (A) SEQ ID NO:183, SEQ ID NO:182, and SEQ ID NO:126 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 126 or its complement; (B) SEQ ID NO:183, SEQ ID NO: 182, and SEQ ID NO:127 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 127 or its complement; (C) SEQ ID NO: 183, SEQ ID NO: 182, and SEQ ID NO: 128 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 128 or its complement; (D) SEQ ID NO:183, SEQ ID NO:182, and SEQ ID NO: 143 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 143 or its complement; (E) SEQ ID NO: 183, SEQ ID NO: 182, and SEQ ID NO: 129 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 129 or its complement; or (F) SEQ ID NO:184, SEQ ID NO: 182, and SEQ ID NO: 126 or its complement, or a DNA equivalent or DNA/RNA chimeric of SEQ ID NO: 126 or its complement.

[0045] In some embodiments of an oligomer combination as above further including at least one detection probe oligomer, the detection probe oligomer comprises a 2' methoxy modification on at least one of a nucleotide residue member of the detection probe oligomer nucleotide sequence.

[0046] In some embodiments of an oligomer combination as above further including at least one detection probe oligomer, the detection probe oligomer further includes a detectable label such as, for example, a fluorescent or chemiluminescent label. A particularly suitable chemiluminescent label is a chemiluminescent acridinium ester (AE) compound linked between two nucleobases of the detection probe oligomer. In some embodiments comprising a detectably labeled probe oligomer, the detectable label is a fluorescent label and the detection probe oligomer further includes a non-fluorescent quencher; particularly suitable detection probe oligomers comprising a fluorescent label and a quencher including molecular torches, molecular beacons, and TaqMan detection probes.

[0047] In some embodiments of an oligomer combination as above further including at least one detection probe oligomer, the detection probe further includes a non-target-hybridizing sequence. In particular variations, a detection probe oligomer comprising a non-target-hybridizing sequence is a hairpin detection probe such as, *e.g.,* a molecular beacon or a molecular torch.

[0048] In certain embodiments, an oligomer combination for detecting *Plasmodium* species nucleic acid in a sample as above includes at least two detection probe oligomers. In some such embodiments, the at least two detection probe oligomers comprise first and second detection probe oligomers, where (A) the first detection probe oligomer comprises a target-hybridizing sequence that is (i) contained in the sequence of SEQ ID NO: 197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO:175 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (B) the second detection probe oligomer comprises a target-hybridizing

sequence that is (i) contained in the sequence of SEQ ID NO: 197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO:176 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof. In a more specific variation, the first detection probe oligomer comprises the target-hybridizing sequence of SEQ ID NO:157 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and the second detection probe oligomer comprises a target-hybridizing sequence selected from SEQ ID NO:148 and SEQ ID NO:152, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0049] In another aspect, the present invention provides use of a combination of at least two oligomers as above for specifically amplifying *Plasmodium* species nucleic acid in a sample.

[0050] In another aspect, the present invention provides a detection probe oligomer for specifically detecting a *Plasmodium* species target nucleic acid in a sample. In some embodiments, the detection probe oligomer comprises a target-hybridizing sequence that is from about 13 to about 40 nucleotides in length and configured to specifically hybridize to a target sequence contained within a *Plasmodium* species target region amplifiable by an oligomer combination comprising first and second *Plasmodium-specific* amplification oligomers, where (a) the first amplification oligomer comprises a target-hybridizing sequence (i) that is from about 14 to about 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:162, and includes the sequence of SEQ ID NO:163, or (ii) that is from about 14 to about 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:166, and includes the sequence of SEQ ID NO:167 or SEQ ID NO:168; and (b) the second amplification oligomer comprises a target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, is contained in SEQ ID NO:169 and includes the sequence of SEQ ID NO:171, SEQ ID NO:172, or SEQ ID NO:173. In some such embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, and SEQ ID NO:178, including complements, DNA equivalents, and DNA/RNA equivalents thereof. Suitable detection probe oligomer target-hybridizing sequences include SEQ ID NOs:131, 132, 135, 140, 145, 147-157, and 159-161, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In certain embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO:174 or SEQ ID NO:175, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in some such variations comprising the sequence of SEQ ID NO:174 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 148-155 and 159, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in other such variations comprising SEQ ID NO:175 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 147, 156, 157, 160, and 161, including complements, DNA equivalents, and DNA/RNA chimerics thereof. In certain embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO:177 and SEQ ID NO:178, including complements, DNA equivalents, and DNA/RNA chimerics thereof; in some such variations, the detection probe oligomer target-hybridizing sequence is selected from SEQ ID NOs: 131, 132, 135, 140, 147, 156, 157, 160, and 161, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0051] In other embodiments of a detection probe oligomer for specifically detecting a *Plasmodium* species target nucleic acid in a sample, the detection probe oligomer comprises a target-hybridizing sequence that is at least about 13 nucleotides in length and configured to specifically hybridize to a target sequence contained within a *Plasmodium* species target region amplifiable by an oligomer combination comprising first and second *Plasmodium-specific* amplification oligomers, where (a) the first amplification oligomer comprises a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:185 and includes the sequence of SEQ ID NO:37, SEQ ID NO:46, or SEQ ID NO:187; and (b) the second amplification oligomer comprises a target-hybridizing sequence that is contained in SEQ ID NO:188 and includes the sequence of SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:182. In some such embodiments, the detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:189 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes a sequence selected from SEQ ID NO: 190 and SEQ ID NO:191, including complements, DNA equivalents, and DNA/RNA equivalents thereof. In more specific variations, the detection probe oligomer target-hybridizing sequence is selected from the SEQ ID NOs: 125-130 and 143, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0052] In some embodiments of a detection probe oligomer as above, the detection probe oligomer comprises a 2' methoxy modification on at least one of a nucleotide residue member of the detection probe oligomer nucleotide sequence.

[0053] In some embodiments of a detection probe oligomer as above, the detection probe oligomer further includes a detectable label such as, for example, a fluorescent or chemiluminescent label. A particularly suitable chemiluminescent label is a chemiluminescent acridinium ester (AE) compound linked between two nucleobases of the detection probe oligomer. In some embodiments comprising a detectable label, the detectable label is a fluorescent label and the detection probe oligomer further includes a non-fluorescent quencher; particularly suitable detection probe oligomers comprising

a fluorescent label and a quencher including molecular torches, molecular beacons, and TaqMan detection probes.

[0054] In some embodiments of a detection probe oligomer as above, the detection probe further includes a non-target-hybridizing sequence. In particular variations, a detection probe oligomer comprising a non-target-hybridizing sequence is a hairpin detection probe such as, *e.g.,* a molecular beacon or a molecular torch.

[0055] In another aspect, the present invention provides a combination of at least two oligomers for detecting a *Plasmodium* species target nucleic acid in a sample, the oligomer combination comprising at least two detection probe oligomers as above. In some embodiments, the at least two detection probe oligomers comprise a (A) a first detection probe oligomer comprising a target-hybridizing sequence that (i) is contained in the sequence of SEQ ID NO: 197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO: 175 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (B) a second detection probe oligomer comprising a target-hybridizing sequence that (i) is contained in the sequence of SEQ ID NO: 197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and (ii) includes the sequence of SEQ ID NO: 176 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof. In more specific variations, the first detection probe oligomer comprises the target-hybridizing sequence of SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and the second detection probe oligomer comprises a target-hybridizing sequence selected from SEQ ID NO:148 and SEQ ID NO:152, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0056] In another aspect, the present invention provides use of a detection probe oligomer or oligomer combination according as above for specifically detecting *Plasmodium* species nucleic acid in a sample.

[0057] In another aspect, the present invention provides a capture probe oligomer for specifically isolating *Plasmodium* species nucleic acid from a sample. In some embodiments, the capture probe oligomer comprises a target-hybridizing sequence covalently attached to a sequence or moiety that binds to an immobilized probe, where the target-hybridizing sequence is up to about 30 contiguous nucleotides in length and includes a sequence selected from SEQ ID NOs: 11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof. In more specification variations, the capture probe oligomer target-hybridizing sequence is selected from SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof.

[0058] In another aspect, the present invention provides a combination of at least two oligomers for specifically isolating *Plasmodium* species nucleic acid from a sample, the oligomer combination comprising at least two capture probe oligomers as above. In some embodiments, the at least two capture probe oligomers comprise a first capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:19, or a DNA equivalent or DNA/RNA chimeric thereof, and a second capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:20, or a DNA equivalent or DNA/RNA chimeric thereof.

[0059] In another aspect, the present invention provides use of a capture probe oligomer or oligomer combination as above for specifically capturing *Plasmodium* species nucleic acid from a sample.

[0060] In another aspect, the present invention provides a kit comprising a combination of at least two oligomers as above.

[0061] In another aspect, the present invention a reaction mixture comprising a combination of at least two oligomers as above.

[0062] These and other aspects of the invention will become evident upon reference to the following detailed description of the invention and the attached drawings.

DEFINITIONS

[0063] 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.

[0064] The terms "a," "an," and "the" include plural referents, unless the context clearly indicates otherwise.

[0065] "Sample" includes any specimen that may contain, or is suspected of containing, *Plasmodium* species or components thereof, such as nucleic acids or fragments of *Plasmodium* nucleic acids. The sample may be an isolated sample. Samples include "biological samples" which include any tissue or material derived from a living or dead human that may contain the *Plasmodium* parasite or components thereof *(e.g.,* a target nucleic acid derived therefrom), including, *e.g.*, blood, peripheral blood and red blood cells. The use of other sample types that may contain the *Plasmodium* parasite or components thereof *(e.g.,* a target nucleic acid derived therefrom) - such as plasma, serum, lymph node, gastrointestinal tissue, faeces, urine, semen or other body fluids or materials - is also contemplated. The biological sample may be treated to physically or mechanically disrupt tissue or cell structure, thus releasing intracellular components into a solution which may further contain enzymes, buffers, salts, detergents and the like, which are used to prepare, using standard methods, a biological sample for analysis. For example, a sample may be treated with cell lysis reagent such as, *e.g.,* a lysis reagent as described in US Pat. No. 10,093,989 or PCT Pub. No. WO 2017/189746. Also, samples may include processed samples, such as those obtained from passing samples over or through a filtering

device, or following centrifugation, or by adherence to a medium, matrix, or support.

[0066] "Nucleic acid" refers to a multimeric compound comprising two or more covalently bonded nucleosides or nucleoside analogs having nitrogenous heterocyclic bases, or base analogs, where the nucleosides are linked together by phosphodiester bonds or other linkages to form a polynucleotide. Nucleic acids include RNA, DNA, or chimeric DNA-RNA polymers or oligonucleotides, and analogs thereof. A nucleic acid "backbone" may be made up of a variety of linkages, including one or more of sugar -phosphodiester linkages, peptide-nucleic acid bonds (in "peptide nucleic acids" or PNAs, *see* PCT Pub. No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of the nucleic acid may be either ribose or deoxyribose, or similar compounds having known substitutions, *e.g.,* 2' methoxy substitutions and 2' halide substitutions *(e.g.,* 2'-F). Nitrogenous bases may be conventional bases (A, G, C, T, U), analogs thereof (*e.g*., inosine, 5-methylisocytosine, isoguanine; The Biochemistry of the Nucleic Acids 5-36, Adams et al., ed., 11th ed., 1992, BioTechniques (2007) 43:617-24), which include derivatives of purine or pyrimidine bases (*e.g*., N4-methyl deoxygaunosine, deaza- or aza-purines, deaza- or azapyrimidines, pyrimidine bases having substituent groups at the 5 or 6 position, purine bases having an altered or replacement substituent at the 2, 6 and/or 8 position, such as 2-amino-6-methylaminopurine, 06-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and 04-alkyl-pyrimidines, and pyrazolo-compounds, such as unsubstituted or 3-substituted pyrazolo[3,4-d]pyrimidine; US Pat. Nos. 5,378,825, 6,949,367 and PCT Pub. No. WO 93/13121). Nucleic acids may include "abasic" residues in which the backbone does not include a nitrogenous base for one or more residues (US Pat. No. 5,585,481). A nucleic acid may comprise only conventional sugars, bases, and linkages as found in RNA and DNA, or may include conventional components and substitutions (*e.g*., conventional bases linked by a 2' methoxy backbone, or a nucleic acid including a mixture of conventional bases and one or more base analogs). Nucleic acids may include "locked nucleic acids" (LNA), in which one or more nucleotide monomers have a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhances hybridization affinity toward complementary sequences in single-stranded RNA (ssRNA), single-stranded DNA (ssDNA), or double-stranded DNA (dsDNA) (Biochemistry (2004) 43:13233-41). Nucleic acids may include modified bases to alter the function or behavior of the nucleic acid, *e.g.,* addition of a 3'-terminal dideoxynucleotide to block additional nucleotides from being added to the nucleic acid. Synthetic methods for making nucleic acids in vitro are well-known in the art.

[0067] 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. Standard nucleic acids, *e.g.,* DNA and RNA, are typically synthesized "5'-to-3'," *i.e.,* by the addition of nucleotides to the 3'-terminus of a growing nucleic acid.

[0068] A "nucleotide," as used herein, is a subunit of a nucleic acid consisting of a phosphate group, a 5-carbon sugar and a nitrogenous base. The 5-carbon sugar found in RNA is ribose. In DNA, the 5-carbon sugar is 2'-deoxyribose. The term also includes analogs of such subunits, such as a methoxy group at the 2' position of the ribose (2'-0-Me).

[0069] A "nucleic-acid-based detection assay," as used herein, is an assay for the detection of a target sequence within a target nucleic acid and utilizing one more oligonucleotides that specifically hybridize to the target sequence.

[0070] In certain embodiments, a nucleic-acid-based detection assay is an "amplification-based assay," *i.e.,* an assay that utilizes one or more steps for amplifying a nucleic acid target sequence. Various amplification methods for use in detection assays are known in the art, several of which are summarized further herein. For the sake of clarity, an amplification-based assay may include one or more steps that do not amplify a target sequence, such as, for example, steps used in non-amplification-based assay methods (*e.g.,* a hybridization assay or a cleavage-based assay).

[0071] In other embodiments, a nucleic-acid-based detection assay is a "non-amplification-based assay," *i.e.,* an assay that does not rely on any step for amplifying a nucleic acid target sequence. For the sake of clarity, a nucleic-acid-based detection assay that includes a reaction for extension of a primer in the absence of any corresponding downstream amplification oligomer (*e.g*., extension of a primer by a reverse transcriptase to generate an RNA:DNA duplex followed by an RNase digestion of the RNA, resulting in a single-stranded cDNA complementary to an RNA target but without generating copies of the cDNA) is understood to be a non-amplification-based assay.

[0072] An exemplary non-amplification-based assay is a "cleavage-based assay," which is an assay that relies on the specific cleavage, by a flap endonuclease, of a linear duplex cleavage structure formed by the specific hybridization of overlapping oligonucleotides to a target nucleic acid. In these assays, a probe oligonucleotide containing a non-target-hybridizing flap region is cleaved in an overlap-dependent manner by the flap endonuclease to release a cleavage product that is then detected. The principles of cleavage-based assays are well-known in the art, and exemplary assays are described in, for example, Nat. Biotechnol. (1999) 17:292-296, Mol. Diagn. (1999) 4: 135-144, J. Clin. Microbiol. (2006) 44:3443-3447, and US Patent Nos. 5,846,717, 6,706,471 and 5,614,402. Cleavage-based assays include, *e.g.,* the commercially available Invader® assays (Hologic, Inc., Madison, WI).

[0073] A "target nucleic acid," as used herein, is a nucleic acid comprising a target sequence to be detected. Target nucleic acids may be DNA or RNA as described herein, and may be either single-stranded or double-stranded. The target nucleic acid may include other sequences besides the target sequence.

[0074] By "isolated" it is meant that a sample containing a target nucleic acid is taken from its natural milieu, but the term does not connote any degree of purification.

[0075] The term "target sequence," as used herein, refers to the particular nucleotide sequence of a target nucleic acid that is to be detected. The "target sequence" includes the complexing sequences to which oligonucleotides (*e.g.,* probe oligonucleotide, priming oligonucleotides and/or promoter oligonucleotides) complex during a detection process (*e.g.,* an amplification-based detection assay such as, for example, TMA or PCR, or a non-amplification-based detection assay such as, for example, a cleavage-based assay). 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. In choosing a target sequence, the skilled artisan will understand that a "unique" sequence should be chosen so as to distinguish between unrelated or closely related target nucleic acids.

[0076] "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 the various strains *of Plasmodium.* It is understood that other reasons exist for configuring a target-hybridizing sequence to have less than 100% complementarity to a target nucleic acid.

[0077] The term "targets a sequence," as used herein in reference to a region of *Plasmodium sp.* nucleic acid, refers to a process whereby an oligonucleotide hybridizes to the target sequence in a manner that allows for detection as described herein. In one embodiment, the oligonucleotide is complementary with the targeted *Plasmodium sp.* nucleic acid sequence and contains no mismatches. In another embodiment, the oligonucleotide is complementary but contains 1, 2, 3, 4, or 5 mismatches with the targeted *Plasmodium sp.* nucleic acid sequence. Preferably, the oligonucleotide that hybridizes to the target 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.

[0078] The term "configured to" denotes an actual arrangement of the polynucleotide sequence configuration of a referenced oligonucleotide target-hybridizing sequence. For 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.

[0079] The term "configured to specifically hybridize to" as used herein means that the target-hybridizing region of an oligonucleotide is designed to have a polynucleotide sequence that could target a sequence of the referenced *Plasmodium sp.* target region. 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 *Plasmodium sp.* target nucleic acid. The oligonucleotide is designed to function as a component of an assay for detection *of Plasmodium sp.* from a sample, and therefore is designed to target *Plasmodium sp.* 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. The term "configured to" denotes an actual arrangement of the polynucleotide sequence configuration of the oligonucleotide target-hybridizing sequence.

[0080] The term "fragment," as used herein in reference to a *Plasmodium sp.* targeted nucleic acid, refers to a piece of contiguous nucleic acid.

[0081] The term "region," as used herein, refers to a portion of a nucleic acid wherein the portion is smaller than the entire nucleic acid. For example, when the nucleic acid in reference is an oligonucleotide promoter primer, the term "region" may be used refer to the smaller promoter portion of the entire oligonucleotide. As a non-limiting example, when the nucleic acid in reference is an amplicon, the term region may be used to refer to the smaller nucleotide sequence identified for hybridization by the target-hybridizing sequence of a probe.

[0082] 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 500 to 900 nt residues. In some embodiments, oligonucleotides are in a size range having a lower limit of about 12 to 15 nt and an upper limit of about 50 to 600 nt, and other embodiments are in a range having a lower limit of about 15 to 20 nt and an upper limit of about 22 to 100 nt. 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).

[0083] As used herein, an oligonucleotide can "substantially correspond to" a specified reference nucleic acid sequence, which 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 target nucleic acid sequence. It is also understood that a first nucleic acid corresponding to a second nucleic acid includes the RNA and DNA 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%. In more preferred embodiments, this percentage is from 100% to about 90%; in other preferred embodiments, this percentage is from 100% to about 95%. 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.

[0084] Exemplary sequences for *Plasmodium* sp. target nucleic acid are shown in Table 19, *infra*. Specifically, SEQ ID NOs:180 and 192-195 are reference sequences corresponding to ribosomal RNA sequences for *Plasmodium falciparum, Plasmodium vivax, Plasmodium knowlesi, Plasmodium ovale,* and *Plasmodium malariae,* respectively. Where a target region of *Plasmodium* sp. is described herein as "corresponding to" a defined region of SEQ ID NO: 180, it is understood that such reference includes homologous regions of any one or more of SEQ ID NOs: 192-195. It is also understood that such reference to a region "corresponding to" a defined region of SEQ ID NO: 180 includes homologous regions of naturally occurring variants of any one or more of SEQ ID NOs: 180 and 192-195 that may be present in a sample.

[0085] 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. An example of an amplification oligomer is a "primer" that hybridizes to a target nucleic acid and contains a 3' OH end that is extended by a polymerase in an amplification process. Another example of an amplification oligomer is an oligomer that is not extended by a polymerase (*e.g.*, because it has a 3' blocked end) but participates in or facilitates amplification. For example, the 5' region of an amplification oligonucleotide may include a promoter sequence that is non-complementary to the target nucleic acid (which may be referred to as a "promoter primer" or "promoter provider"). Those skilled in the art will understand that an amplification oligomer that functions as a primer may be modified to include a 5' promoter sequence, and thus function as a promoter primer. Incorporating a 3' blocked end further modifies the promoter primer, which is now capable of hybridizing to a target nucleic acid and providing an upstream promoter sequence that serves to initiate transcription, but does not provide a primer for oligo extension. Such a modified oligo is referred to herein as a "promoter provider" oligomer. Size ranges for amplification oligonucleotides include those that are about 10 to about 70 nt long (not including any promoter sequence or poly-A tails) and contain at least about 10 contiguous bases, or even at least 12 contiguous bases that are complementary to a region of the target nucleic acid sequence (or a complementary strand thereof). The contiguous bases are at least 80%, or at least 90%, or completely complementary to the target sequence to which the amplification oligomer binds. An amplification oligomer may optionally include modified nucleotides or analogs, or additional nucleotides that participate in an amplification reaction but are not complementary to or contained in the target nucleic acid, or template sequence. It is understood that when referring to ranges for the length of an oligonucleotide, amplicon, or other nucleic acid, that the range is inclusive of all whole numbers (*e.g.,* 19-25 contiguous nucleotides in length includes 19, 20, 21, 22, 23, 24 & 25).

