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(54) **Title:** DETECTING *BABESIA* SPECIES NUCLEIC ACID IN A SAMPLE

(57) **Abstract:** There is described herein a method for specifically detecting *Babesia* species nucleic acid in a sample, which in one aspect comprises: (1) contacting a sample, said sample suspected of containing *Babesia* species nucleic acid, with at least two oligomers for amplifying a target region of a *Babesia* species target nucleic acid, wherein the at least two amplification oligomers comprise: (a) a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or 57; or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101; (iv) comprises or consists of SEQ ID NO:8; (v) comprises or consists of SEQ ID NO:83 and (b) a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and (i) is contained in SEQ ID NO:68 and comprises SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, or SEQ ID NO:85; or (ii) is contained in SEQ ID NO:67 and comprises SEQ ID NO:45 or SEQ ID NO:52;or (iii) is contained in SEQ ID NO:70 and comprises SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or SEQ ID NO:51; (2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Babesia* 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 *Babesia* species target nucleic acid in said sample.

WO 2018/226798 A1

DETECTING *BABESIA* SPECIES NUCLEIC ACID IN A SAMPLE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application Nos. 62/516,530, filed June 7, 2017, and 62/520,793, filed June 16, 2017 the entire contents of each are incorporated herein by reference in their entirety.

BACKGROUND

[0002] Babesiosis is caused by infection of red blood cells by species of protozoan parasites of the genus *Babesia*. The species *Babesia microti* is responsible for most human babesiosis infections reported in the United States. Infections are typically asymptomatic in human individuals but can lead to severe illness or death, especially in elderly or immunosuppressed individuals. The parasite is transmitted to humans by exposure to deer ticks in endemic areas or by blood transfusion (transfusion-transmitted babesiosis (TTB)). Over 100 cases of transfusion-transmitted babesiosis have been reported to the FDA since 1979. Despite being reported as the most unaddressed infectious risk to the United States blood supply, there is still no licensed test for screening for *B. microti* in donated blood (*N Engl J Med.* (2016) 8, 375(23):2236-2245). The threat of TTB has led to a consensus by the Food and Drug Administration (FDA) and the American Association of Blood Banks (AABB) that screening of blood donations for *Babesia* is urgently required for blood safety. Initiation of blood donor screening to prevent TTB should be given high priority (*Curr Opin Hematol.* (2016) 23(6):573-580).

[0003] Therefore a specific and sensitive assay for detecting *Babesia* species in a sample is needed.

SUMMARY

[0004] In one aspect, there is provided a method for specifically detecting *Babesia* species nucleic acid in a sample, said method comprising: (1) contacting a sample, said sample suspected of containing *Babesia* species nucleic acid, with at least two oligomers for amplifying a target region of a *Babesia* species target nucleic acid, wherein the at least two amplification oligomers comprise: (a) a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of

SEQ ID NO:97 and comprises SEQ ID NO:101; (iii) that comprises or consists of SEQ ID NO:8; (iv) that comprises or consists of SEQ ID NO:83 and (b) a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and (i) is contained in SEQ ID NO:68 and comprises SEQ ID NO:85; or (ii) is contained in SEQ ID NO:70 and comprises SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, or SEQ ID NO:51, or (iii) comprises or consists of SEQ ID NO:84; (2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Babesia* 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 *Babesia* species target nucleic acid in said sample.

[0004A] In another aspect, there is provided a method for specifically detecting Babesia species nucleic acid in a sample, said method comprising: contacting a sample, said sample suspected of containing Babesia species nucleic acid, with at least two oligomers for amplifying a target region of a Babesia species target nucleic acid, wherein the at least two amplification oligomers comprise: a first amplification oligomer comprising a first target-hybridizing sequence that consists of SEQ ID NO:8; or that consists of SEQ ID NO:83; wherein the first amplification oligomer further comprises a promoter sequence joined to the 5'-end of the first target-hybridizing sequence; and a second amplification oligomer comprising a second target-hybridizing sequence that consists of SEQ ID NO: 34; SEQ ID NO:84; or SEQ ID NO:86; performing an in vitro nucleic acid amplification reaction, wherein any Babesia target nucleic acid present in said sample is used as a template for generating an amplification product; and detecting the presence or absence of the amplification product, thereby indicating the presence or absence of Babesia species target nucleic acid in said sample.