[0086] 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.

[0087] As used herein, a "promoter provider" or "provider" refers to an oligonucleotide comprising first and second regions and which is modified to prevent the initiation of DNA synthesis from its 3' -terminus. The "first region" of a promoter provider oligonucleotide comprises a base sequence which hybridizes to a DNA template, where the hybridizing sequence is situated 3', but not necessarily adjacent to, a promoter region. The hybridizing portion of a promoter oligonucleotide is typically at least 10 nucleotides in length and may extend up to 50 or more nucleotides in length. The "second region" comprises a promoter sequence for an RNA polymerase. A promoter oligonucleotide is engineered so that it is incapable of being extended by an RNA- or DNA-dependent DNA polymerase, *e.g.*, reverse transcriptase, preferably comprising a blocking moiety at its 3'-terminus as described above. As referred to herein, a "T7 Provider" is a blocked promoter provider oligonucleotide that provides an oligonucleotide sequence that is recognized by T7 RNA

polymerase.

[0088] "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 both thermal cycling and isothermal amplification methods. In some embodiments, isothermal amplification methods are preferred. Replicase-mediated amplification, polymerase chain reaction (PCR), ligase chain reaction (LCR), strand-displacement amplification (SDA), and transcription-mediated or transcription-associated amplification are non-limiting examples of nucleic acid amplification methods. Replicase-mediated amplification uses self-replicating RNA molecules, and a replicase such as QB-replicase (*e.g.,* US Pat. No. 4,786,600). PCR amplification uses a DNA polymerase, pairs of primers, and thermal cycling to synthesize multiple copies of two complementary strands of dsDNA or from a cDNA *(e.g.,* US Pat. Nos. 4,683,195, 4,683,202, and 4,800, 159). LCR amplification uses four or more different oligonucleotides to amplify a target and its complementary strand by using multiple cycles of hybridization, ligation, and denaturation (*e.g*., US Pat. No. 5,427,930 and US Pat. No. 5,516,663). SDA uses a primer that contains a recognition site for a restriction endonuclease and an endonuclease that nicks one strand of a hemimodified DNA duplex that includes the target sequence, whereby amplification occurs in a series of primer extension and strand displacement steps (*e.g*., US Pat. No. 5,422,252; US Pat. No. 5,547,861; and US Pat. No. 5,648,211). Preferred embodiments use an amplification method suitable for the amplification of RNA target nucleic acids, such as transcription-mediated amplification (TMA) or NASBA, but it will be apparent to persons of ordinary skill in the art that oligomers disclosed herein may be readily used as primers in other amplification methods.

[0089] "Transcription-associated amplification," also referred to herein as "transcription-mediated amplification" (TMA), refers 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 are embodiments of amplification methods used for amplifying and detecting *Plasmodium* target sequences as described herein. Variations of transcription-associated amplification are well-known in the art as previously disclosed in detail (*e.g*., US Pat. Nos. 4,868,105; 5,124,246; 5,130,238; 5,437,990; 5,554,516; and 7,374,885; and PCT 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.

[0090] As used herein, the term "real-time TMA" refers to transcription-mediated amplification ("TMA") of target nucleic acid that is monitored by real-time detection means.

[0091] The term "amplicon," which is used interchangeably with "amplification product," refers to the nucleic acid molecule generated during an amplification procedure that is complementary or homologous to a sequence contained within the target sequence. These terms can be used to refer to a single strand amplification product, a double strand amplification product, or one of the strands of a double strand amplification product.

[0092] "Probe," "detection probe," "detection oligonucleotide," and "detection probe oligomer" are used interchangeably herein 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). Probes may be DNA, RNA, analogs thereof, or combinations thereof and they may be labeled or unlabeled. A probe's "target sequence" generally refers to a smaller nucleic acid sequence within a larger nucleic acid sequence that hybridizes specifically to at least a portion of a probe oligomer by standard base pairing. A probe may comprise target-specific sequences and other sequences that contribute to the three-dimensional conformation of the probe *(e.g.,* US Pat. Nos. 5,118,801; 5,312,728; 6,849,412; 6,835,542; 6,534,274; and 6,361,945; and US Pub. No. 20060068417). In a preferred embodiment, the detection probe comprises a 2' methoxy backbone, which can result in a higher signal being obtained.

[0093] The term "TaqMan® probe" refers to detection oligonucleotides that contain a fluorescent dye, typically on the 5' base, and a non-fluorescent quenching dye (quencher), typically on the 3' base. When irradiated, the excited fluorescent dye transfers energy to the nearby quenching dye molecule rather than fluorescing, resulting in a non-fluorescent substrate. During amplification, the exonuclease activity of the polymerase cleaves the TaqMan probe to separate the fluorophore from the quencher, thereby allowing an unquenched signal to be emitted from the fluorophore as an indicator of amplification.

[0094] 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, including covalent bonds or non-covalent interactions, *e.g*., hydrogen bonds, hydrophobic and ionic interactions, or formation of chelates or coordination complexes. 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 in which bound labeled probe in a mixture exhibits a detectable change different from that of an unbound labeled probe, *e.g.*, instability or differential degradation properties. A "homogeneous detectable label" can be detected without physically removing bound from unbound forms of the label or labeled probe *(e.g.,* US Pat. 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 (*e.g.*, US Pat. 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 *(e.g.,* Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), Chapter 10; US Pat. 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 *(e.g.,* US Pat. Nos. 6, 180,340 and 6,350,579).

[0095] As used herein, structures referred to as "molecular torches" are designed to include distinct regions of self-complementarity ("the closing domain") which are connected by a joining region ("the target-binding domain") and which hybridize to one another under predetermined hybridization assay conditions. All or part of the nucleotide sequences comprising closing domains may also function as target-binding domains. Thus, closing domains can include target-binding sequences, non-target binding sequences, and combinations thereof.

[0096] As used herein, structures referred to as "molecular beacons" are designed to include a target-binding sequence flanked on both its 5' and 3' ends by sequences that are complementary to each other and which hybridize to each other under predetermined hybridization assay conditions. The flanking, complementary regions may be referred to as "switch sequences."

[0097] "Capture probe," "capture oligonucleotide," "target capture oligonucleotide," and "capture probe oligomer" are used interchangeably herein 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 an oligonucleotide comprising two binding regions: a target hybridizing sequence and an immobilized probe-binding region. A variation of this example, the two regions may be present on two different oligomers joined together by one or more linkers. In another embodiment of a capture oligomer, the target-hybridizing sequence is a sequence 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 *(see, e.g.,* PCT Pub. No. WO 2008/016988). The immobilized probe-binding region can be a nucleic acid sequence, referred to as a tail. Tails include a substantially homopolymeric tail of about 10 to 40 nucleotides (*e.g.*, A10 to A40), or of about 17 to 33 nt *(e.g.,* T3A14 to T3A30), that bind to a complementary immobilized sequence attached to the support particle or support matrix. Thus, a non-limiting example of preferred nucleic acid tails can in some embodiments include $T_{0\text{-}4}A_{10\text{-}40}$ sequences. Another example of a capture oligomer comprises two regions, a target-hybridizing sequence and a binding pair member that is not a nucleic acid sequence.

[0098] As used herein, an "immobilized oligonucleotide," "immobilized probe," or "immobilized nucleic acid" refers to a nucleic acid binding partner that joins a capture oligomer to a support, directly or indirectly. An immobilized probe joined to a support facilitates separation of a capture probe bound target from unbound material in a sample. One embodiment of an immobilized probe is an oligomer joined to a support that facilitates separation of bound target sequence from unbound material in a sample. Supports may include known materials, such as matrices and particles free in solution, which may be made of nitrocellulose, nylon, glass, polyacrylate, mixed polymers, polystyrene, silane, polypropylene, metal, or other compositions, of which one embodiment is magnetically attractable particles. Supports may be monodisperse magnetic spheres (*e.g.*, uniform size + 5%), to which an immobilized probe is joined directly (via covalent linkage, chelation, or ionic interaction), or indirectly (via one or more linkers), where the linkage or interaction between the probe and support is stable during hybridization conditions.

DESCRIPTION

[0099] The present invention is generally directed to methods and compositions for determining the presence or absence of the protozoan parasite *Plasmodium* sp. in a sample, such as, *e.g.,* a blood sample. Suitably, the methods and compositions described herein are able to detect the presence or absence of *Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale* and/or *Plasmodium vivax.* In some embodiments, the present invention provides methods for the detection of *Plasmodium* sp. in a sample, where the method includes performing amplification-based detection of a target nucleic from *Plasmodium* sp. The present invention further provides compositions (including reaction mixtures) and kits comprising a combination of oligomers for detecting *Plasmodium* sp. - including *Plasmodium falciparum* and/or *Plasmodium knowlesi* and/or *Plasmodium malariae* and/or *Plasmodium ovale* and/or *Plasmodium vivax* - in a sample. The oligomer combination generally includes at least two amplification oligomers for detecting *Plasmodium* sp. - including *Plasmodium falciparum* and/or *Plasmodium knowlesi* and/or *Plasmodium malariae*

and/or *Plasmodium ovale* and/or *Plasmodium vivax* - in a sample, and may further include one or more additional oligomers as described herein for performing amplification-based detection of *Plasmodium* sp. - including *Plasmodium falciparum* and/or *Plasmodium knowlesi* and/or *Plasmodium malariae* and/or *Plasmodium ovale* and/or *Plasmodium vivax* - such as, for example, a capture probe and/or a detection probe.

[0100] Methods for detecting the presence or absence of *Plasmodium* sp. in a sample from a subject generally include performing a nucleic-acid-based detection assay for the specific detection in the sample of *Plasmodium* sp. nucleic acid. Nucleic-acid-based detection assays generally utilize oligonucleotides that specifically hybridize to a target nucleic acid of *Plasmodium* sp. with minimal cross-reactivity to other nucleic acids suspected of being in a sample. In some variations, an oligonucleotide or combination of oligonucleotides for nucleic-acid-based detection of *Plasmodium* sp. has minimal cross-reactivity to *Babesia* sp. (*e.g., B. microti*) nucleic acids.

[0101] In certain aspects of the invention, a combination of at least two oligomers is provided for determining the presence or absence of *Plasmodium* species in a sample. Typically, the oligomer combination includes at least first and second amplification oligomers for amplifying a *Plasmodium* sp. target region corresponding to a region of SEQ ID NO:180. In such embodiments, at least one amplification oligomer comprises a target-hybridizing sequence in the sense orientation ("sense THS") and at least one amplification oligomer comprises a target-hybridizing sequence in the antisense orientation ("antisense THS"), where the sense THS and antisense THS of the amplification oligomers are each configured to specifically hybridize to a *Plasmodium* sp. target sequence corresponding to a sequence contained within SEQ ID NO: 180, and where the target-hybridizing sequences are selected such that the *Plasmodium* sequence targeted by the antisense THS is situated downstream of the *Plasmodium* sequence targeted by the sense THS (*i.e.,* the at least two amplification oligomers are situated such that they flank the target region to be amplified).

[0102] In some embodiments, the *Plasmodium* sp. target region corresponds to a region of SEQ ID NO:180 from about nucleotide position 844 or about nucleotide position 910 to about nucleotide position 1038, about nucleotide position 1051, about nucleotide position 1060, or about nucleotide position 1077. In other embodiments, the *Plasmodium* sp. target region corresponds to a region of SEQ ID NO: 180 from about nucleotide position 1153, about nucleotide position 1169, or about nucleotide position 1182 to about nucleotide position 1327, about nucleotide position 1354, or about nucleotide position 1382.

[0103] In some embodiments, a composition includes an amplification oligomer comprising a *Plasmodium-specific* target-hybridizing sequence substantially corresponding to, or identical to, the sequence shown in any one of SEQ ID NOs:21-56, 80-102, and 182-184. In such variations, the oligomer combination includes at least one amplification oligomer comprising *a Plasmodium-specific* target-hybridizing sequence of the opposite polarity (sense vs. antisense or *vice versa*) as the target-hybridizing sequence of the oligomer as above, such that at least two amplification oligomers flank a target region to be amplified.

[0104] In some embodiments, a composition includes (1) at least one amplification oligomer comprising a *Plasmodium-specific* target-hybridizing region substantially corresponding to at least one sense oligomer sequence depicted in Table 1 below, and (2) at least one amplification oligomer comprising a *Plasmodium-specific* target hybridizing region substantially corresponding to at least one antisense oligomer sequence depicted in Table 1. In particular variations, the sense and/or antisense target-hybridizing sequence(s) of an amplification oligomer combination comprises or consists of the sense and/or antisense sequence(s) selected from Table 1.

Table 1. Exemplary Sense and Antisense Amplification Oligomer Target-hybridizing Sequences for Amplification *of Plasmodium* species Target Regions

SEQ ID NO	Sequence (5' → 3')	Sense/Antisense[1]
21	AATACTACAGCATGG	Sense
22	GGAAGGCAGCAGGCGCGTA	Sense
23	AATACTACAGCATGGA	Sense
24	AATACTACAGCATGGAA	Sense
25	ATACTACAGCATGGAATA	Sense
26	ATTCAGATGTCAGAGGTGA	Sense
27	GTATTCAGATGTCAGAGGTGA	Sense
28	GTTACGATTAATAGGAGT	Sense
29	GTTACGATTAATAGGAGTA	Sense
30	GTTACGATTAATAGGAGTAG	Sense

(continued)

SEQ ID NO	Sequence (5' → 3')	Sense/Antisense[1]
31	GTTACGATTAATAGGAGTAGC	Sense
32	AATACTACAGCATGGAAT	Sense
33	AATACTACAGCATGGAATA	Sense
34	TACGATTAATAGGAGT	Sense
35	TACTACAGCATGGAATA	Sense
36	TATTCAGATGTCAGAGGTGA	Sense
37	TCAGTNCCTTATGAGAAATC	Sense
38	TGGCTTAGTTACGATT	Sense
39	TGGCTTAGTTACGATTAATAG	Sense
40	TTAATAGGAGTAGCTTGGGG	Sense
41	TTACGATTAATAGGAGT	Sense
42	TTCAGATGTCAGAGGTGA	Sense
43	TTGGCTTAGTTACGAT	Sense
44	TTGGCTTAGTTACGATTA	Sense
45	TTGGGGACATTCGTATTCAGA	Sense
46	TTTAGATTGCTTCCTTCAGT	Sense
47	TTTGAATACTANAGCA	Sense
48	ACATTCGTATTCAGATGTCAG	Sense
49	CTTAGTTACGATTAATAGGA	Sense
50	CGATTAATAGGAGTAGCTTGG	Sense
51	CTTAGTTACGATTAATAGGAGTAG	Sense
52	CTTGAATACTNCAGCA	Sense
53	GGCTTAGTTACGATTA	Sense
54	AATACTANAGCATGG	Sense
55	AATACTANAGCATGGAATA	Sense
56	AATTCTAAAGAAGAGAG	Sense
80	TTCACTCCCTTAACTTTCGTTCTTG	Antisense
81	CTTGATTAATGGAAGTATTTTAGA	Antisense
82	CTTAACTTTCGTTCTTGATTAATGGAAGT	Antisense
83	CCTACTCTTGTCTTAAACTA	Antisense
84	AAACGGCCATGCATCACCATCCAAGA	Antisense
85	CTCCCTTAACTTTCGTTCTTGATTAATGGAAGT	Antisense
86	CGACGGTATCTGATCGTCTTCACTCCC	Antisense
87	CTTAACTTTCGTTCTTGATTAATGGAAG	Antisense
88	CTTAACTTTCGTTCTTGATTAATGGAAGTA	Antisense
89	CACTCCCTTAACTTTCGTTCTTGATTAATG	Antisense
90	CACTCCCTTAACTTTCGTTCTTGATTAATGG	Antisense
91	CTTCACTCCCTTAACTTTCGTTCTTGATT	Antisense

(continued)

SEQ ID NO	Sequence (5' → 3')	Sense/Antisense[1]
92	CTTCACTCCCTTAACTTTCGTTCTTGAT	Antisense
93	ATCGTCTTCACTCCCTTAACTTTCGTTC	Antisense
94	CTCCCTTAACTTTCGTTCTTGATTAATG	Antisense
95	TCACTCCCTTAACTTTCGTTCTTGAT	Antisense
96	CCCTTAACTTTCGTTCTTGATTAATG	Antisense
97	CTTAACTTTCGTTCTTGATTAATG	Antisense
98	TAACTTTCGTTCTTGATTAATG	Antisense
99	ACTCCCTTAACTTTCGTTCTTGAT	Antisense
100	TCCCTTAACTTTCGTTCTTGAT	Antisense
101	AGGCAAATGCTTTCGCAGTTGTTNGTCT	Antisense
102	AGGCAAATGCTTTCGCAGTTGTTTGTCT	Antisense
182	TCAAGAAAGAGCTATNAATCTGTCAATCC	Antisense
183	GAAATCAAAGTCTTTGGGTTCTG	Sense
184	CAAAGTCTTTGGGTTCTGG	Sense
[1]The Sense/Antisense designation of these sequences is for exemplary purposes only. Such designation does not necessarily limit a sequence to the accompanying designation.		

[0105] In some embodiments, an oligomer combination comprises (a) an amplification oligomer comprising a target-hybridizing sequence (i) that is from about 14 to about 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:162, and includes the sequence of SEQ ID NO:163, or (ii) that is from about 14 to about 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 166, and includes SEQ ID NO: 167 or SEQ ID NO:168; and (b) an amplification oligomer comprising a target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, is contained in SEQ ID NO:169 and includes the sequence of SEQ ID NO:171, SEQ ID NO:172, or SEQ ID NO:173. In some such embodiments, the oligomer combination comprises an amplification oligomer of (a)(i) where the target-hybridizing sequence of is selected from SEQ ID NOs:21, 23-25, 32, 33, 35, 54, and 55, or where the target-hybridizing sequence is contained in the sequence of SEQ ID NO: 164 and includes the sequence of SEQ ID NO:165 *(e.g.,* a target-hybridizing sequence selected from SEQ ID NOs:21, 23-25, 32, 33, and 35). In yet other embodiments, the oligomer combination comprises an amplification oligomer of (a)(ii) where the target-hybridizing sequence of includes the sequence of SEQ ID NO:167 *(e.g.,* a target-hybridizing sequence selected from the SEQ ID NOs:28-31, 34, 40, 41, and 49-51), or where the target-hybridizing sequence includes the sequence of SEQ ID NO: 168 *(e.g.,* a target-hybridizing sequence selected from SEQ ID NOs:38, 39, 43, 44, and 53). In certain embodiments of an oligomer combination as above, the target-hybridizing sequence of (b) is selected from SEQ ID NOs:80-82 and 85-100. In other embodiments, the target-hybridizing sequence of (b) is contained in the sequence of SEQ ID NO:170 and includes the sequence of SEQ ID NO:171 *(e.g.,* a target-hybridizing sequence selected from SEQ ID NOs:81, 82, 85, 87-90, 94, and 96-98), or is contained in the sequence of SEQ ID NO:170 and includes the sequence of SEQ ID NO: 172 *(e.g.,* a target-hybridizing sequence selected from SEQ ID NOs:80, 82, 85, and 87-100).

[0106] In some embodiments, an oligomer combination comprises (a') an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:185 and includes the sequence of SEQ ID NO:37, SEQ ID NO:46, or SEQ ID NO:187; and (b') an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:188 and includes the sequence of SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:182. In some embodiments, the amplification oligomer of (a') comprises a target-hybridizing sequence selected from SEQ ID NOs:37, 46, 183, and 184, or a target-hybridizing sequence contained in the sequence of SEQ ID NO:186 *(e.g.,* a target-hybridizing sequence of SEQ ID NO: 183 or SEQ ID NO: 184). In certain embodiments, the amplification oligomers of (b') comprises a target-hybridizing sequence selected from SEQ ID NOs:83, 84, and 182.

[0107] In certain embodiments, an amplification oligomer as described herein is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence and which is non-complementary to the *Plasmodium* sp. target nucleic acid. For example, in some embodiments of an oligomer combination as described

herein, an amplification oligomer of (b) or (b') as described above is a promoter primer further comprising 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, an amplification oligomer is a promoter primer having the sequence shown selected from SEQ ID NOs:57-77 and 181.

[0108] Table 2 shows particularly suitable combinations of amplification oligomer target-hybridizing sequences ("Amp 1" and "Amp 2") for detection of *Plasmodium* species target nucleic acid.

Table 2. Exemplary Combinations of Amplification Oligomer Target-hybridizing Sequences.

Amp 1 (SEQ ID NO)	Amp 2 (SEQ ID NO)
30	5
33	8
49	11
21	14
30	17
33	20
49	23
21	26
30	29
21	32
34	35
53	38
21	41
34	44
53	46
183	182
184	182

[0109] In some embodiments, an oligomer combination as above includes at least two sense amplification oligomers and/or at least two antisense amplification oligomers flanking a *Plasmodium* sp. target region. For example, an oligomer combination may include (a) at least two amplification oligomer (*e.g.,* two or three amplification oligomers) each comprising a target-hybridizing sequence that is from about 14 to about 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 166, and includes SEQ ID NO: 167 or SEQ ID NO: 16 and/or (b) at least two amplification oligomers (*e.g.,* two or three amplification oligomers) each comprising a target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, is contained in SEQ ID NO: 169 and includes the sequence of SEQ ID NO:171, SEQ ID NO: 172, or SEQ ID NO:173; in some such variations, the oligomer combination includes (a) a first amplification oligomer comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO: 167 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:34) and a second amplification oligomer comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO: 168 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:53). In other embodiments comprising at least two sense amplification oligomers and/or at least two antisense amplification oligomers, an oligomer combination comprises (a)(i) an amplification oligomer comprising a target-hybridizing sequence that is from about 14 to about 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:162, and includes the sequence of SEQ ID NO:163, and (ii) an amplification oligomer comprising a target-hybridizing sequence that is from about 14 to about 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 166, and includes SEQ ID NO: 167 or SEQ ID NO: 168; and/or (b) at least two amplification oligomers (*e.g.*, two or three amplification oligomers) each comprising a target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, is contained in SEQ ID NO: 169 and includes the sequence of SEQ ID NO: 171, SEQ ID NO: 172, or SEQ ID NO: 173; in some such variations, the oligomer combination includes (a)(i) an amplification oligomer comprising a target-hybridizing sequence that is contained in the sequence of SEQ ID NO: 164 and includes the sequence of SEQ

ID NO: 165 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:21), and (a)(ii) an amplification comprising a target-hybridizing sequence that includes the sequence of SEQ ID NO: 167 *(e.g.,* the target-hybridizing sequence of SEQ ID NO:34). In some embodiments of an oligomer combination as above comprising at least two amplification oligomers of (b), oligomer combination comprise first and second amplification oligomers of (b), each comprising a target-hybridizing sequence that is contained in SEQ ID NO:170 and includes the sequence of SEQ ID NO:171 or SEQ ID NO: 172 *(e.g.,* a first amplification oligomer comprising the target-hybridizing sequence of SEQ ID NO:94 and a second amplification oligomer comprising the target-hybridizing sequence of SEQ ID NO:95).

[0110] In some embodiments, an oligomer combination as described herein further comprises at least one capture probe oligomer comprising a target-hybridizing sequence configured to specifically hybridize to *Plasmodium* species target nucleic acid. In some such embodiments, the capture probe oligomer comprises a target-hybridizing sequence a sequence substantially corresponding to to a sequence contained in the complement of SEQ ID NO:180. In some embodiments, a capture probe oligomer target-hybridizing sequence is covalently attached to a sequence or moiety that binds to an immobilized probe. Suitable capture probe oligomer target-hybridizing sequences include sequences that are up to about 30 contiguous nucleotides in length and include a sequence substantially corresponding to a sequence selected from SEQ ID NOs:11-15, 17, 19, and 20 *(e.g.,* a target-hybridizing sequence comprising or consisting of a sequence selected from SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof). In some embodiments, a capture probe oligomer comprises or consists of a sequence selected from SEQ ID NOs: 1-5, 7, 9, and 10. In some embodiments, the oligomer combination includes at least two capture probe oligomers *(e.g.,* at least two capture probe oligomers as above). A first capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:19 *(e.g.,* a capture probe oligomer comprising the sequence of SEQ ID NO:9) and a second capture probe oligomer comprising the target-hybridizing sequence of SEQ ID NO:20 *(e.g.,* a capture probe oligomer comprising the sequence of SEQ ID NO:10) are particularly suitable for use together in oligomer combinations as described herein.

[0111] In certain variations, an oligomer combination as described herein further comprises at least one detection probe oligomer configured to specifically hybridize to a *Plasmodium* sp. target sequence that is amplifiable using the at least two amplification oligomers targeting a *Plasmodium* sp. target region. In some embodiments where a *Plasmodium* sp. target region corresponds to a region of SEQ ID NO:180 from about nucleotide position 844 or about nucleotide position 910 to about nucleotide position 1038, about nucleotide position 1051, about nucleotide position 1060, or about nucleotide position 1077, the oligomer combination includes a detection probe oligomer that specifically hybridizes to a target region corresponding to a region of SEQ ID NO:180 from about nucleotide position 951 to about nucleotide position 998 or the full complement thereof. For example, a detection probe oligomer may include a target-hybridizing sequence that is from about 13 to about 40 nucleotides in length and is (i) contained in the sequence of SEQ ID NO:196 or its complement and (ii) includes a sequence selected from SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, and SEQ ID NO:178, including complements thereof. In more specific variations, a detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:197 or its complement and (ii) includes the sequence of SEQ ID NO:174 or SEQ ID NO:175, including complements thereof. In other variations, a detection probe oligomer target-hybridizing sequence is (i) contained in the sequence of SEQ ID NO:196 or its complement and (ii) includes a sequence selected from SEQ ID NO:177 and SEQ ID NO:178, including complements thereof. Particularly suitable detection probe oligomer target-hybridizing sequences include SEQ ID NOs:131, 132, 135, 140, 145, 147-157, and 159-161, including complements thereof. Suitable detection probes further include DNA equivalents and DNA/RNA chimerics of any of the above

[0112] In some embodiments where a *Plasmodium* sp. target region corresponds to a region of SEQ ID NO:180 from about nucleotide position 1153, about nucleotide position 1169, or about nucleotide position 1182 to about nucleotide position 1327, about nucleotide position 1354, or about nucleotide position 1382, the oligomer combination includes a detection probe oligomer that specifically hybridizes to a target region corresponding to a region of SEQ ID NO:180 from about nucleotide position 1210 to about nucleotide position 1233 or the full complement thereof. For example, a detection probe oligomer may include a target-hybridizing sequence that is at least about 13 nucleotides in length and is (i) contained in the sequence of SEQ ID NO:189 or its complement and (ii) includes a sequence selected from SEQ ID NO:190 and SEQ ID NO:191, including complements thereof. Particularly suitable detection probe oligomer target-hybridizing sequences include SEQ ID NOs:125-130 and 143, including complements thereof. Suitable detection probes further include DNA equivalents and DNA/RNA chimerics of any of the above.

[0113] Table 3 shows exemplary combinations of detection probe target hybridizing sequences together with first and second amplification oligomer target-hybridizing sequences ("Amp 1" and "Amp 2") for detection of *Plasmodium* species target nucleic acid.

Table 3. Exemplary Combinations of Amplification Oligomer and Detection Probe Target-hybridizing Sequences.

Amplification Oligomer THSs		Detection Probe THS (SEQ ID NO)
Amp 1 (SEQ ID NO)	Amp 2 (SEQ ID NO)	
30	82	151
30	82	157
33	82	155
49	82	150
49	82	155
21	89	148
21	89	152
30	89	148
30	89	152
33	89	158
49	89	150
21	92	148
21	92	152
30	92	148
30	92	152
21	94	148
21	94	152
34	94	148
34	94	152
34	94	157
53	94	148
53	94	152
53	94	157
21	95	148
21	95	152
34	95	148
34	95	152
34	95	157
53	95	148
53	95	152
53	95	157
183	182	126
183	182	127
183	182	128
183	182	143
183	182	129
184	182	126

[0114] In some variations, an oligomer combination includes at least two detection probe oligomers *(e.g.,* at least two specific detection probes as described herein). For example, where a *Plasmodium* sp. target region corresponds to a region of SEQ ID NO:180 from about nucleotide position 844 or about nucleotide position 910 to about nucleotide position 1038, about nucleotide position 1051, about nucleotide position 1060, or about nucleotide position 1077, an oligomer combination may include (A) a first detection probe oligomer comprising a target-hybridizing sequence that is (i) contained in the sequence of SEQ ID NO:197 or its complement and (ii) includes the sequence of SEQ ID NO: 175 or its complement, and (B) a second detection probe oligomer comprising a target-hybridizing sequence that is (i) contained in the sequence of SEQ ID NO:197 or its complement and (ii) includes the sequence of SEQ ID NO:176 or its complement (including DNA equivalents or DNA/RNA chimerics of the foregoing). Particularly suitable combinations of first and second detection probe oligomers include a first detection probe oligomer comprising the target-hybridizing sequence of SEQ ID NO: 157 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and a second detection probe oligomer comprising a target-hybridizing sequence selected from SEQ ID NO: 148 and SEQ ID NO: 152, including complements, DNA equivalents, and DNA/RNA chimerics thereof.

[0115] A detection probe oligomer may contain a 2'-methoxy backbone at one or more linkages in the nucleic acid backbone. In some embodiments, the at least one detection probe oligomer is provided in an amplicon detection reaction mixture.

[0116] Typically, a detection probe oligomer in accordance with the present invention 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) 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;).

[0117] A detection probe oligomer in accordance with the present invention may further include a non-target-hybridizing sequence. Specific embodiments of such detection probes include, for example, probes that form conformations held by intramolecular hybridization, such as conformations generally referred to as hairpins. Particularly suitable hairpin probes include 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., supra;* US 5,118,801 and US 5,312,728, *supra).* Methods for using such hairpin probes are well-known in the art.

[0118] In yet other embodiments, a detection probe is a linear oligomer that does not substantially form conformations held by intramolecular bonds. In specific variations, a linear detection probe oligomer includes a chemiluminescent compound as the label, preferably an acridinium ester (AE) compound.

[0119] Also provided by the present invention are detection probe oligomers, capture probe oligomers, and combinations thereof as described herein.

[0120] In other aspects, the present invention provides methods for detecting the presence or absence of *Plasmodium* sp. in a sample from a subject. Such methods generally include performing a nucleic-acid-based detection assay for the specific detection in the sample of *Plasmodium* sp. nucleic acid. A nucleic-acid-based detection assay for specific detection of *Plasmodium* sp. may use any one or more *Plasmodium* sp.-specific oligomers as described herein *(e.g.,* an oligomer combination as described herein comprising at least two amplification oligomers; or a detection probe or combination of detection probes as described herein). A positive signal from a nucleic-acid-based detection assay in accordance with the present invention is indicative of the presence of one or more of *Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale* and/or *Plasmodium vivax* in a sample.

[0121] In some embodiments of a method comprising the use of a nucleic-acid-based detection assay, an amplification-based assay is used to detect *Plasmodium* sp. Such amplification-based assay methods generally include performing a nucleic acid amplification of an *Plasmodium* sp. target region and detecting the amplified product *(e.g.,* by specifically hybridizing the amplified product with a nucleic acid detection probe that provides a signal to indicate the presence of *Plasmodium* sp. in the sample). The amplification step includes contacting the sample with one or more amplification oligomers specific for a target sequence in a *Plasmodium* sp. target nucleic acid to produce an amplified product if *Plasmodium* sp. nucleic acid is present in the sample. In particular embodiments, a combination of at least two amplification oligomers as described herein are used at the amplification step. 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). 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 *(see, e.g.,* discussion of amplification methods in Definitions section, *supra)* and are readily used in accordance with the methods of the present disclosure.

[0122] Detection of the amplified products may be accomplished by a variety of methods to detect a signal specifically associated with the amplified target sequence. 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 uses a hybridizing step that includes contacting the amplified product with at least one detection probe 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 some embodiments, a method utilizing an amplification-based assay for detection of *Plasmodium* species utilizes one or more detection probe oligomers as described herein for detection of an amplified product.

[0123] 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 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. In some embodiments of a method for detecting *Plasmodium* species, such as in certain embodiments using transcription-mediated amplification (TMA), the detection probe is a linear chemiluminescently labeled probe such as, *e.g.,* a linear acridinium ester (AE) 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 region, *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).

[0124] 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).

[0125] 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 primer 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.

[0126] In some embodiments, the method utilizes 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 region. 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 region, 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.

[0127] In some embodiments of a method comprising the use of a nucleic-acid-based detection assay, a non-amplification-based assay is used to detect *Plasmodium* sp. In some such embodiments, the non-amplification-based assay is a hybridization assay comprising the hybridization of a specific detection probe to a target nucleic acid. Methods for conducting polynucleotide hybridization assays have been well developed in the art. Hybridization assay procedures and conditions will vary depending on the application and are selected in accordance with the general binding methods known, including those referred to in, *e.g.,* Maniatis et al, Molecular Cloning: A Laboratory Manual (3rd ed. Cold Spring Harbor, N.Y., 2002), and Berger and Kimmel, Methods in Enzymology, Vol. 152 Guide to Molecular Cloning Techniques (Academic Press, Inc., San Diego, Calif., 1987,). Generally, the probe and sample are mixed under conditions that will permit specific nucleic acid hybridization, and specific hybridization of the probe to its respective target is then detected. Nucleic acid hybridization is adaptable to a variety of assay formats. One suitable format is the sandwich assay format, which is particularly adaptable to hybridization under non-denaturing conditions. A primary component of a sandwich-type assay is a solid support, which has adsorbed to it or covalently coupled to it immobilized nucleic acid probe that is unlabeled and complementary to one portion of the DNA sequence. Target nucleic acid is hybridized to the immobilized probe, and a second, labeled detection probe - which is complementary to a second and different region of the same DNA strand to which the immobilized, unlabeled nucleic acid probe is hybridized - is hybridized to the [target nucleic acid]: [immobilized probe] duplex to detect the target nucleic acid. Another exemplary format utilizes electrochemical detection of target nucleic acids hybridized to unlabeled detection probes immobilized on a suitable electrode surface as a signal transducer. *See, e.g.,* Drummond et al., Nat. Biotechnol. 21: 1192, 2003; Gooding, Electroanalysis 14: 1149, 2002; Wang, Anal. Chim. Acta 469:63, 2002; Cagnin et al., Sensors 9:3122, 2009; Katz and Willner, Electroanalysis 15:913, 2003; Daniels and Pourmand, Electroanalysis 19: 1239, 2007.

[0128] In certain embodiments of a method for detecting *Plasmodium* species comprising a hybridization assay, the hybridization assay utilizes one or more detection probe oligomers as described herein.

[0129] In some embodiments, a non-amplification-based assay for detection of *Plasmodium* sp. is a cleavage-based assay, in which a probe oligonucleotide containing a non-target-hybridizing flap region is cleaved in an overlap-dependent manner by a flap endonuclease to release a cleavage product that is then detected. Exemplary cleavage-based assay reagents are described in, *e.g.,* Lyamichev et al. (Nat. Biotechnol. 17:292-296, 1999), Ryan et al. (Mol. Diagn. 4: 135-144, 1999), and Allawi et al. (J. Clin. Microbiol. 44:3443-3447, 2006).

[0130] Appropriate conditions for flap endonuclease reactions are either known or can be readily determined using methods known in the art *(see, e.g.,* Kaiser et al., J. Biol. Chem. 274:2138-721394, 1999). Exemplary flap endonucleases that may be used in the method include *Thermus aquaticus* DNA polymerase I, *Thermus thermophilus* DNA polymerase I, mammalian FEN-1, *Archaeoglobus fulgidus* FEN-1, *Methanococcus jannaschii* FEN-1, *Pyrococcus fiiriosus* FEN-1, *Methanobacterium thermoautotrophicum* FEN-1, *Thermus thermophilus* FEN-1, CLEAVASE® (Hologic, Inc., Madison, WI), *S. cerevisiae* RTH1, S. *cerevisiae* RAD27, *Schizosaccharomyces pombe* rad2, bacteriophage T5 5' -3' exonuclease, *Pyrococcus horikoshii* FEN-1, human endonuclease 1, calf thymus 5'-3' exonuclease, including homologs thereof in eubacteria, eukaryotes, and archaea, such as members of the class II family of structure-specific enzymes, as well as enzymatically active mutants or variants thereof. Descriptions of flap endonucleases can be found in, for example,

Lyamichev et al., Science 260:778-783, 1993; Eis et al., Nat. Biotechnol. 19:673-676, 2001; Shen et al., Trends in Bio. Sci. 23: 171 -173, 1998; Kaiser et al., J. Biol. Chem. 274:21387-21394, 1999; Ma etal., J. Biol. Chem. 275:24693-24700, 2000; Allawi et al., J. Mol. Biol. 328:537-554, 2003; Sharma et al., J. Biol. Chem. 278:23487-23496, 2003; and Feng et al., Nat. Struct. Mol. Biol. 11 :450-456, 2004.

[0131] In certain variations, a cleavage-based assay detects an RNA target nucleic acid of *Plasmodium* sp., and the cleavage-based assay utilizes a flap endonuclease that is capable of cleaving and RNA:DNA linear duplex structure. In some alternative embodiments, a cleavage-based assay detects a DNA target nucleic acid *of Plasmodium* sp., and the cleavage-based assay utilizes a flap endonuclease that is capable of cleaving and DNA:DNA linear duplex structure. Exemplary flap endonucleases capable of cleaving RNA:DNA duplexes include polymerasedeficient 5' nucleases of the genus *Thermus* as well as certain CLEAVASE® enzymes (Hologic, Inc., Madison, WI) such as, for example, CLEAVASE® BN (BstX-NotI deletion of Taq polymerase, see US Patent No. 5,614,402), CLEAVASE® II ("AG" mutant of full length Taq polymerase, see US Patent No. 5,614, 402), CLEAVASE® VII (synthesis-deficient mutation of full length *Thermus thermophilus* polymerase), CLEAVASE® IX (polymerase deficient mutant of the Tth DNA polymerase), and CLEAVASE® XII (polymerase deficient chimeric polymerase constructed from fragments of taq DNA polymerase and Tth DNA polymerase). Exemplary flap endonucleases capable of cleaving DNA:DNA duplexes include the flap endonucleases indicated above, as well as CLEAVASE® 2.0 (*Archaeoglobus fulgidus* FEN-1), CLEAVASE® 2.1 (*Archaeoglobus fulgidus* FEN-1 with 6 histidines on the C-terminus), CLEAVASE® 3.0 (*Archaeoglobus veneficus* FEN-1), and CLEAVASE® 3.1 (*Archaeoglobus veneficus* FEN-1 with 6 histidines on the C-terminus).

[0132] In some embodiments, a cleavage-based assay detects an RNA target nucleic acid of *Plasmodium* sp., and the assay includes a step for synthesizing a DNA complement of an RNA target region, which cDNA strand is then hybridized to overlapping first and second probe oligonucleotides to form a linear duplex cleavage structure for cleavage by the flap endonuclease. Reaction conditions for synthesizing cDNA from an RNA template, using an RNA-dependent DNA polymerase (reverse transcriptase), are well-known in the art.

[0133] In certain embodiments utilizing a nucleic-acid-based detection assay, the method further includes purifying the *Plasmodium* sp. target nucleic acid from other components in the sample. 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 *Plasmodium* sp. nucleic acid and other sample components. In some embodiments, purification includes lysing a sample of cells such as, for example, blood cells *(e.g.,* red blood cells) and purifying any *Plasmodium* sp. target nucleic acid from the lysed cell sample. Exemplary lysis reagents and methods for used in accordance with the present invention are described in US Pat. No. 10,093,989 and PCT Pub. No. WO 2017/189746.

[0134] In some embodiments, a target nucleic acid of *Plasmodium* sp. is separated from other sample components by hybridizing the target nucleic acid to a capture probe oligomer. The capture probe oligomer comprises a target-hybridizing sequence configured to specifically or non-specifically hybridize to a target nucleic acid so as to form a [target nucleic acid]: [capture probe] complex that is separated from other sample components. Capture probes comprising target-hybridizing sequences suitable for non-specific capture of target nucleic acids are described in, *e.g.,* PCT Pub. No. WO 2008/016988. In some specific variations comprising target-hybridizing sequence(s) configured to specifically hybridize to a *Plasmodium* sp. target nucleic acid, a *Plasmodium* sp.-specific capture probe comprises a target-hybridizing sequence that is up to about 30 contiguous nucleotides in length and includes a sequence substantially corresponding to a sequence selected from SEQ ID NOs:11-15, 17, 19, and 20 *(e.g.,* a target-hybridizing sequence comprising or consisting of a sequence selected from SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof). In a preferred variation, the capture probe binds the [target nucleic acid]:[capture probe] complex to an immobilized probe to form a [target nucleic acid]:[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 sequence or moiety that binds 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.

[0135] In more specific embodiments, the capture probe oligomer includes a tail portion *(e.g.,* a 3' tail) that is not complementary to target nucleic acid 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.,* A10 to A40), more preferably about 14 to 33 nt (*e.g.,* A14 to A30 or T3A14 to T3A30), that bind to a complementary immobilized sequence (*e.g.*, poly-T) attached to a solid support, *e.g.*, a matrix or particle. In some such embodiments comprising target-hybridizing sequence(s) configured to specifically hybridize to *Plasmodium* sp. target nucleic acid, a *Plasmodium*

sp.-specific capture probe comprises or consists of a nucleotide sequence selected from SEQ ID NOs:1-5, 7, 9, and 10.