[0005] Suitably, the first target-hybridizing sequence comprises or consists of a sequence selected from the group consisting of: SEQ ID NOs:2, and 4 and 6 and 8, suitably, wherein the first target-hybridizing sequence comprises or consists of a sequence selected from the group consisting of: SEQ ID NOs:2, and 4 and 8.

[0006] Suitably, the second amplification oligomer comprises or consists of the sequence selected from the group consisting of: SEQ ID NOs:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35, 36, 84 and 86.

[0007] Suitably, the second amplification oligomer sequence comprises or consists of SEQ ID NO:21 or SEQ ID NO:27 or SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86.

[0008] Suitably, the first amplification oligomer is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the first target-hybridizing sequence.

Suitably, the promoter sequence is a T7 promoter sequence. Suitably, the T7 promoter sequence comprises or consists of SEQ ID NO:58.

[0009] Suitably, the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 5 and 7 and 82, suitably, wherein the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 7 and 82.

[0010] Suitably, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:SEQ ID NO:2 or 6 and SEQ ID NO:11; (b) SEQ ID NO:4 or 6 and SEQ ID NO:13; (c) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17; (d) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19; (e) S

NO:4 and SEQ ID NO:20; (f) SEQ ID NO:4 or 6 or 8 and SEQ ID NO:21; (g) SEQ ID NO:2 or 4 or 8 and SEQ ID NO:27; (h) SEQ ID NO:4 and SEQ ID NO:28; (i) SEQ ID NO:4 and SEQ ID NO:29; (j) SEQ ID NO:4 and SEQ ID NO:31; (k) SEQ ID NO:8 and SEQ ID NO:32; (l) SEQ ID NO:8 and SEQ ID NO:33; (m) SEQ ID NO:8 and SEQ ID NO:34; (n) SEQ ID NO:8 and SEQ ID NO:35; (o) SEQ ID NO:8 and SEQ ID NO:36; (p) SEQ ID NO:8 and SEQ ID NO:84; (q) SEQ ID NO:8 and SEQ ID NO:86; (r) SEQ ID NO:83 and SEQ ID NO:34; (s) SEQ ID NO:83 and SEQ ID NO:35; (t) SEQ ID NO:83 and SEQ ID NO:36; (u) SEQ ID NO:83 and SEQ ID NO:84; (v) SEQ ID NO:83 and SEQ ID NO:86.

[0011] Suitably, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27; (b) SEQ ID NO:4 and SEQ ID NO:21; (c) SEQ ID NO:8 and SEQ ID NO:21; (d) SEQ ID NO:8 and SEQ ID NO:34; (e) SEQ ID NO:8 and SEQ ID NO:84; (f) SEQ ID NO:8 and SEQ ID NO:86; (g) SEQ ID NO:83 and SEQ ID NO:34; (h) SEQ ID NO:83 and SEQ ID NO:84; (i) SEQ ID NO:83 and SEQ ID NO:86.

[0012] Suitably, the method further comprises purifying the target nucleic acid from other components in the sample before step (1).

[0013] Suitably, 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 (i) is from about 15 to 21 contiguous nucleotides contained in the sequence of SEQ ID NO:78; or (ii) is from about 21 to 30 contiguous nucleotides comprising the sequence of SEQ ID NO:78; or (iii) is the sequence is SEQ ID NO:44. Suitably, the capture probe oligomer sequence comprises or consists of SEQ ID NO:43.

[0014] Suitably, the detecting step (3) comprises contacting said *in vitro* nucleic acid amplification reaction with a detection probe oligomer 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 *Babesia* species in said sample.

[0015] Suitably, the detection probe oligomer comprises a target-hybridizing sequence that is from about 14 to about 40 nucleotides in length and is configured to specifically hybridize to a target sequence comprising or consisting of SEQ ID NO:59, the RNA equivalent of SEQ ID NO:59, the complement of SEQ ID NO:59, the RNA equivalent of the complement of SEQ ID NO:59, or SEQ ID NO:65, the DNA equivalent of SEQ ID NO:65, the

complement of SEQ ID NO:65, or the DNA equivalent of the complement of SEQ ID NO:65.