[0136] Target capture typically occurs in a solution phase mixture that contains one or more capture probe oligomers that hybridize to the target nucleic acid under hybridizing conditions, usually at a temperature higher than the Tm of the [tail sequence]:[immobilized probe sequence] duplex. For embodiments comprising a capture probe tail, the [target nucleic acid]:[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]: [target nucleic acid] 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 [target nucleic acid]:[capture probe]:[immobilized probe] complex may be suspended in a washing solution and retrieved from the washing solution, preferably by using magnetic attraction. In embodiments of the method comprising the use of an amplification-based detection assay, to limit the number of handling steps, a target nucleic acid may be amplified by simply mixing the target nucleic acid in the complex on the support with amplification oligomers and proceeding with amplification steps.

[0137] In accordance with the present disclosure, detecting the presence or absence of *Plasmodium* sp. may be performed separately *(e.g.,* in a separate reaction vessel), or performed together with another assay as a multiplex reaction system. Accordingly, in some embodiments, a method as described herein utilizes a multiplex reaction, where the reaction mix contains reagents for assaying multiple (*e.g.,* at least two, three, four, or more) different target sequences in parallel. In these cases, a reaction mix may contain multiple different target-specific oligonucleotides for performing the detection assay. For example, in a method utilizing an amplification-based detection assay, a multiplex reaction may contain multiple sets (*e.g.,* multiple pairs) of amplification oligomers (for example, multiple pairs of PCR primers or multiple pairs of TMA amplification oligomers (*e.g.*, for TMA, multiple pairs of promoter primer and non-promoter primer, or multiple pairs of promoter provider and non-promoter primer)). In other embodiments utilizing a cleavage-based detection assay, a multiplex reaction may contain multiple probe oligonucleotides having different flaps, multiple different overlapping probe oligonucleotides, and multiple different FRET cassettes for detecting the different flaps, once they are cleaved.

[0138] The oligomer combination described herein may be in the form of a reaction mixture or a kit comprising the oligomers. The reaction mixture or kit may further include a number of optional components such as, for example, capture probe nucleic acids or 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 *Plasmodium* sp. target nucleic acid may or may not be present. A kit comprising an oligomer combination for amplification of *Plasmodium* sp. may also 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). For an oligomer combination (*e.g.*, reaction mixture or kit) that includes a detection probe together with an amplification oligomer combination targeting a common target nucleic acid, selection of amplification oligomers and detection probe oligomers are linked by a common target region (*i.e.,* the combination will include a probe that binds to a sequence amplifiable by the amplification oligomer combination).

[0139] The compositions, methods, reaction mixtures, systems, kits and the like for detection of *Plasmodium* nucleic acids are further illustrated by the following non-limiting examples.

EXAMPLES

[0140] "Parasite Transport Solution" generally refers to a solution formulated to preserve a sample, and in some instances formulated to at least partially lyse one or more cell types in a sample. One exemplary parasite transport solution comprises 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. Another exemplary parasite transport solution comprises an aqueous solution of 100 mM TRIS, 30 mM magnesium chloride, and 6% (v/v) LLS, at pH 7.5. A further exemplary parasite transport solution comprises an aqueous solution of 14 mM sodium bicarbonate, 250 mM ammonium chloride, 5% (v/v) LLS, and 0.1 mM EDTA, at a pH of 7.4. Other formulations of parasite transport solutions may function equally well.

[0141] "Target Capture Reagent" generally refers to a solution containing a number of components that facilitate capture of a nucleic acid from a solution. One exemplary Target Capture Reagent comprises 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. Another exemplary Target Capture Reagent comprises 790 mM HEPES, 453 mM lithium hydroxide, 10% w/v LLS, 230 mM Succinic Acid, 0.03% w/v Foam Ban MS-575, and 0.0125% w/v of magnetic particles (1 micron SERA-MAC^TM MG-CM particles, GE Healthcare Lifesciences) with dT_{14} oligomers covalently bound. Other formulations of Target Capture Reagent may function equally as well.

[0142] "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.

[0143] "Probe Reagent" generally refers to a solution containing one or more labeled detection probes. One exemplary Probe Reagent is a solution made up of from about 75 to about 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. Another exemplary Probe Reagent is a solution made up of from about 75 to about 100 mM succinic acid, 3.5% (w/v) LLS, 75 mM lithium hydroxide, 15 mM aldrithiol-2, 1.0 M lithium chloride, 1 mM EDTA, and 3.0% (v/v) ethanol, at pH 4.1-4.3. Other formulations may perform equally as well.

[0144] "Amplification Reagent" generally refers to a concentrated mixture of reaction components to facilitate amplification reactions. An Amplification Reagent will comprise a number of different reagents at various concentrations depending on factors such as for example amplification type (PCR, TMA, etc.), target nucleic acids (GC content), and the like. One exemplary Amplification Reagent comprises 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. Another exemplary Amplification Reagent comprises 19.1 mM Trizma Base, 7.5mM Trizma Hydrochloride, 23.3 mM KCl, 21.5 mM MgCl2, 1 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 6.5 mM rATP, 4.0 mM rCTP, 4.0 mM UTP, 6.5 mM rGTP, 3.33% v/v glycerol, 0.05 mM Zinc Acetate, 6 ppm Pro Clin 300 preservative, at pH 8.25-8.45. 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 37C 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 37C using a DNA template containing a T7 promoter.

[0145] "Hybridization Reagent" generally refers to a solution made up of reagents having concentrations in the range of about: 75-100 mM succinic acid, 2%-3.5% (w/v) LLS, 75-100 mM lithium hydroxide, 14-16 mM aldrithiol-2, 1.0-1.2 M lithium chloride, 20-1000 mM EDTA, and 2.0-4.0% (v/v) ethanol, at pH 4-5 Other formulations for a Hybridization Reagent may function equally well.

[0146] "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.

[0147] "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.

Example 1

[0148] Primer screening was performed using Transcription-Mediated Amplification (TMA) on the manual Procleix Enhanced Semi-automated System (eSAS) using *Plasmodium falciparum in vitro* transcript (IVT) . An assay rack consisted of 10 rows of Ten-tube units (TTUs). Seventy five microliters (75 μL) of Amplification Reagent and 5 picomoles of each T7 promoter provider oligonucleotide and non-T7 primer oligonucleotide were added to the appropriate tubes on the rack such that each combination of amplification oligomers were tested with three replicates of *P. falciparum* IVT at 30 and 10 copies per reaction and two replicates of *B. microti* IVT at 1,000,000 copies per reaction, where applicable. *B. microti* was included in initial screening as a cross reactivity specimen due to the conserved regions between *Babesia* and *Plasmodium.* It is necessary to determine that amplification and detection systems are specific to *Plasmodium.* To achieve the target copies per reaction, 10 μL of *P. falciparum* IVT at 3 c/μL or 1 c/μL diluted in a buffer was spiked into the appropriate tubes, and 10 μL of *B. microti* IVT at 100,000 c/μL diluted in a buffer were spiked into the appropriate tubes. Various combinations of primers were tested. This set-up allows for 10 primer combinations to be tested per rack. Once the primer combinations and IVTs were spiked, 200 μL of oil was added to each tube and then the rack was covered with sealing cards and vortexed for a minimum of 20 seconds.

[0149] The rack was then incubated in a water bath at 60±1°C for 10±1 minutes followed by incubation in a 41.5±1°C water bath between 9 and 20 minutes. While the rack remained in the water bath, the sealing cards were removed and 25μL of commercially available Procleix Ultrio Plus enzyme reagent (Grifols Diagnostic Solutions Inc.) was added to each reaction tube and then covered again with sealing cards. The rack was gently shaken to mix and then covered again with sealing cards and incubated for another 60±5 minutes in the 41.5±1°C water bath.

[0150] After incubation completed, the rack was transferred to the hybridization protection assay (HPA) area where the sealing cards were removed. 100μL of Probe reagent consisting of an Acridinium-Ester (AE) labeled probe added at a total desired concentration of at least 2.5e6 Relative Light Units (RLU) per reaction to a Hybridization reagent. Probe

reagent was then added to the appropriate reaction tubes. The tubes were covered with sealing cards and the rack was vortexed for a minimum of 20 seconds after which the rack was incubated in a water bath at 61±2°C for 15±1 minutes.

[0151] The rack was removed from the water bath, the sealing cards removed, and 250 μL of commercially available Procleix Ultrio Plus Selection reagent (Grifols Diagnostic Solutions Inc.) was added to each tube. The tubes were covered with sealing cards and vortexed for a minimum of 20 seconds and then returned to the 61±2°C water bath and incubated for 10±1 minutes. After incubation, the rack was allowed to cool in a 23±4°C water bath for a minimum of 10 minutes.

[0152] For detection the TTUs are removed from the rack and loaded on to the automated Leader instrument for subsequent light off using commercially available Procleix Auto Detect 1 and 2 reagents (Grifols Diagnostic Solutions Inc.) and the results were exported for analysis of the signal in Relative Light Units (RLU).

[0153] AE-labeled probes screened in this example are shown in Table 4 below. Probes were screened with amplification oligomer pairs in four experimental groups: Group 1, Groups 2a and 2b, and Group 3.

Table 4.

Probe #	SEQ ID NO:	2MeAE linker site
1	159	7,8
3	159	9,10
4	151	6,7
5	151	7,8
6	151	8,9
7	150	8,9
8	155	7,8
12	160	13,14
14	147	11,12
15	157	10,11
16	156	10,11
20	158	6,7
21	158	7,8
22	158	10,11

[0154] *Group 1*. Probes 1, 3, 4-6, 12, and 14-16 were each tested with SEQ ID NO:59 (T7 promoter provider oligomer) and SEQ ID NO:30 (non-T7 oligomer). The results of this probe screen are shown in Table 5 below. Candidates showed no cross-reactivity with *B. microti* IVT at 1e6 copies per reaction. In addition, probes having the same nucleotide sequence but with different 2MeAE linker sites *(see, e.g.,* probes 1 and 3; *see also* probes 4, 5, and 6) performed well irrespective of their different labelling.

Table 5.

	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	B.mic 1e6 c/rxn	B.mic 1e6 c/rxn	B.mic 1e6c/c/rxn
Probe 1	1,222,972	1,157,299	1,201,553	927,342	1,093,944	98,711	919	985	871
Probe 3	1,179,477	1,192,980	1,155,140	1,130,568	854,803	1,206,816	1,515	1,640	1,708
Probe 5	816,162	815,694	774,947	708,438	745,288	519,080	1,562	1,253	1,322
Probe 4	965,152	1,019,364	1,043,712	767,525	957,437	905,300	1,203	1,565	1,276
Probe 6	1,179,590	1,182,350	1,136,391	1,034,114	1,098,623	856,584	2,211	3,484	2,473
Probe 12	1,801,146	1,739,366	1,606,426	698,553	1,610,398	1,515,342	1,540	1,278	2,523
Probe 14	1,017,636	997,356	1,015,960	951,505	830,243	909,076	870	865	1,470
Probe 15	1,116,536	1,203,192	1,038,531	1,093,438	958,900	891,744	1,174	1,019	1,803
Probe 16	182,425	195,626	73,184	141,723	47,725	52,841	4,155	2,440	2,629
NEG	**Probe 1**			**Probe 3**			**Probe 4**		
	1,108	1,046	1,310	1,935	1,749	1,784	1,319	1,489	1,388
	Probe 4			**Probe 6**			**Probe 12**		
	2,614	1,194	2,321	2,673	2,935	4,364	1,473	1,474	2,528
	Probe 14			**Probe 15**			**Probe 16**		
	1,3111	1,597,	978	1,359,	1,171	1,475	3,940	1,430	1,869

[0155] *Groups 2a and 2b.* In Group 2a, the following primer/probe combinations were tested: each of probes 8 and 20-22 paired with SEQ ID NO:59 and SEQ ID NO:33 (T7/NT7), each of probes 7 and 20-22 paired with SEQ ID NO:59 and SEQ ID NO:52 (T7/NT7), and each of probes 7 and 8 paired with SEQ ID NO:59 and SEQ ID NO:49 (T7/NT7). In Group 2b, the following primer/probe combinations were tested: each of probes 20-22 paired with SEQ ID NO:66 and SEQ ID NO:33 (T7/NT7), each of probes 7 and 20-22 paired with SEQ ID NO:66 and SEQ ID NO:52 (T7/NT7), and probe 7 paired with SEQ ID NO:66 and SEQ ID NO:49 (T7/NT7).

[0156] The results of this probe screen are shown in Tables 6 and 7 below. Systems with the non-T7 oligomer of SEQ ID NO:52 did not amplify *Plasmodium.* While systems with the T7/NT7 oligomer pair of SEQ ID NO:59/SEQ ID NO:33 paired with any of Probes 20-22 did not detect *Plasmodium,* these probes detect *Plasmodium* when used with the T7/NT7 oligomer pair of SEQ ID NO:66/SEQ ID NO:33.

Table 6.

T7/NT7 + Probe	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	B. mic 1e6 c/rxn	B.mic 1e6 c/rxn	NEG	NEG
59/49 +Probe 7	1,414,377	1,429,342	1,423,366	1,293,402	1,271,942	1,347,111	1,967	1,113	1,651	1,603
59/52 +Probe 7	15,998	92,089	4,310	676	1,465	3,151	1,473	3,105	793	1,315
59/49 +Probe 8	1,556,915	1,585,955	1,594,418	1,523,106	1,419,616	1,564,211	7,048	2,935	3,710	5,601
59/33 +Probe 8	1,026,164	1,188,939	1,511,173	1,022,388	1,030,495	421,293	2,804	2,963	4,303	6,879
59/33 +Probe 20	438,708	543,560	635,481	385,704	410,210	728,374	1,263	1,475	1,151	1,295
59/52	1,058	1,196	875	9,308	69,190	686	926	930	1,169	1,771
+Probe 20										
59/33 +Probe 21	1,131,775	1,206,597	957,441	1,558	260,318	653,321	1,217	3,177	1,160	2,281
59/52 +Probe 21	2,260	8,505	1,529	901	1,109	2,917	1,314	1,386	1,911	1,978
59/33 +Probe 22	475,040	486,044	474,661	342,995	713	427,613	1,257	729	585	1,143
59/52 +Probe 22	53,691	57,052	1,205	1,225	2,526	2,948	4,203	677	5,728	1,344

Table 7.

T7/NT7 + Probe	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	B. mic 1e6 c/rxn	B.mic 1e6 c/rxn	NEG	NEG
66/49 +Probe 7	1,697,508	1,654,623	1,702,775	1,632,642	1,670,622	1,710,421	4,352	6,899	1,718	780
66/52 +Probe 7	698,772	9,692	1,394,770	1,732	926	198,284	3,588	4,249	1,173	890
66/33 + Probe 20	2,085,094	1,917,183	1,977,048	2,166,352	1,898,001	1,529,882	1,294	1,645	2,378	1,368
66/52 + Probe 20	5,387	6,054	1,395,984	78,381	13,600	1,825	2,979	5,688	5,636	2,165
66/33 + Probe 21	2,787,188	2,641,943	2,691,394	2,189,645	1,834,205	2,528,971	5,627	3,547	3,817	4,417
66/52 + Probe 21	103,016	1,944,005	8,745	2,694,902	1,167,241	5,856	3,229	4,437	6,515	2,704
66/33 + Probe 22	1,976,930	2,048,269	2,206,921	2,248,244	1,576,131	1,897,000	1,619	5,010	2,252	2,978
66/52 + Probe 22	314,430	1,222,503	36,814	1,596	60,021	110,944	1,831	2,930	2,213	1,337

[0157] *Group 3.* Re-designs of the non-T7 primer of SEQ ID NO:33 were tested. Each of probes 7 and 8 were paired with each of SEQ ID NO:66/SEQ ID NO:25 and SEQ ID NO 66/SEQ ID NO:35 (T7/NT7). In addition, probe 20 was paired with SEQ ID NO:66/SEQ ID NO:33 (T7/NT7).

[0158] The results of this probe screen are shown in Table 8 below. The T7/NT7 oligomer pair of SEQ ID NO:66/SEQ ID NO:33 performed well with probe 20. Redesigns for the non-T7 oligomer of SEQ ID NO:33 (SEQ ID NO:25 and SEQ ID NO:35) induced false positives with probes 7 and 8.

Table 8.

T7/NT7 + Probe	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 30 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	P. fal 10 c/rxn	NEG	NEG	NEG	NEG
66/33 +Probe 20	2,259,738	2,251,254	2,259,806	2,193,185	2,229,852	2,305,634	**10,987**	2,278	2,517	833
66/25 +Probe 7	1,639,345	1,634,590	1,094,963	1,577,109	1,447,969	1,443,799	**659,001**	**907,064**	**955,394**	**106,595**
69/25 +Probe 7	1,604,438	1,518,505	1,606,930	1,473,808	1,431,398	1,542,777	**873,665**	**963,803**	**849,156**	**705,831**
66/35 +Probe 7	1,590,743	1,608,076	1,615,693	1,595,727	1,351,299	1,582,092	**759,553**	**622,876**	**495,724**	**743,057**
69/35 +Probe 7	1,669,062	1,684,886	1,610,911	1,604,953	1,279,697	1,584,979	**816,245**	**874,237**	**467,269**	**1,302,044**
66/25 +Probe 8	1,690,077	1,673,850	1,597,686	1,601,896	1,359,389	1,572,372	**1,172,325**	**693,937**	**286,919**	**536,015**
69/25 +Probe 8	1,607,570	1,637,678	1,622,369	1,613,782	1,538,278	1,613,946	**93,383**	**1,029,827**	**1,213,750**	**1,192,320**
66/35 +Probe 8	1,622,258	1,609,624	1,572,273	1,600,610	1,570,232	1,611,927	**1,235,255**	**947,640**	**841,628**	**1,143,229**
69/35 +Probe 8	1,623,329	1,679,140	1,634,462	1,628,953	1,509,017	1,583,660	**604,251**	**705,198**	**1,259,683**	**966,102**

Example 2

[0159] This example describes materials and methods for screening of candidate amplification systems using TMA on the full automated Procleix Panther system (Grifols Diagnostic Solutions Inc.).

[0160] Specimens included *P. falciparum, P. knowlesi, P. malariae, P. ovale,* and/or *P. vivax* IVT diluted in buffer. Specimens may also include *B. microti* IVT as a cross-reactivity specimen. It is necessary to determine that amplification and detection systems are specific to *Plasmodium.* An assay calibrator comprising a *P. falciparum* IVT panel at 500 c/mL was included to determine the analyte cutoff for the run. The assay software uses the analyte cutoff to determine if samples are reactive or non-reactive. Samples with a signal to cutoff ratio of ≥ 1 are considered reactive, while those < 1 are non-reactive. Assay Reagents used included the following: a Target Capture Reagent (TCR) comprising of at least one target capture oligomer (TCO); an Amplification reagent comprising at least one T7 promoter provider and at least one non-T7 primer; a Probe reagent consisting of at least one AE labeled probe; Ultrio Plus Enzyme reagent; and Selection reagent.

Example 3: ***Plasmodium*** **Target Capture Probe Screening**

[0161] Candidate target capture probes (TCOs) were screened with samples containing *P. falciparum* IVT at 500 c/mL prepared in 3 mL Parasite Transport Medium (PTM; 100 mM TRIS, 30 mM magnesium chloride, and 6% (v/v) LLS, at pH 7.5) with 1 mL whole blood. TMA reactions were performed on the fully automated Procleix Panther system substantially as described in Example 2. TCOs of SEQ ID NOs. 1-5, 7, 9, and 10 were tested in reactions using T7 oligomers of SEQ ID NO:66 and SEQ ID NO:69 (5 pmol/rxn each), non-T7 oligomers of SEQ ID NO:21 and SEQ ID NO:30 (5 pmol/rxn each), and detection probes of SEQ ID NO:148 and SEQ ID NO: 152 (1.9e6 RLU/rxn each).

[0162] Results are shown in Tables 9 and 10 below. Assay performance was best with TCOs SEQ ID NO:9 and SEQ ID NO: 10. SEQ ID NO: 3 was not optimal in this assay.

Table 9.

TCO	Mean Total RLU*
SEQ ID NO: 3	663,034
SEQ ID NO:1	873,914
SEQ ID NO:9	1,474,284
SEQ ID NO:2	985,039
SEQ ID NO:10	1,455,387
SEQ ID NO:7	793,219
SEQ ID NO:5	951,504
SEQ ID NO:4	952,023
* N=10 for all TCOs except SEQ ID NOT (N=20)	

Table 10.

TCO	%CV Total RLU*
SEQ ID NO: 3	19.2
SEQ ID NO:1	19.7
SEQ ID NO:9	2.6
SEQ ID NO:2	11.2
SEQ ID NO:10	3.6
SEQ ID NO:7	28.1
SEQ ID NO:5	14.4

(continued)

TCO	%CV Total RLU*
SEQ ID NO:4	14.4
* N=10 for all TCOs except SEQ ID NOT (N=20)	

Example 4: Analytical Sensitivity - LoD of RNA Copies/mL

[0163] Limit of detection (LoD) for a candidate amplification system was assessed by probit analysis using *in vitro* synthesized transcripts for *P. falciparum, P. knowlesi, P. malariae, P. ovale,* and *P. vivax.* TMA reactions were performed on the fully automated Procleix Panther system substantially as described in Example 2. The IVT for each species was serially diluted in buffer to 100, 30, 10, 3, 1 and 0 copies/mL and tested in 32 replicates for each level per species. Specimens were tested using TMA on the fully automated Procleix Panther system (Grifols Diagnostic Solutions Inc.). An assay calibrator comprising a *P. falciparum* IVT panel at 500 c/mL was included to determine the analyte cutoff for the run. The assay software uses the analyte cutoff to determine if samples are reactive or non-reactive. Samples with a signal to cutoff ratio of ≥ 1 are considered reactive, while those < 1 are nonreactive. For this assay, the following oligomers were used: T7 oligomers of SEQ ID NO:66 and SEQ ID NO:69 (5 pmol/rxn each), non-T7 oligomers of SEQ ID NO:21 and SEQ ID NO:30 (5 pmol/rxn each), detection probes of SEQ ID NO:148 and SEQ ID NO:152 (1.9e6 RLU/rxn each), and TCOs of SEQ ID NO:9 and SEQ ID NO:10.

[0164] Results are shown in Table 11 below. Similar LoD values were observed for the five species tested. 95% LoD ranged from 9.4 to 14.8 copies/mL.

Table 11.

In vitro transcript (N=32)	50% LoD in Copies/mL (Fiducial Limits)	95% LoD Copies/mL (Fiducial Limits)
P. falciparum	2.1 (1.3 - 2.9)	9.4 (6.5 - 17.3)
P. knowlesi	3.3 (2.2 - 4.5)	14.8 (10.2 - 27.3)
P. malariae	3.3 (2.2 - 4.4)	13.1 (9.1 - 24.4)
P. ovale	2.5 (1.5 - 3.5)	13.6 (9.0 - 27.8)
P. vivax	4.5 (3.4 - 5.7)	11.6 (8.9 - 17.7)

Example 5: Analytical Sensitivity LoD of RNA Parasites/mL

[0165] Limit of detection (LoD) for a candidate amplification system was assessed by probit analysis using cultured parasite-infected cells. Specifically, conditions were tested using a lysed negative whole blood specimen and diluted cultured *P. falciparum*-infected erythrocytes. The cultured sample was received with a known percent parasitemia and RBC count determined by Fluorescence-Activated Cell Sorting (FACS) to estimate the concentration (parasite per mL value). Based upon the estimated parasite per mL value, the sample was diluted in normal negative human whole blood to an estimated 6, 4, 2, 1, and 0.5 parasites per mL. The diluted *Plasmodium*-infected whole blood was lysed at a ratio of 0.9 mL of whole blood in 2.7mL of Parasite Transport Medium (PTM; 100 mM TRIS, 30 mM magnesium chloride, and 6% (v/v) LLS, at pH 7.5). Specimens were tested using TMA on the fully automated Procleix Panther system (Grifols Diagnostic Solutions Inc.). An assay calibrator comprising a *P. falciparum* IVT panel at 500 c/mL was included to determine the analyte cutoff for the run. The assay software uses the analyte cutoff to determine if samples are reactive or non-reactive. Samples with a signal to cutoff ratio of ≥ 1 are considered reactive, while those < 1 are non-reactive. Assay reagents used included the following: a Target Capture Reagent (TCR) comprising TCOs of SEQ ID NO:9 and SEQ ID NO:10; an Amplification reagent comprising T7 oligomers of SEQ ID NO:66 and SEQ ID NO:69 (5 pmol/rxn each), non-T7 oligomers of SEQ ID NO:21 and SEQ ID NO:30 (5 pmol/rxn each); a Probe reagent comprising AE-labeled detection probes of SEQ ID NO:148 and SEQ ID NO:152 (1.9e6 RLU/rxn each); Ultrio Plus Enzyme reagent; and Selection reagent.

[0166] Results are shown in Table 12 below. 95% LoD was 2.14 parasites/mL. 480 replicates each of internal control buffer, PTM, and negative lysate were evaluated with no false positives (0/1,440).

Table 12.

	50% LoD in Parasites/mL (Fiducial Limits)	95% LoD in Parasites/mL (Fiducial Limits)
P. falciparum	0.35 (0.14-0.56)	2.14 (1.38-5.17)

Example 6: Interference and Cross-reactivity with *Babesia*

[0167] Interference and cross-reactivity with *Babesia microti* (a homologous protozoan) was assessed using *in vitro* synthesized transcripts. Testing was performed in TMA reactions performed on the fully automated Procleix Panther system substantially as described in Example 2, with *P. falciparum* IVT dilutions between 100-1 c/mL with and without the addition of *B. microti* IVT at 1e6 c/mL. The following oligomers were used: T7 oligomers of SEQ ID NO:71 and SEQ ID NO:72 (5 pmol/rxn each), non-T7 oligomers of SEQ ID NO:34 and SEQ ID NO:53 (5 pmol/rxn each), AE-labeled detection probes of SEQ ID NO: 148 and SEQ ID NO: 157 (1.9e6 RLU/rxn each), and TCOs of SEQ ID NO:9 and SEQ ID NO: 10.

[0168] Results are shown in Tables 13 and 14 below. Comparable reactivity and analyte RLU was observe for *P. falciparum* IVT with and without the presence of *B. microti* IVT.

Table 13.

		***P. falciparum* (copies)**					
		100	**30**	**10**	**3**	**1**	**0**
% Reactive (N=24)	**Control**	100	100	100	58	17	0
	Presence of *B. microti*	100	100	96	58	17	0

Table 14.

		***P. falciparum* (copies)**					
		100	**30**	**10**	**3**	**1**	**0**
Mean RLU (N=24)	**Control**	2,043,991	2,031,427	1,993,527	1,992,755	2,001,796	2,518
	Presence of *B. microti*	2,055,344	2,029,613	1,925,711	1,777,978	1,815,085	855

Example 7: Evaluation of Redundant Probes

[0169] Redundant probe combinations were assessed using *in vitro* synthesized transcripts. Testing was performed in TMA reactions performed on the fully automated Procleix Panther system substantially as described in Example 2. The following oligomers were used: (i) T7 oligomers of SEQ ID NO:71 and SEQ ID NO:72 (5 pmol/rxn each), (ii) non-T7 oligomers of SEQ ID NO:34 and SEQ ID NO:53 (5 pmol/rxn each), (iii) AE-labeled detection probes of SEQ ID NO:148 (4,5 2MeAE linker) and SEQ ID NO:157 (1.27e6 RLU/rxn each) with either SEQ ID NO:148 (5,6 2MeAE linker) or SEQ ID NO:148 (6,7 2MeAE linker), and (iv) TCOs of SEQ ID NO:9 and SEQ ID NO:10.

[0170] Results are shown in Table 15 below. "C1" refers to the probe combination containing SEQ ID NO:148 (5,6 2MeAE linker); "C2" refers to the probe combination containing SEQ ID NO: 148 (6,7 2MeAE linker). Comparable reactivity was observed for all *Plasmodium* species at 30 and 10 c/mL with the redundant probe combinations. Consistent with results in Example 1, probes having the same nucleotide sequence (SEQ ID NO:148) but with different 2MeAE linker sites performed well irrespective of their different labelling.

Table 15.

		***P. falciparum* (copies)**		***P. knowlesi* (copies)**		***P. malariae* (copies)**		***P. ovale* (copies)**		***P. vivax* (copies)**	
		30	**10**	**30**	**10**	**30**	**10**	**30**	**10**	**30**	**10**
% Reactive (N=8)	**C1**	100	88	100	100	100	100	100	75	100	100
	C2	100	75	100	88	100	100	100	88	100	100

Example 8: Analytical Sensitivity - LoD of RNA Parasites/mL

[0171] Limit of detection (LoD) for a candidate amplification system was assessed by probit analysis using cultured *P. falciparum*-infected erythrocytes. Testing was performed in TMA reactions performed on the fully automated Procleix Panther system substantially as described in Example 5. The following oligomers were used: (i) T7 oligomers of SEQ ID NO:71 and SEQ ID NO:72 (5 pmol/rxn each), (ii) non-T7 oligomers of SEQ ID NO:34 and SEQ ID NO:53 (5 pmol/rxn each), (iii) AE-labeled detection probes of SEQ ID NO:148 (4,5 2MeAE linker), SEQ ID NO:157, and SEQ ID NO:148 (5,6 2MeAE linker) (1.17e6, 7.92e5, and 2.03e6 RLU/rxn, respectively), and (iv) TCOs of SEQ ID NO:9 and SEQ ID NO: 10.

[0172] Results are shown in Table 16 below. 95% LoD was 2.31 parasites/mL. There were no false positives in the negative specimens tested. These results are comparable to the system evaluated in Example 5 where the 95% LoD was 2.14 parasites/mL.

Table 16.

	50% LoD in Parasites/mL (Fiducial Limits)	95% LoD in Parasites/mL (Fiducial Limits)
P. falciparum	0.70 (0.33-0.97)	2.31 (1.61-5.82)

Example 9

[0173] Primer screening was performed using procedures for a manual Biphasic Real-Time TMA Assay. For the target capture step, 400 μL of Target Capture Reagent (TCR) comprising at least 1 Target Capture Oligo (TCO) and 1 T7 promoter provider was added to a 2mL Deep Well 96-well plate (Thermo Scientific Cat. No. 95040450), followed by 500 μL of specimen. Specimens consisted of *Plasmodium* species *in vitro* transcript (IVT) diluted in buffer for *Plasmodium* detection and *Babesia* species IVT diluted in buffer for *Babesia* detection. Specimens may also include *B. microti* IVT or *P. falciparum* IVT as a cross-reactivity specimen for opposing detection systems due to the conserved regions between *Babesia* and *Plasmodium.* It is necessary to determine that amplification and detection systems are specific to the analyte system. The plate was covered with a sealing card and loaded on to a Torrey Pines plate incubator and covered with the lid. Incubation steps for the Torrey Pines incubator included 7 minutes at 80°C followed by 17 minutes at 62°C and between 15 to 25 minutes at 25°C, respectively.

[0174] After target capture incubation steps, the plate was loaded with a deep well comb tip (Thermo Scientific Cat. No. 97002534) and placed on to a Kingfisher 96 instrument (Thermo Scientific Type 710 REF 5400500) fitted with deep well magnets. The Kingfisher instrument was additionally loaded with a wash plate consisting of a 2mL 96-well plate (Nunc Deep Well plate Cat. No. 278752) prepared with 500 μL of commercially available Procleix Wash Buffer reagent (Grifols Diagnostic Solutions Inc.) and a second wash plate consisting of a 200μL 96-well plate (Thermo Scientific Cat. No. 97002540) containing 200 μL of the wash buffer. For the deep well wash steps, the plate containing the hybridized TCR-sample mixture was mixed for 5 minutes before collecting magnetic beads for 20 counts and eluting for 20 seconds to the plate containing 500 μL of wash buffer. The 500 μL wash plate was mixed for 1 minute before collecting magnetic beads for 10 counts and eluting for 20 seconds to the wash plate containing 200 μL of wash buffer.

[0175] The second wash plate containing the mixture of hybridized magnetic beads and wash buffer was removed from the Kingfisher instrument, loaded with a small PCR tip comb (Thermo Scientific 97002514) and transferred to a second Kingfisher 96 instrument (Thermo Scientific Type 710 REF 5400500) fitted with PCR magnets. The Kingfisher instrument was additionally loaded with a 96-well PCR plate (Axygen Cat. No. PCR-96-HS-C) containing 30 μL of Amplification Reagent (without Phenol Red) containing at least 1 non-T7 primer (amplification plate). To transfer the hybridized magnetic beads to the amplification plate the wash plate was mixed for 5 minutes before collecting magnetic beads for 30 counts and eluting for 30 seconds to the amplification plate. The wash plate was mixed again for 1 minute before collecting magnetic beads for 30 counts and eluting for 30 seconds to the amplification plate to complete the transfer.

[0176] The amplification plate was covered with a sealing card and loaded on to a Stratagene instrument (Mx3005P Multiplex Quantitative PCR System) to incubate for 5 minutes at 43°C. The plate was transferred to a heat block set to 42°C and uncovered to add 10 μL of commercially available Ultrio Plus Enzyme reagent (Grifols Diagnostic Solutions Inc.) and re-covered with a sealing card. The plate was mixed on the heat block for 1 minute at 1400 RMP and reloaded on the Stratagene instrument to incubate for 5 minutes at 43°C. The plate was transferred again to the heat block and uncovered to add 15 μL of Promoter reagent (Amplification Reagent without Phenol Red) containing a mixture of at least 1 T7 promoter provider and at least 1 Fluorescent labeled molecular Torch or Beacon (5'-Hexochloro-Fluorescein (HEX) for *Plasmodium* or 5'-Fluorescein (FAM) for *Babesia*) and sealed with a clear adhesive plate cover. The plate was mixed on the heat block for 1 minute at 1400 RMP and reloaded on the Stratagene for the read protocol. The read protocol consisted of incubation at 43°C and read every 30 seconds for cycles of 120 or 150.

[0177] The raw data exported from the Stratagene instrument was analyzed using an inhouse software tool. Fluorescent curves were analyzed using a threshold of 1,000 Relative Fluorescent Units (RFU). The time for specimens to meet or exceed the threshold (TTime) was determined by the software. Specimens with a TTime were considered Reactive for *Plasmodium* in the HEX channel or *Babesia* in the FAM channel. Lower TTimes indicated better performance of the tested systems. Specimens with no TTime, or under the threshold, were considered non-reactive.

[0178] In this example, combinations of molecular torch or beacon probes and amplification oligomer pairs were tested in three groups (Groups 1, 2, and 3). Tested oligomer combinations are shown in Table 17 below.

Table 17. Combinations of Amplification Oligomers and Torch Probes.

T7 Primer	Non-T7 Primer	Probe (Torch/Beacon)	Group
SEQ ID NO:181	SEQ ID NO:183	SEQ ID NO:104 (torch)	Group 1
SEQ ID NO:181	SEQ ID NO:183	SEQ ID NO:105 (torch)	
SEQ ID NO:181	SEQ ID NO:183	SEQ ID NO:106 (torch)	
SEQ ID NO:181	SEQ ID NO:183	SEQ ID NO:121 (torch)	
SEQ ID NO:181	SEQ ID NO:183	SEQ ID NO:107 (beacon)	
SEQ ID NO:181	SEQ ID NO:183	SEQ ID NO:108 (beacon)	
SEQ ID NO:181	SEQ ID NO:184	SEQ ID NO:104 (torch)	Group 2
SEQ ID NO:59	SEQ ID NO:51	SEQ ID NO:123 (torch)	Group 3
SEQ ID NO:59	SEQ ID NO:31	SEQ ID NO:123 (torch)	
SEQ ID NO:59	SEQ ID NO:50	SEQ ID NO:123 (torch)	

[0179] *Group 1 Results.* Probes of SEQ ID NOs: 104-107 and 121 successfully detected *Plasmodium* with fluorescent curves generating average TTimes of less than or equal to 30.69 minutes at 10 c/mL. No fluorescent curves with TTimes were generated for the probe of SEQ ID NO: 108 concluding it was unable to detect *Plasmodium.*

[0180] *Group 2 Results.* Amplification oligomers of SEQ ID NOs:181 and 184 in combination with the torch probe of SEQ ID NO:104 successfully detected *Plasmodium,* demonstrated by fluorescent curves generating TTimes of less than or equal to 20.89 minutes at 10 c/mL, and showed no cross-reactivity with *Babesia* IVT, demonstrated by the absence of a fluorescent curve with a TTime.

[0181] *Group 2 Results.* Non-T7 amplification oligomers of SEQ ID NOs:51, 31, and 50 each in combination with the T7 amplification oligomer of SEQ ID NO:59 and the torch probe of SEQ ID NO:123 successfully detected *Plasmodium* as demonstrated by fluorescent curves generating TTimes of less than or equal to 22.82 minutes.

Example 10

[0182] A candidate amplification system was tested using Real-time TMA on the fully automated Panther system installed with Real-time Fluorometers (Hologic Inc.). Specimens consisted of *Plasmodium* species IVT diluted in buffer for *Plasmodium* detection and *Babesia* species IVT diluted in buffer for *Babesia* detection. Specimens may also include *B. microti* IVT or *P. falciparum* IVT as a cross reactivity specimen. It is necessary to determine that amplification and detection systems are specific to the analyte system. Assay Reagents used included the following: a TCR comprising of at least one TCO and one T7 promoter provider; an Amplification Reagent comprising of at least one non-T7 primer; a Promoter reagent (Amplification Reagent without Phenol Red) consisting of at least one T7 promoter provider and at least one Fluorescent labeled molecular Torch or Beacon (5'-Hexochloro-Fluorescein (HEX) for Plasmodium or 5'-Fluorescein (FAM) for *Babesia*); and Ultrio Plus Enzyme reagent.

[0183] For analysis of the raw data exported from the Panther system, the Panther RT-Dev Tool (Hologic Inc.) was used. Fluorescent curves were analyzed using a threshold of 1,000 Relative Fluorescent Units (RFU). The time for specimens to meet or exceed the threshold (TTime) was determined by the software. Specimens with a TTime were considered Reactive for *Plasmodium* in the HEX channel or *Babesia* in the FAM channel. Lower TTimes indicated better performance of the tested systems. Specimens with no TTime, or under the threshold, were considered non-reactive.

[0184] The oligomers tested in this example were a T7 oligomer of SEQ ID NO:59, a non-T7 oligomer of SEQ ID NO:30, and a torch probe of SEQ ID NO: 123. Results demonstrated detection of 8 out of 8 replicates tested at 30 c/mL of *Plasmodium* IVT and 3 out of 8 replicates tested at 10 c/mL. Fluorescent curves generated TTimes of less than or equal to 33.18 minutes for the detected replicates at 10 c/mL.

Example 11: Detection of ***Plasmodium*** **in Clinical Specimens**

[0185] Candidate amplification systems were tested using Real-time TMA on the fully automated Panther system installed with Real-time Fluorometers (Hologic Inc.). Specimens consisted of positive controls for *B. microti* and *P. falciparum* IVT diluted in buffer to 300 c/mL and a negative control consisting of negative buffer. Clinical specimens consisted of *P. falciparum* and *P. ovale* whole blood and plasma specimens. Whole blood specimens were prepared by manual addition of 100 μL to 3 mL of a lysis buffer (14 mM sodium bicarbonate, 250 mM ammonium chloride, 5% (v/v) LLS, and 0.1 mM EDTA, at a pH of 7.4). Plasma specimens were prepared by manual addition of 100 μL to 3 mL of processed human plasma. Specimens were tested on the Panther system. Assay Reagents used included the following: a TCR comprising of at least one TCO and one T7 promoter provider; an Amplification Reagent comprising of at least one non-T7 primer; a Promoter reagent (Amplification Reagent without Phenol Red) consisting of at least one T7 promoter provider and at least one Fluorescent labeled molecular Torch or Beacon (5'-Hexochloro-Fluorescein (HEX) for *Plasmodium* or 5'-Fluorescein (FAM) for *Babesia*); and Enzyme reagent.

[0186] For analysis of the raw data exported from the Panther system, the Panther RT-Dev Tool (Hologic Inc.) was used. Fluorescent curves were analyzed using a threshold of 1,000 Relative Fluorescent Units (RFU). The time for specimens to meet or exceed the threshold (TTime) was determined by the software. Specimens with a TTime were considered Reactive for *Plasmodium* in the HEX channel or *Babesia* in the FAM channel Lower TTimes indicated better performance of the tested systems. Specimens with no TTime, or under the threshold, were considered Nonreactive.

[0187] The oligomers tested in this example were a T7 oligomer of SEQ ID NO:59, a non-T7 oligomer of SEQ ID NO:30, and a torch probe of SEQ ID NO:123. Results are shown in Table 18 below. Real-time TMA results with the tested oligomers showed 100% concordance with PCR results. Based on the calibration curve generated using *in vitro Plasmodium falciparum* infected erythrocytes, the tested sample have between 7.14E6 and 2.14E8 parasites/mL in whole blood. No cross reactivity was observed with *Babesia.*

Table 18. Detection of Plasmodium positive Clinical Specimens.