[0016] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:65 and includes at least the sequence of SEQ ID NO:37 or SEQ ID NO:42.

[0017] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:41 and includes at least the sequence of SEQ ID NO:38 or SEQ ID NO:39.

[0018] Suitably, the detection probe consists of the sequence selected from the group consisting of: SEQ ID NO:37, 38, 39, 41, 42, 91, 92, 94, or 99.

[0019] Suitably, the detection probe comprises or consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 and 99.

[0020] Suitably, the detection probe oligomer further comprises a 2' methoxy modification on at least one of a nucleotide residue member of the nucleotide sequence.

[0021] Suitably, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:11 and SEQ ID NO:39 or SEQ ID NO:37; (b) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38 or SEQ ID NO:39; (c) SEQ ID NO:4 and SEQ ID NO:13 and SEQ ID NO:39 or SEQ ID NO:37; (d) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17 and SEQ ID NO:39; (e) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19 and SEQ ID NO:39 or SEQ ID NO:37; (f) SEQ ID NO:4 and SEQ ID NO:20 and SEQ ID NO:39 or SEQ ID NO:37; (g) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37; (h) SEQ ID NO:4 and SEQ ID NO:27 and SEQ ID NO:39 or SEQ ID NO:38; (i) SEQ ID NO:4 and SEQ ID NO:28 and SEQ ID NO:39; (j) SEQ ID NO:4 and SEQ ID NO:29 and SEQ ID NO:39 or SEQ ID NO:37; (k) SEQ ID NO:4 and SEQ ID NO:31 and SEQ ID NO:39; (l) SEQ ID NO:6 and SEQ ID NO:11 and SEQ ID NO:37; (m) SEQ ID NO:6 and SEQ ID NO:13 and SEQ ID NO:37; (n) SEQ ID NO:6 and SEQ ID NO:21 and SEQ ID NO:37; (o) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37 or SEQ ID NO:42; (p) SEQ ID NO:8 and SEQ ID NO:27 and SEQ ID NO:39; (q) SEQ ID NO:8 and SEQ ID NO:32 and SEQ ID NO:37 or SEQ ID NO:42; (r) SEQ ID NO:8 and SEQ ID NO:33 and SEQ ID NO:37 or SEQ ID NO:42; (s) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (t) SEQ ID NO:8 and SEQ ID NO:35 and SEQ ID NO:37 or SEQ ID NO:42; (u) SEQ ID NO:8

and SEQ ID NO:36 and SEQ ID NO:37 or SEQ ID NO:42; (v) SEQ ID NO:8, and SEQ ID NO:34, 84, or 86, and SEQ ID NO:91, 92, or 93; (v) SEQ ID NO:8, and SEQ ID NO:34, 84, or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99; or (x) SEQ ID NO:83, and SEQ ID NO:34, 84, or, 86, and SEQ ID NO:91, 92, or 93.

[0022] Suitably, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38; (b) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39; (c) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:37 or SEQ ID NO:39; (d) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37, 42, 91, 92 or 93; (e) SEQ ID NO:8 and SEQ ID NO:34, or 84 or 86 and SEQ ID NO:37, 42, 91, 92 or 93; (f) SEQ ID NO:83 and SEQ ID NO:34, 84 or 86 and SEQ ID NO:37, 42, 91, 92 or 93; (g) SEQ ID NO:8 and SEQ ID NO:34, 84 or 86 and SEQ ID NO:37, 42, 91, 92 or 93; (h) SEQ ID NO:83 and SEQ ID NO:34, 84 or 86 and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99; or (i) SEQ ID NO:83 and SEQ ID NO:34, 84 or 86 and SEQ ID NO:37, 42, 91, 92 or 93.