		Whole Blood		Plasma	Whole Blood	Plasma	
Sample	**Organism**	**PCR Result (Wadsworth)**	**Real-time TMA #reactive/ #tested**	**Real-time TMA #reactive/ #tested**	**Est. # par/mL**	**Est. # par/mL**	**Relative amounts of parasites (WB/ plasma)**
1	*P. fal*	Positive	4/4	4/4	2.14E+08	3.18E+02	672,841
2	*P. fal*	Positive	4/4	4/4	1.49E+08	2.63E+02	566,724
3	*P. fal*	Positive	4/4	4/4	1.11E+08	2.55E+03	43,685
4	*P. fal*	Positive	4/4	4/4	7.28E+07	4.60E+02	158,340
5	*P. fal*	Positive	4/4	4/4	5.49E+07	1.08E+02	508,948
6	*P. fal*	Positive	4/4	4/4	3.07E+07	1.96E+01	1,568,437
7	*P. fal*	Positive	4/4	4/4	2.27E+07	2.22E+01	1,026,604
8	*P. fal*	Positive	4/4	4/4	1.09E+07	2.38E+01	457,997
9	*P. ova*	Positive	4/4	4/4	1.05E+07	3.21E+04	329
10	*P. fal*	Positive	4/4	4/4	7.14E+06	1.74E+01	411,025

Example 12

[0188] Candidate amplification systems were tested using Real-time TMA on the fully automated Panther system installed with Real-time Fluorometers (Hologic Inc.). Specimens consisted of 5 strains of *Plasmodium* infected erythrocytes: US 05 F Benin I, US 05 F Santa Lucia, US 08 F Nigeria XII, US05 F FC27/A3, and US 05 F PH1. Infected erythrocytes were provided with an estimated parasite/mL value. Each strain was serially diluted in normal negative human whole blood to estimated values of 10 parasites/mL. The diluted *Plasmodium*-infected whole blood was manually lysed at a ratio of 1 mL of whole blood in 3 mL of a lysis buffer (14 mM sodium bicarbonate, 250 mM ammonium chloride, 5% (v/v) LLS, and 0.1 mM EDTA, at a pH of 7.4). Specimens were tested on the Panther system. Assay Reagents used

included the following a TCR comprising of at least one TCO and one T7 promoter provider; an Amplification Reagent comprising of at least one non-T7 primer; a Promoter reagent (Amplification Reagent without Phenol Red) consisting of at least one T7 promoter provider and at least one Fluorescent labeled molecular Torch or Beacon (5'-Hexochloro-Fluorescein (HEX) for Plasmodium or 5'-Fluorescein (FAM) for *Babesia*); and Enzyme reagent.

[0189] For analysis of the raw data exported from the Panther system, the Panther RT-Dev Tool (Hologic Inc.) was used. Fluorescent curves were analyzed using a threshold of 1,000 Relative Fluorescent Units (RFU). The time for specimens to meet or exceed the threshold (TTime) was determined by the software. Specimens with a TTime were considered Reactive for *Plasmodium* in the HEX channel or *Babesia* in the FAM channel. Lower TTimes indicated better performance of the tested systems. Specimens with no TTime, or under the threshold, were considered Nonreactive.

[0190] The oligomers tested in this example were a T7 oligomer of SEQ ID NO:59, a non-T7 oligomer of SEQ ID NO:30, and a torch probe of SEQ ID NO:123. Results demonstrated that all 5 strains of *Plasmodium* infected erythrocytes were detected in 6 out of 6 replicates tested at 10 parasites/mL with fluorescent curves generating TTimes ranging from 14.29 to 17.17 minutes.

Table 19. Exemplary Sequences.

SEQ ID NO:	Sequence (5' to 3')	Comments
1	GGAUUGGGUAAUUUGCGCGCCCTTTAAAAAAAAAAAAAAA AAAAAAAAAAAAAAA	Target capture oligomer
2	CAAGAAAGAGCUAUCAAUCUGUCAAUCCTTTAAAAAAAAA AAAAAAAAAAAAAAAAAAAAA	Target capture oligomer
3	CCCGUGUUGAGUCAAAUUAAGCCGCATTTAAAAAAAAAAA AAAAAAAAAAAAAAAAAAA	Target capture oligomer
4	GGGUAAUUUGCGCGCCUGCUGCTTTAAAAAAAAAAAAAAA AAAAAAAAAAAAAAA	Target capture oligomer
5	UUUCUCAGGCUCCCUCUCCGGAAUCGTTTAAAAAAAAAAA AAAAAAAAAAAAAAAAAAA	Target capture oligomer
6	ACAUCUGAAUACGAAUGUCCCCAATTTAAAAAAAAAAAAA AAAAAAAAAAAAAAAAA	Target capture oligomer
7	CUAGUCGGCAUAGUUUAUGGUUATTTAAAAAAAAAAAAA AAAAAAAAAAAAAAAA	Target capture oligomer
8	AAAAACGGCCAUGCAUCACCAUCCTTTAAAAAAAAAAAAA AAAAAAAAAAAAAAAAA	Target capture oligomer
9	UAGGCCAAUACCCUACCGUCCTTTAAAAAAAAAAAAAAA AAAAAAAAAAAAAA	Target capture oligomer
10	AAAGACUUUGAUUUCUCUCAAGGTTTAAAAAAAAAAAAA AAAAAAAAAAAAAAAA	Target capture oligomer
11	GGAUUGGGUAAUUUGCGCGCCC	THS of SEQ ID NO: 1
12	CAAGAAAGAGCUAUCAAUCUGUCAAUCC	THS of SEQ ID NO:2
13	CCCGUGUUGAGUCAAAUUAAGCCGCA	THS of SEQ ID NO:3
14	GGGUAAUUUGCGCGCCUGCUGC	THS of SEQ ID NO:4
15	UUUCUCAGGCUCCCUCUCCGGAAUCG	THS of SEQ ID NO:5
16	ACAUCUGAAUACGAAUGUCCCCAA	THS of SEQ ID NO:6
17	CUAGUCGGCAUAGUUUAUGGUUA	THS of SEQ ID NO:7
18	AAAAACGGCCAUGCAUCACCAUCC	THS of SEQ ID NO:8
19	UAGGCCAAUACCCUACCGUCC	THS of SEQ ID NO:9

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
20	AAAGACUUUGAUUUCUCUCAAGG	THS of SEQ ID NO: 10
21	AATACTACAGCATGG	Non-T7 amp oligo
22	GGAAGGCAGCAGGCGCGTA	Non-T7 amp oligo
23	AATACTACAGCATGGA	Non-T7 amp oligo
24	AATACTACAGCATGGAA	Non-T7 amp oligo
25	ATACTACAGCATGGAATA	Non-T7 amp oligo
26	ATTCAGATGTCAGAGGTGA	Non-T7 amp oligo
27	GTATTCAGATGTCAGAGGTGA	Non-T7 amp oligo
28	GTTACGATTAATAGGAGT	Non-T7 amp oligo
29	GTTACGATTAATAGGAGTA	Non-T7 amp oligo
30	GTTACGATTAATAGGAGTAG	Non-T7 amp oligo
31	GTTACGATTAATAGGAGTAGC	Non-T7 amp oligo
32	AATACTACAGCATGGAAT	Non-T7 amp oligo
33	AATACTACAGCATGGAATA	Non-T7 amp oligo
34	TACGATTAATAGGAGT	Non-T7 amp oligo
35	TACTACAGCATGGAATA	Non-T7 amp oligo
36	TATTCAGATGTCAGAGGTGA	Non-T7 amp oligo
37	TCAGTNCCTTATGAGAAATC	Non-T7 amp oligo
38	TGGCTTAGTTACGATT	Non-T7 amp oligo
39	TGGCTTAGTTACGATTAATAG	Non-T7 amp oligo
40	TTAATAGGAGTAGCTTGGGG	Non-T7 amp oligo
41	TTACGATTAATAGGAGT	Non-T7 amp oligo
42	TTCAGATGTCAGAGGTGA	Non-T7 amp oligo
43	TTGGCTTAGTTACGAT	Non-T7 amp oligo
44	TTGGCTTAGTTACGATTA	Non-T7 amp oligo
45	TTGGGGACATTCGTATTCAGA	Non-T7 amp oligo
46	TTTAGATTGCTTCCTTCAGT	Non-T7 amp oligo
47	TTTGAATACTANAGCA	Non-T7 amp oligo
48	ACATTCGTATTCAGATGTCAG	Non-T7 amp oligo
49	CTTAGTTACGATTAATAGGA	Non-T7 amp oligo
50	CGATTAATAGGAGTAGCTTGG	Non-T7 amp oligo
51	CTTAGTTACGATTAATAGGAGTAG	Non-T7 amp oligo
52	CTTGAATACTNCAGCA	Non-T7 amp oligo
53	GGCTTAGTTACGATTA	Non-T7 amp oligo
54	AATACTANAGCATGG	Non-T7 amp oligo
55	AATACTANAGCATGGAATA	Non-T7 amp oligo
56	AATTCTAAAGAAGAGAG	Non-T7 amp oligo

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
57	AATTTAATACGACTCACTATAGGGAGATTCACTCCCTTAA CTTTCGTTCTTG	T7 amp oligo
58	AATTTAATACGACTCACTATAGGGAGACTTGATTAATGGA AGTATTTAGA	T7 amp oligo
59	AATTTAATACGACTCACTATAGGGAGACTTAACTTTCGTT CTTGATTAATGGAAGT	T7 amp oligo
60	AATTTAATACGACTCACTATAGGGAGACCTACTCTTGTCT TAAACTA	T7 amp oligo
61	AATTTAATACGACTCACTATAGGGAGAAAACGGCCATGCA TCACCATCCAAGA	T7 amp oligo
62	AATTTAATACGACTCACTATAGGGAGACTCCCTTAACTTT CGTTCTTGATTAATGGAAGT	T7 amp oligo
63	AATTTAATACGACTCACTATAGGGAGACGACGGTATCTGA TCGTCTTCACTCCC	T7 amp oligo
64	AATTTAATACGACTCACTATAGGGAGACTTAACTTTCGTT CTTGATTAATGGAAG	T7 amp oligo
65	AATTTAATACGACTCACTATAGGGAGACTTAACTTTCGTT CTTGATTAATGGAAGTA	T7 amp oligo
66	AATTTAATACGACTCACTATAGGGAGACACTCCCTTAACT TTCGTTCTTGATTAATG	T7 amp oligo
67	AATTTAATACGACTCACTATAGGGAGACACTCCCTTAACT TTCGTTCTTGATTAATGG	T7 amp oligo
68	AATTTAATACGACTCACTATAGGGAGACTTCACTCCCTTA ACTTTCGTTCTTGATT	T7 amp oligo
69	AATTTAATACGACTCACTATAGGGAGACTTCACTCCCTTA ACTTTCGTTCTTGAT	T7 amp oligo
70	AATTTAATACGACTCACTATAGGGAGAATCGTCTTCACTC CCTTAACTTTCGTTC	T7 amp oligo
71	AATTTAATACGACTCACTATAGGGAGACTCCCTTAACTTT CGTTCTTGATTAATG	T7 amp oligo
72	AATTTAATACGACTCACTATAGGGAGATCACTCCCTTAAC TTTCGTTCTTGAT	T7 amp oligo
73	AATTTAATACGACTCACTATAGGGAGACCCTTAACTTTCG TTCTTGATTAATG	T7 amp oligo
74	AATTTAATACGACTCACTATAGGGAGACTTAACTTTCGTT CTTGATTAATG	T7 amp oligo

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
75	AATTTAATACGACTCACTATAGGGAGATAACTTTCGTTCTTGATTAATG	T7 amp oligo
76	AATTTAATACGACTCACTATAGGGAGAACTCCCTTAACTTTCGTTCTTGAT	T7 amp oligo
77	AATTTAATACGACTCACTATAGGGAGATCCCTTAACTTTCGTTCTTGAT	T7 amp oligo
78	AATTTAATACGACTCACTATAGGGAGAAGGCAAATGCTTTCGCAGTTGTTNGTCT	T7 amp oligo
79	AATTTAATACGACTCACTATAGGGAGAAGGCAAATGCTTTCGCAGTTGTTTGTCT	T7 amp oligo
80	TTCACTCCCTTAACTTTCGTTCTTG	THS of SEQ ID NO:57
81	CTTGATTAATGGAAGTATTTTAGA	THS of SEQ ID NO:58
82	CTTAACTTTCGTTCTTGATTAATGGAAGT	THS of SEQ ID NO:59
83	CCTACTCTTGTCTTAAACTA	THS of SEQ ID NO:60
84	AAACGGCCATGCATCACCATCCAAGA	THS of SEQ ID NO:61
85	CTCCCTTAACTTTCGTTCTTGATTAATGGAAGT	THS of SEQ ID NO:62
86	CGACGGTATCTGATCGTCTTCACTCCC	THS of SEQ ID NO:63
87	CTTAACTTTCGTTCTTGATTAATGGAAG	THS of SEQ ID NO:64
88	CTTAACTTTCGTTCTTGATTAATGGAAGTA	THS of SEQ ID NO:65
89	CACTCCCTTAACTTTCGTTCTTGATTAATG	THS of SEQ ID NO:66
90	CACTCCCTTAACTTTCGTTCTTGATTAATGG	THS of SEQ ID NO:67
91	CTTCACTCCCTTAACTTTCGTTCTTGATT	THS of SEQ ID NO:68
92	CTTCACTCCCTTAACTTTCGTTCTTGAT	THS of SEQ ID NO:69
93	ATCGTCTTCACTCCCTTAACTTTCGTTC	THS of SEQ ID NO:70
94	CTCCCTTAACTTTCGTTCTTGATTAATG	THS of SEQ ID NO:71
95	TCACTCCCTTAACTTTCGTTCTTGAT	THS of SEQ ID NO:72
96	CCCTTAACTTTCGTTCTTGATTAATG	THS of SEQ ID NO:73
97	CTTAACTTTCGTTCTTGATTAATG	THS of SEQ ID NO:74
98	TAACTTTCGTTCTTGATTAATG	THS of SEQ ID NO:75
99	ACTCCCTTAACTTTCGTTCTTGAT	THS of SEQ ID NO:76
100	TCCCTTAACTTTCGTTCTTGAT	THS of SEQ ID NO:77
101	AGGCAAATGCTTTCGCAGTTGTTNGTCT	THS of SEQ ID NO:78
102	AGGCAAATGCTTTCGCAGTTGTTTGTCT	THS of SEQ ID NO:79
103	CGCGCAAGCGAGAAAGCGCG	Torch detection probe
104	GCUCGCAUUCGCGCAAGCGAGC	Torch detection probe
105	GCUUGCGAGUAUUCGCGCAAGC	Torch detection probe
106	GGCAAGCGAGAAAGUCUUGCC	Torch detection probe

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
107	CCGAGGUAUUCGCGCAACUCGG	Beacon detection probe
108	GGCUCACUUUCUCGCUUGGAGCC	Beacon detection probe
109	CUCUGGAGACNAGCACCAGAG	Torch detection probe
110	GUCUCAUUUUCUGGAGAC	Torch detection probe
111	GCCUAAAAUACUUCCUAGGC	Torch detection probe
112	CAUGGAAAUACUUCCAUG	Torch detection probe
113	CCGAGAUUUUCUGGAGACCUCGG	Beacon detection probe
114	CCGAGGCCUAAAAUACUUCCCUCGG	Beacon detection probe
115	GGGAAUUUAAAACCUUCCC	Torch detection probe
116	GGAAGGAAUUUAAAACCUUCC	Torch detection probe
117	GUGGGAAUUUAAAACCCCCAC	Torch detection probe
118	UCCAGAAAUUCUUAGAUUUUCUGGA	Torch detection probe
119	ACUCCGAACGAAAGUUAAGGGAGU	Torch detection probe
120	AGGGAGUGAAGACGAUCAUCCCU	Torch detection probe
121	UCGCGCAAGCGAGAAAGGCGCGA	Torch detection probe
122	CCGAAGUGNCUAAAAUACUUCGG	Torch detection probe
123	CACCUCAGAUGUCAGAGGUG	Torch detection probe
124	CUACCUCUAAAGAAGAGAGGUAG	Torch detection probe
125	CGCGCAAGCGAGAAAG	THS of SEQ ID NO:103
126	AUUCGCGCAAGCGAGC	THS of SEQ ID NO:104
127	GAGUAUUCGCGCAAGC	THS of SEQ ID NO:105
128	GGCAAGCGAGAAAGU	THS of SEQ ID NO: 106
129	GUAUUCGCGCAA	THS of SEQ ID NO: 107
130	ACUUUCUCGCUUG	THS of SEQ ID NO:108
131	CUCUGGAGACNAGCA	THS of SEQ ID NO: 109
132	AUUUUCUGGAGAC	THS of SEQ ID NO:110
133	GCCUAAAAUACUUCC	THS of SEQ ID NO:111
134	AAAUACUUCCAUG	THS of SEQ ID NO:112
135	AUUUUCUGGAGAC	THS of SEQ ID NO:113
136	CCUAAAAUACUUC	THS of SEQ ID NO:114
137	GGGAAUUUAAAACC	THS of SEQ ID NO: 115
138	GGAAUUUAAAACCUUCC	THS of SEQ ID NO:116
139	GUGGGAAUUUAAAACC	THS of SEQ ID NO:117
140	GAAAUUCUUAGAUUUUCUGGA	THS of SEQ ID NO: 118
141	GAACGAAAGUUAAGGGAGU	THS of SEQ ID NO:119
142	AGGGAGUGAAGACGAUCA	THS of SEQ ID NO: 120
143	UCGCGCAAGCGAGAAAG	THS of SEQ ID NO:121

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
144	UGNCUAAAAUACUUCGG	THS of SEQ ID NO: 122
145	CAGAUGUCAGAGGUG	THS of SEQ ID NO:123
146	UCUAAAGAAGAGAGGUAG	THS of SEQ ID NO: 124
147	UCUUAGAUUUUCUGGAGAC	Detection probe
148	UUCAGAUGUCAGAGG	Detection probe
149	UUCAGAUGUCAGAGGT	Detection probe
150	UAUUCAGAUGUCAGAGGT	Detection probe
151	UAUUCAGAUGUCAGAGGUG	Detection probe
152	UCAGAUGUCAGAGGT	Detection probe
153	UUCAGAUGUCAGAGGT	Detection probe
154	AUUCAGAUGUCAGAGGT	Detection probe
155	AUUCAGAUGUCAGAGGUG	Detection probe
156	CUUAGAUUUUCUGGAGA	Detection probe
157	CUUAGAUUUUCUGGAGAC	Detection probe
158	CUUAGUUACGAUUAAUAGGA	Detection probe
159	GUAUUCAGAUGUCAGAGGUGA	Detection probe
160	AUUCUUAGAUUUUCUGGAGAC	Detection probe
161	CUAAGAUUUUCUGGAGAC	Detection probe
162	TTTGAATACTANAGCATGGAATA	Amp oligo hybridizing region (SEQ ID NOs:21, 23-25, 32, 33, 35, 54, & 55 are contained within here)
163	TACTANAGCA	Amp oligo core sequence (SEQ ID NOs: 21, 23-25, 32, 33, 35, 54, & 55 share this)
164	TTTGAATACTACAGCATGGAATA	Amp oligo hybridizing region (SEQ ID NOs:21, 23-25, 32, 33, & 35 are contained within here)
165	TACTACAGCA	Amp oligo core sequence (SEQ ID NOs: 21, 23-25, 32, 33, & 35 share this)
166	TTGGCTTAGTTACGATTAATAGGAGTAGCTTGGGG	Amp oligo hybridizing region (SEQ ID NOs:28-31, 34, 38-41, 43, 44, 49-51, & 53 are contained within here)
167	TTAATAGGAGT	Amp oligo core sequence (SEQ ID NOs: 28-31, 34, 40, 41, & 49-51 share this)
168	GGCTTAGTTACGAT	Amp oligo core sequence (SEQ ID NOs: 38, 39, 43, 44, & 53 share this)
169	CGACGGTATCTGATCGTCTTCACTCCCTTAACTTTCGTTC TTGATTAATGGAAGTATTTTAGA	Amp oligo hybridizing region (SEQ ID NOs:80-82, & 85-100 are contained within here)
170	ATCGTCTTCACTCCCTTAACTTTCGTTCTTGATTAATGGA AGTATTTTAGA	Amp oligo hybridizing region (SEQ ID NOs:80-82, 85, & 87-100 are contained within here)

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
171	CTTGATTAATG	Amp oligo core sequence (SEQ ID NOs: 81, 82, 85, 87-90, 94, & 96-98 share this)
172	TAACTTTCGTTC	Amp oligo core sequence (SEQ ID NOs: 80, 82, 85, & 87-100 share this)
173	CTTCACTCCC	Amp oligo core sequence (SEQ ID NOs: 86 & 91-93 share this)
174	UCAGAUGUCAGAGG	Detection probe core sequence (SEQ ID NOs: 148-155 & 159 share this)
175	CUAAGAUUUUCUGGAGA	Detection probe core sequence (SEQ ID NOs: 147, 156, 157, 160, & 161 share this)
176	CAGAUGUCAGAGG	Detection probe core sequence (SEQ ID NOs: 145, 148-155, & 159 share this)
177	YUCUGGAGAC	Detection probe core sequence (SEQ ID NOs: 131, 132, 135, 147, 156, 157, 160, & 161 share this)
178	AUUUUCUGGA	Detection probe core sequence (SEQ ID NOs: 132, 135, 140, 147, 156, 157, 160, & 161 share this)
179	AATTTAATACGACTCACTATAGGGAGA	Exemplary T7 promoter sequence for use in an isothermal amplification reaction

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
180	aacctggttgatcttgccagtagtcatatgcttgtctcaa agattaagccatgcaagtgaaagtatatatatattttata tgtagaaactgcgaacggctcattaaaacagttatagtct acttgacatttttattataaggataactacggaaaagctg tagctaatacttgctttattatccttttgatttttatcttt ggataagtatttgttaggccttataagaaaaaagttatta acttaaggaattataacaaagaagtaacacgtaataaatt tattttatttagtgtgtatcaatcgagtttctgacctatc agcttttgatgttagggtattggcctaacatggctatgac gggtaacggggaattagagttcgattccggagagggagcc tgagaaatagctaccacatctaaggaaggcagcaggcgcg taaattacccaattctaaagaagagaggtagtgacaagaa ataacaatgcaaggccaatttttggttttgtaattggaat ggtgggaatttaaaaccttcccagagtaacaattggaggg caagtctggtgccagcagccgcggtaattccagctccaat agcgtatattaaaattgttgcagttaaaacgctcgtagtt gaatttcaaagaatcgatattttattgtaactattctagg ggaactattttagctttcgctttaatacgcttcctctatt attatgttctttaaataacaaagattctttttaaaatccc cacttttgcttttgcttttttggggattttgttactttgag taaattagagtgttcaaagcaaacagttaaagcatttact gtgtttgaatactatagcatggaataacaaaattgaacaa gctaaaatttttttgttctttttttcttattttggcttagtt acgattaataggagtagcttggggacattcgtattcagat gtcagaggtgaaattcttagattttctggagacgaacaac tgcgaaagcatttgtctaaaatacttccattaatcaagaa cgaaagttaagggagtgaagacgatcagataccgtcgtaa tcttaaccataaactatgccgactaggtgttggatgaaag tgttaaaaataaaagtcatctttcgaggtgacttttagat tgcttccttcagtaccttatgagaaatcaaagtctttggg	Plasmodium falciparum 3D7 18S ribosomal RNA (PF3D7 _0725600), rRNA NCBI Reference Sequence: XR_ 002273081.2
	ttctggggcgagtattcgcgcaagcgagaaagttaaaaga attgacggaagggcaccaccaggcgtggagcttgcggctt aatttgactcaacacggggaaactcactagtttaagacaa gagtaggattgacagattaatagctctttcttgatttctt ggatggtgatgcatggccgtttttagttcgtgaatatgat ttgtctggttaattccgataacgaacgagatcttaacctg ctaattagcggcgagtacactatattcttatttgaaattg aacataggtaactatacatttattcagtaatcaaattagg atatttttattaaaatatccttttccctgttctactaata atttgtttttttactctatttctctcttctttttaagaatgt acttgcttgattgaaaagcttcttagaggaacattgtgtg tctaacacaaggaagtttaaggcaacaacaggtctgtgat gtccttagatgaactaggctgcacgcgtgctacactgata tatataacgagtttttaaaaatatgcttatatttgtatct ttgcttatattttgcatacttttcctccgccgaaaggcgt aggtaatctttatcaatatatatcgtgatggggatagatt attgcaattattaatcttgaacgaggaatgcctagtaagc atgattcatcagattgtgctgactacgtccctgccctttg tacacaccgcccgtcgctcctaccgattgaaagatatgat gaattgtttggacaagaaaaattgaattatattcttttttt tttctggaaaaaccgtaaatcctatcttttaaaggaagga gaagtcgtaacaaggtttccgtaggtgaacctgcggaagg atcatta	
181	AATTTAATACGACTCACTATAGGGAGATCAAGAAAGAGCT ATNAATCTGTCAATCC	T7 amp oligo

(continued)

SEQ ID NO:	Sequence (5' to 3')	Comments
182	TCAAGAAAGAGCTATNAATCTGTCAATCC	THS of SEQ ID NO:181
183	GAAATCAAAGTCTTTGGGTTCTG	Non-T7 amp oligo
184	CAAAGTCTTTGGGTTCTGG	Non-T7 amp oligo
185	TTTAGATTGCTTCCTTCAGTNCCTTATGAGAAATCAAAGTCTTTGGGTTCTGG	Amp oligo hybridizing region (SEQ ID NOs:37, 46, 183, & 184 are contained within here)
186	GAAATCAAAGTCTTTGGGTTCTGG	Amp oligo hybridizing region (SEQ ID NOs: 183 & 184 are contained within here)
187	CAAAGTCTTTGGGTTCTG	Amp oligo core sequence (SEQ ID NOs: 183 & 184 share this)
188	AAACGGCCATGCATCACCATCCAAGAAATCAAGAAAGAGCTATNAATCTGTCAATCCTACTCTTGTCTTAAACTA	Amp oligo hybridizing region (SEQ ID NOs:83, 84, & 182 are contained within here)
189	GAGUAUUCGSGCAAGCGAGAAAGU	Detection probe hybridizing region (SEQ ID NOs:125-130 & 143 are contained within here)
190	AUUCGCGCAA	Detection probe core sequence (SEQ ID NOs: 126, 127, & 129 share this)
191	CAAGCGAGC	Detection probe core sequence (SEQ ID NOs: 125, 126, 128, 130, & 143 share this)
192	[GenBank Accession JQ627153.1]	Plasmodium vivax isolate SV1 18S ribosomal RNA gene, partial sequence
193	[GenBank Accession L07560.1]	Plasmodium knowlesi small subunit ribosomal RNA sequence
194	[GenBank Accession AB182491.1]	Plasmodium ovale gene for small subunit ribosomal RNA, complete sequence, variant type 1
195	[GenBank Accession AF487999.1]	Plasmodium cf. malariae type 1 small subunit ribosomal RNA gene, complete sequence
196	GUAUUCAGAUGUCAGAGGUGAAAUUCUUAGAUUYUCUGGAGACNAGCA	Detection probe hybridizing region (SEQ ID NOs:131, 132, 135, 140, 145, 147-157 & 159-161 are contained within here)
197	GUAUUCAGAUGUCAGAGGUGAAAUUCUUAGAUUUUCUGGAGAC	Detection probe hybridizing region (SEQ ID NOs:132, 135, 140, 145, 147-157 & 159-161 are contained within here)

Claims

1. A method for specifically detecting *Plasmodium* species nucleic acid in a sample, said method comprising:

(1) contacting a sample, said sample suspected of containing *Plasmodium* species nucleic acid, with at least

two oligomers for amplifying a target region of a *Plasmodium* species target nucleic acid, wherein the at least two amplification oligomers comprise:

(a) an amplification oligomer comprising a target-hybridizing sequence

(i) that is from 14 to 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 162, and comprises the sequence of SEQ ID NO: 163; or
(ii) that is from 14 to 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO: 166, and comprises the sequence of SEQ ID NO: 167 or SEQ ID NO: 168; and

(b) an amplification oligomer comprising a target-hybridizing sequence that is from 15 to 33 contiguous nucleotides in length, is contained in SEQ ID NO: 169 and comprises the sequence of SEQ ID NO: 171, SEQ ID NO: 172, or SEQ ID NO: 173;

(2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Plasmodium* target nucleic acid present in said sample is used as a template for generating an amplification product; and
(3) detecting the presence or absence of the amplification product, thereby indicating the presence or absence of *Plasmodium* species target nucleic acid in said sample.

2. The method of claim 1, wherein the amplification oligomer of (b) is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence of (b), optionally wherein the promoter sequence is a T7 promoter sequence, optionally wherein the amplification oligomer of (b) comprises a sequence selected from the group consisting of SEQ ID NOs:57-59 and 62-77.

3. The method of any one of the preceding claims, further comprising purifying the target nucleic acid from other components in the sample before step (1), optionally wherein the purifying step comprises contacting the sample with at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a sequence or moiety that binds to an immobilized probe, wherein said target-hybridizing sequence is up to 30 contiguous nucleotides in length and comprises a sequence selected from the group consisting of SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof.

4. The method of any one of the preceding claims, wherein the detecting step (3) comprises contacting the *in vitro* nucleic acid amplification reaction with at least one detection probe oligomer comprising a target-hybridizing sequence configured to specifically hybridize to the amplification product under conditions whereby the presence or absence of the amplification product is determined, thereby indicating the presence or absence of *Plasmodium* species in said sample.

5. A combination of at least two oligomers for determining the presence or absence of *Plasmodium* species in a sample, said oligomer combination comprising at least two oligomers for amplifying a target region of *Plasmodium* species target nucleic acid, wherein the at least two amplification oligomers comprise:

(a) an amplification oligomer comprising a target-hybridizing sequence

(i) that is from 14 to 20 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:162, and comprises the sequence of SEQ ID NO:163; or
(ii) that is from 14 to 25 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:166, and comprises the sequence of SEQ ID NO:167 or SEQ ID NO:168; and

(b) an amplification oligomer comprising a target-hybridizing sequence that is from 15 to 33 contiguous nucleotides in length, is contained in SEQ ID NO:169 and comprises the sequence of SEQ ID NO:171, SEQ ID NO:172, or SEQ ID NO:173.

6. The oligomer combination of claim 5, wherein the at least two amplification oligomers comprise the amplification oligomer of (a)(i);

wherein the target-hybridizing sequence of (a)(i) is selected from the group consisting of SEQ ID NOs:21, 23-25, 32, 33, 35, 54, and 55,
or wherein the target-hybridizing sequence of (a)(i) is contained in the sequence of SEQ ID NO: 164, comprises

the sequence of SEQ ID NO: 165, and is optionally selected from the group consisting of SEQ ID NOs:21, 23-25, 32, 33, and 35.

7. The oligomer combination of claim 5, wherein the at least two amplification oligomers comprise the amplification oligomer of (a)(ii),

wherein the target-hybridizing sequence of (a)(ii) comprises the sequence of SEQ ID NO: 167 and is optionally selected from the group consisting of SEQ ID NOs:28-31, 34, 40, 41, and 49-51; or
wherein the target-hybridizing sequence of (a)(ii) comprises the sequence of SEQ ID NO: 168 and is optionally selected from the group consisting of SEQ ID NOs:38, 39, 43, 44, and 53.

8. The oligomer combination of any one of claims 5 to 7, wherein the target-hybridizing sequence of (b) is selected from the group consisting of SEQ ID NOs:80-82 and 85-100,
or wherein the target-hybridizing sequence of (b) is contained in SEQ ID NO: 170, comprises the sequence of SEQ ID NO: 171 or SEQ ID NO: 172, and is optionally selected from (i) the group consisting of SEQ ID NOs:81, 82, 85, 87-90, 94, and 96-98 or (ii) the group consisting of SEQ ID NOs:80, 82, 85, and 87-100.

9. The oligomer combination of any one of claims 5 to 8, wherein the amplification oligomer of (b) is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the target-hybridizing sequence of (b), optionally wherein the promoter sequence is a T7 promoter sequence, optionally wherein the amplification oligomer of (b) comprises a sequence selected from the group consisting of SEQ ID NOs:57-59 and 62-77.

10. The oligomer combination of claim 5, wherein the at least two amplification oligomers comprise first and second amplification oligomers as in (a)(ii), optionally wherein

the first amplification oligomer as in (a)(ii) comprises a target-hybridizing sequence comprising the sequence of SEQ ID NO: 167, optionally wherein the first amplification oligomer target-hybridizing sequence is SEQ ID NO:34; and
the second amplification oligomer as in (a)(ii) comprises a target-hybridizing sequence comprising the sequence of SEQ ID NO:168, optionally wherein the second amplification oligomer target-hybridizing sequence is SEQ ID NO:53.

11. The oligomer combination of claim 5, wherein the at least two amplification oligomers comprise an amplification oligomer as in (a)(i) and an amplification oligomer as in (a)(ii), optionally wherein

the amplification oligomer as in (a)(i) comprises a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:164 and comprises the sequence of SEQ ID NO:165, optionally wherein the amplification oligomer as in (a)(i) comprises the target-hybridizing sequence of SEQ ID NO:21; and
the amplification oligomer as in (a)(ii) comprises the sequence of SEQ ID NO:167, optionally wherein the amplification oligomer as in (a)(ii) comprises the target-hybridizing sequence of SEQ ID NO:34.

12. The oligomer combination of any one of claims 10 and 11, wherein the at least two amplification oligomers comprise first and second amplification oligomers of (b), optionally wherein each of the first and second amplification oligomers of (b) comprises a target-hybridizing sequence that is contained in SEQ ID NO:170 and comprises the sequence of SEQ ID NO:171 or SEQ ID NO:172,
optionally wherein the first amplification oligomer as in (b) comprises the target-hybridizing sequence of SEQ ID NO:94 and the second amplification oligomer as in (b) comprises the target-hybridizing sequence of SEQ ID NO:95.

13. The oligomer combination of any one of claims 5 to 12, further comprising at least one capture probe oligomer comprising a target-hybridizing sequence covalently attached to a sequence or moiety that binds to an immobilized probe, wherein said target-hybridizing sequence is up to 30 contiguous nucleotides in length and comprises a sequence selected from the group consisting of SEQ ID NOs:11-15, 17, 19, and 20, including DNA equivalents and DNA/RNA chimerics thereof.

14. The oligomer combination of any one of claims 5 to 13, further comprising at least one detection probe oligomer comprising a target-hybridizing sequence configured to specifically hybridize to a *Plasmodium* species amplicon amplifiable by the at least two amplification oligomers.

15. The oligomer combination of claim 14, wherein the detection probe oligomer comprises:

(a) a target-hybridizing sequence that is from 13 to 40 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:196 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and comprises the sequence of SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177, or SEQ ID NO:178;
(b) a target-hybridizing sequence that is contained in the sequence of SEQ ID NO:197 or its complement, or a DNA equivalent or DNA/RNA chimeric thereof, and comprises the sequence of SEQ ID NO: 174, SEQ ID NO:175, or SEQ ID NO:176;
(d) a target-hybridizing sequence comprising the sequence of any of SEQ ID NO:131-146; or
(e) the sequence of any of SEQ ID NO:147-161.

Patentansprüche

1. Verfahren zum spezifischen Nachweis von Plasmodium-Spezies-Nukleinsäure in einer Probe, wobei das Verfahren umfasst:

(1) Kontaktieren einer Probe, die vermutlich Plasmodium-Spezies-Nukleinsäure enthält, mit mindestens zwei Oligomeren zur Verstärkung einer Zielregion einer Plasmodium-Spezies-Zielnukleinsäure, wobei die mindestens zwei Verstärkungs-Oligomere umfassen:

(a) ein Verstärkungs-Oligomer, das eine zielhybridisierende Sequenz enthält

(i) die eine Länge von 14 bis 20 zusammenhängenden Nukleotiden aufweist, in der Sequenz SEQ ID NO: 162 enthalten ist und die Sequenz SEQ ID NO: 163 umfasst; oder
(ii) die eine Länge von 14 bis 25 zusammenhängenden Nukleotiden aufweist, in der Sequenz SEQ ID NO: 166 enthalten ist und die Sequenz SEQ ID NO: 167 oder SEQ ID NO: 168 umfasst; und

(b) ein Verstärkungs-Oligomer, das eine zielhybridisierende Sequenz mit einer Länge von 15 bis 33 zusammenhängenden Nukleotiden umfasst, die in SEQ ID NO: 169 enthalten ist und die Sequenz SEQ ID NO: 171, SEQ ID NO: 172 oder SEQ ID NO: 173 umfasst;

(2) Durchführen einer In-vitro-Nukleinsäure-Verstärkungsreaktion, wobei jede in der Probe vorhandene Plasmodium-Zielnukleinsäure als Vorlage zur Erzeugung eines Verstärkungsprodukts verwendet wird; und
(3) Detektieren der Anwesenheit oder der Abwesenheit des Verstärkungsprodukts, wodurch die Anwesenheit oder die Abwesenheit von Plasmodium-Spezies-Zielnukleinsäure in der Probe angezeigt wird.

2. Verfahren nach Anspruch 1, wobei das Verstärkungs-Oligomer nach (b) ein Promotor- Primer oder Promotor-Provider ist, der ferner eine Promotorsequenz umfasst, die sich 5 Zoll bis zur Zielhybridisierungssequenz von (b) befindet, wobei die Promotorsequenz gegebenenfalls eine T7-Promotorsequenz ist, wobei das Verstärkungs-Oligomer nach (b) gegebenenfalls eine Sequenz umfasst, die aus der Gruppe ausgewählt ist, die aus SEQ ID NOs: 57-59 und 62-77 besteht.

3. Verfahren nach einem der vorhergehenden Ansprüche, das vor Schritt (1) ferner das Reinigen der Zielnukleinsäure von anderen Komponenten in der Probe umfasst, wobei der Reinigungsschritt gegebenenfalls das Kontaktieren der Probe mit mindestens einem Einfangsonden-Oligomer umfasst, das eine Ziel-hybridisierende Sequenz umfasst, die kovalent an eine Sequenz oder Einheit gebunden ist, die an eine immobilisierte Sonde bindet, wobei die Zielhybridisierende Sequenz bis zu 30 zusammenhängende Nukleotide lang ist und eine Sequenz umfasst, die aus der Gruppe ausgewählt ist, die aus SEQ ID NOs:11-15, 17, 19 und 20 besteht, einschließlich DNA-Äquivalenten und DNA/RNA-Chimären davon. enthalten ist.

4. Verfahren nach einem der vorhergehenden Ansprüche, wobei der Nachweisschritt (3) das Kontaktieren der *In-vitro*-Nukleinsäure-Verstärkungsreaktion mit mindestens einem Nachweissonden-Oligomer umfasst, das eine zielhybridisierende Sequenz umfasst, die so konfiguriert ist, dass sie spezifisch mit dem Verstärkungsprodukt unter Bedingungen hybridisiert, unter denen die Anwesenheit oder die Abwesenheit des Verstärkungsprodukts bestimmt wird, wodurch die Anwesenheit oder die Abwesenheit von *Plasmodium*-Spezies in der Probe angezeigt wird.

5. Kombination von mindestens zwei Oligomeren zur Bestimmung der des Vorhandenseins oder der Abwesenheit von

Plasmodium-Spezies in einer Probe, wobei die Oligomerkombination mindestens zwei Oligomere zur Verstärkung einer Zielregion von Plasmodium-Spezies-Zielnukleinsäure umfasst, wobei die mindestens zwei Verstärkungs-Oligomere umfassen:

(a) ein Verstärkungs-Oligomer, das eine zielhybridisierende Sequenz enthält

(i) die eine Länge von 14 bis 20 zusammenhängenden Nukleotiden aufweist, in der Sequenz von SEQ ID NO:162 enthalten ist und die Sequenz von SEQ ID NO:163 umfasst; oder
(ii) die eine Länge von 14 bis 25 zusammenhängenden Nukleotiden aufweist, in der Sequenz von SEQ ID NO:166 enthalten ist und die Sequenz von SEQ ID NO:167 oder SEQ ID NO:168 umfasst; und

(b) ein Verstärkungs-Oligomer, das eine zielhybridisierende Sequenz mit einer Länge von 15 bis 33 zusammenhängenden Nukleotiden umfasst, die in SEQ ID NO:169 enthalten ist und die Sequenz von SEQ ID NO:171, SEQ ID NO:172 oder SEQ ID NO:173 umfasst.

6. Oligomerkombination nach Anspruch 5, wobei die mindestens zwei Verstärkungs-Oligomere das Verstärkungs-Oligomer nach (a)(i) umfassen;

wobei die Ziel-Hybridisierungssequenz nach (a)(i) ausgewählt ist aus der Gruppe bestehend aus SEQ ID NOs:21, 23-25, 32, 33, 35, 54 und 55,
oder wobei die Ziel-Hybridisierungssequenz nach (a)(i) in der Sequenz SEQ ID NO: 164 enthalten ist, die Sequenz SEQ ID NO: 165 umfasst und optional aus der Gruppe bestehend aus SEQ ID NOs:21, 23-25, 32, 33 und 35 ausgewählt ist.

7. Oligomerkombination nach Anspruch 5, wobei die mindestens zwei Verstärkungs-Oligomere das Verstärkungs-Oligomer nach (a)(ii) umfassen,

wobei die Ziel-Hybridisierungssequenz nach (a)(ii) die Sequenz SEQ ID NO: 167 umfasst und optional aus der Gruppe bestehend aus SEQ ID NOs:28-31, 34, 40, 41 und 49-51 ausgewählt ist; oder
wobei die Ziel-Hybridisierungssequenz von (a)(ii) die Sequenz von SEQ ID NO: 168 umfasst und optional aus der Gruppe bestehend aus SEQ ID NOs:38, 39, 43, 44 und 53 ausgewählt ist.

8. Oligomerkombination nach einem der Ansprüche 5 bis 7, wobei die zielhybridisierende Sequenz nach (b) ausgewählt ist aus der Gruppe bestehend aus SEQ ID NOs: 80-82 und 85-100,
oder worin die Ziel-Hybridisierungssequenz nach (b) in SEQ ID NO:170 enthalten ist, die Sequenz von SEQ ID NO:171 oder SEQ ID NO:172 umfasst und optional ausgewählt ist aus (i) der Gruppe bestehend aus SEQ ID NOs:81, 82, 85, 87-90, 94 und 96-98 oder (ii) der Gruppe bestehend aus SEQ ID NOs:80, 82, 85 und 87-100.

9. Oligomerkombination nach einem der Ansprüche 5 bis 8, wobei das Verstärkungs-Oligomer nach (b) ein Promotor-Primer oder Promotor-Provider ist, der ferner eine Promotorsequenz umfasst, die sich 5 Zoll bis zur Zielhybridisierungssequenz nach (b) befindet, wobei die Promotorsequenz optional eine T7-Promotorsequenz ist, wobei das Verstärkungs-Oligomer nach (b) optional eine Sequenz umfasst, die aus der Gruppe ausgewählt ist, die aus SEQ ID NOs: 57-59 und 62-77 besteht.

10. Oligomerkombination nach Anspruch 5, wobei die mindestens zwei Verstärkungs-Oligomere ein erstes und ein zweites Verstärkungs-Oligomer gemäß (a)(ii) umfassen, wobei optional

das erste Verstärkungs-Oligomer nach (a)(ii) eine zielhybridisierende Sequenz umfasst, die die Sequenz SEQ ID NO:167 umfasst, wobei es sich bei der zielhybridisierenden Sequenz des ersten Verstärkungs-Oligomers optional um SEQ ID NO:34 handelt; und
das zweite Verstärkungs-Oligomer nach (a)(ii) eine zielhybridisierende Sequenz umfasst, die die Sequenz SEQ ID NO:168 umfasst, wobei die zielhybridisierende Sequenz des zweiten Verstärkungs-Oligomers optional SEQ ID NO:53 ist.

11. Oligomerkombination nach Anspruch 5, wobei die mindestens zwei Verstärkungs-Oligomere ein Verstärkungs-Oligomer nach (a)(i) und ein Verstärkungs-Oligomer wie in (a)(ii) umfassen, wobei optional

das Verstärkungs-Oligomer nach (a)(i) eine zielhybridisierende Sequenz umfasst, die in der Sequenz SEQ ID

NO:164 enthalten ist und die Sequenz SEQ ID NO:165 umfasst, wobei das Verstärkungs-Oligomer nach (a)(i) optional die zielhybridisierende Sequenz SEQ ID NO:21 umfasst; und
das Verstärkungs-Oligomer nach (a)(ii) die Sequenz von SEQ ID NO:167 umfasst, wobei das Verstärkungs-Oligomer gemäß (a)(ii) optional die Ziel-Hybridisierungssequenz von SEQ ID NO:34 umfasst.

12. Oligomerkombination nach einem der Ansprüche 10 und 11, wobei die mindestens zwei Verstärkungs-Oligomere erste und zweite Verstärkungs-Oligomere nach (b) umfassen, wobei optional jedes der ersten und zweiten Verstärkungs-Oligomere nach (b) eine Zielhybridisierende Sequenz umfasst, die in SEQ ID NO: 170 enthalten ist und die Sequenz nach SEQ ID NO:171 oder SEQ ID NO:172 umfasst,
wobei das erste Verstärkungs-Oligomere nach (b) die Ziel-Hybridisierungssequenz SEQ ID NO:94 und das zweite Verstärkungs-Oligomer nach (b) die ZielHybridisierungssequenz SEQ ID NO:95 umfasst.

13. Oligomerkombination nach einem der Ansprüche 5 bis 12, die ferner mindestens ein Einfangsonden-Oligomer umfasst, das eine zielhybridisierende Sequenz umfasst, die kovalent an eine Sequenz oder Einheit gebunden ist, die an eine immobilisierte Sonde bindet, wobei die zielhybridisierende Sequenz bis zu 30 zusammenhängende Nukleotide lang ist und eine Sequenz umfasst, die aus der Gruppe ausgewählt ist, die aus SEQ ID NOs: 11-15, 17, 19 und 20 besteht, einschließlich DNA-Äquivalenten und DNA/RNA-Chimären davon.

14. Oligomerkombination nach einem der Ansprüche 5 bis 13, die ferner mindestens ein Nachweissonden-Oligomer umfasst, das eine zielhybridisierende Sequenz umfasst, die so konfiguriert ist, dass sie spezifisch an ein Plasmodium-Spezies-Amplikon hybridisiert, das durch die mindestens zwei Verstärkungs-Oligomere verstärkt wird ist.

15. Oligomerkombination nach Anspruch 14, wobei das Nachweissonden-Oligomer umfasst:

(a) eine Ziel-Hybridisierungssequenz mit einer Länge von 13 bis 40 zusammenhängenden Nukleotiden, die in der Sequenz SEQ ID NO:196 oder ihrem Komplement oder einem DNA-Äquivalent oder DNA/RNA-Chimären davon enthalten ist und die Sequenz SEQ ID NO:175, SEQ ID NO:176, SEQ ID NO:177 oder SEQ ID NO:178 umfasst;
(b) eine zielhybridisierende Sequenz, die in der Sequenz SEQ ID NO:197 oder ihrem Komplement oder ein DNA-Äquivalent oder eine DNA/RNA-Chimäre davon enthalten ist und die Sequenz SEQ ID NO: 174, SEQ ID NO: 175 oder SEQ ID NO: 176 umfasst;
(d) eine Ziel-Hybridisierungssequenz, die die Sequenz einer der SEQ ID NO:131-146 umfasst; oder
(e) die Sequenz einer der SEQ ID NO:147-161.

Revendications

1. Procédé de détection spécifique d'acide nucléique d'espèces de *Plasmodium* dans un échantillon, ledit procédé comprenant les étapes consistant à :

(1) mettre en contact un échantillon, ledit échantillon suspecté de contenir l'acide nucléique d'espèces de *Plasmodium* avec au moins deux oligomères pour amplifier une région cible d'un acide nucléique cible d'espèces de *Plasmodium,* dans lequel lesdits au moins deux oligomères d'amplification comprennent :

(a) un oligomère d'amplification comprenant une séquence d'hybridation de cible

(i) qui est de 14 à 20 nucléotides de longueur contigus, est contenu dans la séquence de SEQ ID NO : 162, et comprend la séquence de SEQ ID NO : 163 ; ou
(ii) qui est de 14 et 25 nucléotides de longueur contigus, est contenu dans la séquence de SEQ ID NO : 166, et comprend la séquence de SEQ ID NO : 167 ou SEQ ID NO : 168 ; et

(b) un oligomère d'amplification comprenant une séquence d'hybridation de cible qui est de 15 à 33 nucléotides de longueur contigus, est contenu dans SEQ ID NO : 169 et comprend la séquence de SEQ ID NO : 171, SEQ ID NO : 172 ou SEQ ID NO : 173 ;

(2) effectuer une réaction d'amplification d'acide nucléique *in vitro,* dans lequel tout acide nucléique cible de *Plasmodium* présent dans ledit échantillon est utilisé comme matrice pour générer un produit d'amplification ; et
(3) détecter la présence ou l'absence du produit d'amplification, indiquant ainsi la présence ou l'absence de

l'acide nucléique cible d'espèces de *Plasmodium* dans ledit échantillon.

2. Procédé de la revendication 1, dans lequel l'oligomère d'amplification de (b) est une amorce de promoteur ou un fournisseur de promoteur comprenant en outre une séquence de promoteur située en 5' de la séquence d'hybridation de cible de (b), éventuellement dans lequel la séquence de promoteur est une séquence de promoteur T7, éventuellement dans lequel l'oligomère d'amplification de (b) comprend une séquence choisie dans le groupe constitué de SEQ ID NOs : 57-59 et 62-77.

3. Procédé de l'une quelconque des revendications précédentes, comprenant en outre la purification d'acide nucléique cible à partir d'autres composants de l'échantillon avant l'étape (1), éventuellement dans lequel l'étape de purification comprend la mise en contact de l'échantillon avec au moins un oligomère de sonde de capture comprenant une séquence d'hybridation de cible attachée de manière covalente à une séquence ou à une fraction qui se lie à une sonde immobilisée, dans lequel ladite séquence d'hybridation de cible a une longueur allant jusqu'à 30 nucléotides contigus et comprend une séquence sélectionnée dans le groupe constitué de SEQ ID NOs : 11-15, 17, 19 et 20, y compris des équivalents ADN et des chimères ADN/ARN de ceux-ci.

4. Procédé de l'une quelconque des revendications précédentes, dans lequel l'étape de détection (3) consiste à mettre en contact la réaction d'amplification d'acide nucléique *in vitro* avec au moins un oligomère de sonde de détection comprenant une séquence d'hybridation de cible configurée pour s'hybrider spécifiquement au produit d'amplification dans des conditions permettant de déterminer la présence ou l'absence du produit d'amplification, indiquant ainsi la présence ou l'absence d'espèces de Plasmodium dans ledit échantillon.

5. Combinaison d'au moins deux oligomères pour déterminer la présence ou l'absence d'espèces de *Plasmodium* dans un échantillon, ladite combinaison d'oligomères comprenant au moins deux oligomères pour amplifier une région cible d'acide nucléique cible d'espèces de *Plasmodium,* dans laquelle lesdits au moins deux oligomères d'amplification comprennent :

(a) un oligomère d'amplification comprenant une séquence d'hybridation de cible

(i) qui est de 14 à 20 nucléotides de longueur contigus, est contenu dans la séquence de SEQ ID NO : 162, et comprend la séquence de SEQ ID NO : 163 ; ou
(ii) qui est de 14 à 25 nucléotides de longueur contigus, est contenu dans la séquence de SEQ ID NO : 166, et comprend la séquence de SEQ ID NO : 167 ou SEQ ID NO : 168 ; et

(b) un oligomère d'amplification comprenant une séquence d'hybridation de cible qui est de 15 à 33 nucléotides de longueur contigus, est contenu dans SEQ ID NO : 169 et comprend la séquence de SEQ ID NO : 171, SEQ ID NO : 172 ou SEQ ID NO : 173.

6. Combinaison d'oligomères de la revendication 5, dans laquelle lesdits au moins deux oligomères d'amplification comprennent l'oligomère d'amplification de (a)(i) ;

dans laquelle la séquence d'hybridation de cible de (a)(i) est choisie dans le groupe constitué des SEQ ID NOs : 21, 23-25, 32, 33, 35, 54 et 55,
ou dans laquelle la séquence d'hybridation de cible de (a)(i) est contenue dans la séquence de SEQ ID NO : 164, comprend la séquence SEQ ID NO : 165, et est éventuellement choisi dans le groupe constitué des SEQ ID NOs : 21, 23-25, 32, 33, et 35.

7. Combinaison d'oligomères de la revendication 5, dans laquelle lesdits au moins deux oligomères d'amplification comprennent l'oligomère d'amplification de (a)(ii),

dans laquelle la séquence d'hybridation de cible de (a)(ii) comprend la séquence de SEQ ID NO : 167 et est éventuellement choisi dans le groupe constitué des SEQ ID NOs : 28-31, 34, 40, 41, et 49-51 ; ou
dans laquelle la séquence d'hybridation de cible de (a)(ii) comprend la séquence de SEQ ID NO : 168 et est éventuellement choisi dans le groupe constitué de SEQ ID NOs : 38, 39, 43, 44 et 53.

8. Combinaison d'oligomères de l'une quelconque des revendications 5 à 7, dans laquelle la séquence d'hybridation de cible de (b) est choisie dans le groupe constitué des SEQ ID NOs : 80-82 et 85-100,
ou dans laquelle la séquence d'hybridation de cible (b) est contenue dans SEQ ID NO : 170, comprend la séquence

de SEQ ID NO : 171 ou SEQ ID NO : 172, et est éventuellement choisi parmi (i) le groupe constitué de SEQ ID NOs : 81, 82, 85, 87-90, 94 et 96-98 ou (ii) le groupe constitué de SEQ ID NOs : 80, 82, 85 et 87-100.

9. Combinaison d'oligomères de l'une quelconque des revendications 5 à 8, dans laquelle l'oligomère d'amplification de (b) est une amorce de promoteur ou un fournisseur de promoteur comprenant en outre une séquence de promoteur située en 5' de la séquence d'hybridation de cible de (b), éventuellement dans laquelle la séquence de promoteur est une séquence de promoteur T7, éventuellement dans laquelle l'oligomère d'amplification de (b) comprend une séquence choisie dans le groupe constitué de SEQ ID NOs : 57-59 et 62-77.

10. Combinaison d'oligomères de la revendication 5, dans laquelle lesdits au moins deux oligomères d'amplification comprennent les premier et second oligomères d'amplification comme dans (a)(ii), éventuellement dans laquelle

le premier oligomère d'amplification comme dans (a)(ii) comprend une séquence d'hybridation de cible comprenant la séquence de SEQ ID NO : 167, éventuellement dans laquelle la première séquence d'hybridation de cible d'oligomère d'amplification est SEQ ID NO : 34 ; et
le second oligomère d'amplification tel que décrit en (a)(ii) comprend une séquence d'hybridation de cible comprenant la séquence de SEQ ID NO : 168, éventuellement dans lequel la seconde séquence d'hybridation de cible d'oligomère d'amplification est SEQ ID NO : 53.

11. Combinaison d'oligomères de la revendication 5, dans laquelle lesdits au moins deux oligomères d'amplification comprennent un oligomère d'amplification tel que défini en (a)(i) et un oligomère d'amplification tel que défini en (a)(ii), éventuellement dans laquelle

l'oligomère d'amplification en (a)(i) comprend une séquence d'hybridation de cible qui est contenue dans la séquence de SEQ ID NO : 164 et comprend la séquence SEQ ID NO : 165, éventuellement dans laquelle l'oligomère d'amplification en (a)(i) comprend la séquence d'hybridation de cible de SEQ ID NO : 21 ; et
l'oligomère d'amplification tel que défini en a) ii) comprend la séquence SEQ ID NO : 167, éventuellement dans laquelle l'oligomère d'amplification en (a)(ii) comprend la séquence d'hybridation de cible de SEQ ID NO : 34.

12. Combinaison d'oligomères de l'une quelconque des revendications 10 et 11, dans laquelle lesdits au moins deux oligomères d'amplification comprennent les premier et second oligomères d'amplification de (b), éventuellement dans laquelle chacun des premier et second oligomères d'amplification de (b) comprend une séquence d'hybridation de cible qui est contenue dans SEQ ID NO : 170 et comprend la séquence SEQ ID NO : 171 ou SEQ ID NO : 172, éventuellement, dans laquelle le premier oligomère d'amplification en (b) comprend la séquence d'hybridation de cible de SEQ ID NO : 94 et le second oligomère d'amplification en (b) comprend la séquence d'hybridation de cible de SEQ ID NO : 95.

13. Combinaison d'oligomères de l'une quelconque des revendications 5 à 12, comprenant en outre au moins un oligomère de sonde de capture comprenant une séquence d'hybridation de cible attachée de manière covalente à une séquence ou à une fraction qui se lie à une sonde immobilisée, dans laquelle ladite séquence d'hybridation de cible a une longueur allant jusqu'à 30 nucléotides contigus et comprend une séquence choisie dans le groupe constitué de SEQ ID NOs : 11-15, 17, 19 et 20, y compris des équivalents ADN et des chimères ADN/ARN de ceux-ci.

14. Combinaison d'oligomères de l'une quelconque des revendications 5 à 13, comprenant en outre au moins un oligomère de sonde de détection comprenant une séquence d'hybridation de cible configurée pour s'hybrider spécifiquement à un amplicon d'espèces de *Plasmodium* amplifiable par lesdits au moins deux oligomères d'amplification.

15. Combinaison d'oligomères de la revendication 14, dans laquelle l'oligomère de la sonde de détection comprend :

(a) une séquence d'hybridation de cible qui est de 13 à 40 nucléotides de longueur contigus, est contenue dans la séquence SEQ ID NO : 196 ou son complément, ou un équivalent ADN ou un chimère ADN/ARN de celui-ci, et comprend la séquence SEQ ID NO : 175, SEQ ID NO : 176, SEQ ID NO : 177 ou SEQ ID NO : 178 ;
(b) une séquence d'hybridation de cible qui est contenue dans la séquence SEQ ID NO : 197 ou son complément, ou un équivalent ADN ou un chimère ADN/ARN de celui-ci, et comprend la séquence SEQ ID NO : 174, SEQ ID NO : 175 ou SEQ ID NO : 176;
(d) une séquence d'hybridation de cible comprenant la séquence de l'un quelconque des numéros SEQ ID NO : 131-146 ; ou

(e) la séquence de l'une quelconque des séquences SEQ ID NO : 147-161.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 62782945 **[0001]**
- CN 102220419 B **[0005]**
- WO 2009074649 A **[0005]**
- US 10093989 B **[0065] [0133]**
- WO 2017189746 A **[0065] [0133]**
- WO 9532305 A **[0066]**
- US 5378825 A **[0066]**
- US 6949367 B **[0066]**
- WO 9313121 A **[0066]**
- US 5585481 A **[0066] [0116]**
- US 5846717 A **[0072]**
- US 6706471 B **[0072]**
- US 5614402 A **[0072] [0131]**
- US 4786600 A **[0088]**
- US 4683195 A **[0088]**
- US 4683202 A **[0088]**
- US 4800159 A **[0088]**
- US 5427930 A **[0088]**
- US 5516663 A **[0088]**
- US 5422252 A **[0088]**
- US 5547861 A **[0088]**
- US 5648211 A **[0088]**
- US 4868105 A **[0089]**
- US 5124246 A **[0089]**
- US 5130238 A **[0089]**
- US 5437990 A **[0089]**
- US 5554516 A **[0089]**
- US 7374885 B **[0089]**
- WO 8801302 A **[0089]**
- WO 8810315 A **[0089]**
- WO 9503430 A **[0089]**
- US 5118801 A **[0092] [0116] [0117] [0124]**
- US 5312728 A **[0092] [0116] [0117] [0124]**
- US 6849412 B **[0092] [0117] [0124]**
- US 6835542 B **[0092] [0117] [0124]**
- US 6534274 B **[0092] [0117] [0124]**
- US 6361945 B **[0092] [0117] [0124]**
- US 20060068417 A **[0092]**
- US 5283174 A **[0094] [0123]**
- US 5656207 A **[0094]**
- US 5658737 A **[0094]**
- US 5639604 A **[0094] [0116] [0123]**
- US 5547842 A **[0094]**
- US 4581333 A **[0094]**
- US 6180340 B **[0094] [0116]**
- US 6350579 B **[0094] [0116]**
- WO 2008016988 A **[0097] [0134]**
- US 5185439 A **[0116]**
- US 5656744 A **[0116]**
- US 5424413 A **[0122]**
- US 5451503 A **[0122]**
- US 5849481 A **[0122]**
- WO 89002476 A **[0124]**
- US 5925517 A **[0124]**
- US 6150097 A **[0124]**
- US 20060068417 A1 **[0124]**
- US 20060194240 A1 **[0124]**
- US 6110678 A **[0134] [0135]**
- US 6280952 B **[0134]**
- US 6534273 B **[0134]**

Non-patent literature cited in the description

- The Biochemistry of the Nucleic Acids. 1992, 5-36 **[0066]**
- *BioTechniques,* vol. 43, 617-24 **[0066]**
- *Biochemistry,* 2004, vol. 43, 13233-41 **[0066]**
- *Nat. Biotechnol.,* 1999, vol. 17, 292-296 **[0072]**
- *Mol. Diagn.,* 1999, vol. 4, 135-144 **[0072]**
- *J. Clin. Microbiol.,* 2006, vol. 44, 3443-3447 **[0072]**
- **SAMBROOK et al.** Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0094]**
- **TYAGI et al.** *Nature Biotechnol,* 1998, vol. 16, 49-53 **[0116]**
- **MANIATIS et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, 2002 **[0127]**
- Guide to Molecular Cloning Techniques. **BERGER ; KIMMEL.** Methods in Enzymology. Academic Press, Inc, 1987, vol. 152 **[0127]**
- **DRUMMOND et al.** *Nat. Biotechnol.,* 2003, vol. 21, 1192 **[0127]**
- **GOODING.** *Electroanalysis,* 2002, vol. 14, 1149 **[0127]**
- **WANG.** *Anal. Chim. Acta,* 2002, vol. 469, 63 **[0127]**
- **CAGNIN et al.** *Sensors,* 2009, vol. 9, 3122 **[0127]**
- **KATZ ; WILLNER.** *Electroanalysis,* 2003, vol. 15, 913 **[0127]**
- **DANIELS ; POURMAND.** *Electroanalysis,* 2007, vol. 19, 1239 **[0127]**
- **LYAMICHEV et al.** *Nat. Biotechnol.,* 1999, vol. 17, 292-296 **[0129]**

- **RYAN et al.** *Mol. Diagn.,* 1999, vol. 4, 135-144 **[0129]**
- **ALLAWI et al.** *J. Clin. Microbiol.,* 2006, vol. 44, 3443-3447 **[0129]**
- **KAISER et al.** *J. Biol. Chem.,* 1999, vol. 274, 2138-721394 **[0130]**
- **LYAMICHEV et al.** *Science,* 1993, vol. 260, 778-783 **[0130]**
- **EIS et al.** *Nat. Biotechnol.,* 2001, vol. 19, 673-676 **[0130]**
- **SHEN et al.** *Trends in Bio. Sci.,* 1998, vol. 23, 171-173 **[0130]**
- **KAISER et al.** *J. Biol. Chem.,* 1999, vol. 274, 21387-21394 **[0130]**
- **MA et al.** *J. Biol. Chem.,* 2000, vol. 275, 24693-24700 **[0130]**
- **ALLAWI et al.** *J. Mol. Biol.,* 2003, vol. 328, 537-554 **[0130]**
- **SHARMA et al.** *J. Biol. Chem.,* 2003, vol. 278, 23487-23496 **[0130]**
- **FENG et al.** *Nat. Struct. Mol. Biol.,* 2004, vol. 11, 450-456 **[0130]**