[0023] Suitably, the detection probe comprises a label. Suitably, the label is a chemiluminescent label or a fluorescent label. Suitably, the detecting step (3) occurs during the amplifying step (2). Suitably, the detection probe comprises a fluorescent label and a quencher. Suitably, the detection probe is selected from the group consisting of a molecular torch, a molecular beacon, and a TaqMan detection probe. Suitably, the detection probe further comprises a non-target-hybridizing sequence. Suitably, the detection probe is a molecular torch or a molecular beacon.

[0024] Suitably, the amplification reaction at step (2) is an isothermal amplification reaction. Suitably, the amplification reaction is a transcription-mediated amplification (TMA) reaction. Suitably, the amplification reaction is a real-time amplification reaction.

[0025] Suitably, the sample is a clinical sample. Suitably, the sample is a blood sample. Suitably, the sample is a lysed blood cell sample. Suitably, the lysed blood cell sample is a lysed red blood cell sample.

[0026] Suitably, the amplification product has a length of from 180 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof.

[0027] In a further aspect, there is described a method for specifically detecting *Babesia* species nucleic acid in a sample, said method comprising: (1) contacting a sample, said sample suspected of containing *Babesia* species nucleic acid, with at least two oligomers for amplifying a target region of a *Babesia* species target nucleic acid, wherein two of said at least

two amplification oligomers are selected from the group consisting of: (a) a first amplification oligomer and a second amplification oligomer, wherein the first amplification oligomer comprises a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101; (iii) that comprises/consists of SEQ ID NO:8; (v) comprises or consists of SEQ ID NO:83; or (b) a first amplification oligomer and a second amplification oligomer, wherein the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and (i) is contained in SEQ ID NO:68 and contains SEQ ID NO:52, SEQ ID NO:53 SEQ ID NO:54, SEQ ID NO:55, or SEQ ID NO:85, or (ii) is contained in SEQ ID NO:70 and contains SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or SEQ ID NO:51, or (iii) comprises or consists of SEQ ID NO:84; (2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Babesia* target nucleic acid present in said sample is used as a template for generating an amplification product, wherein said amplification product has a length of from 180 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof; and (3) detecting the presence or absence of the amplification product, thereby indicating the presence or absence of *Babesia* species target nucleic acid in said sample.

[0028] Suitably, the first target-hybridizing sequence comprises or consists of a sequence selected from the group consisting of: SEQ ID NOs:2, and 4 and 6 and 8, suitably, wherein the first target-hybridizing sequence comprises or consists of a sequence selected from the group consisting of: SEQ ID NOs:2, and 4 and 8.

[0029] Suitably, the second amplification oligomer comprises or consists of the sequence selected from the group consisting of: SEQ ID NOs:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35, 36, 84, and 86.

[0030] Suitably, the second amplification oligomer sequence comprises or consists of SEQ ID NO:21 or SEQ ID NO:27 or SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86.

[0031] Suitably, the first amplification oligomer is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the first target-hybridizing sequence. Suitably, the promoter sequence is a T7 promoter sequence. Suitably, the T7 promoter

sequence comprises or consists of SEQ ID NO:58. Suitably, the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 5 and 7 and 82, suitably, wherein the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 7 and 82.

[0032] Suitably, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 or 6 and SEQ ID NO:11; (b) SEQ ID NO:4 or 6 and SEQ ID NO:13; (c) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17; (d) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19; (e) SEQ ID NO:4 and SEQ ID NO:20; (f) SEQ ID NO:4 or 6 or 8 and SEQ ID NO:21; (g) SEQ ID NO:2 or 4 or 8 and SEQ ID NO:27; (h) SEQ ID NO:4 and SEQ ID NO:28; (i) SEQ ID NO:4 and SEQ ID NO:29; (j) SEQ ID NO:4 and SEQ ID NO:31; (k) SEQ ID NO:8 and SEQ ID NO:32; (l) SEQ ID NO:8 and SEQ ID NO:33; (m) SEQ ID NO:8 and SEQ ID NO:34; (n) SEQ ID NO:8 and SEQ ID NO:35; (o) SEQ ID NO:8 and SEQ ID NO:36; (p) SEQ ID NO:8 and SEQ ID NO:84; (q) SEQ ID NO:8 and SEQ ID NO:86; (r) SEQ ID NO:83 and SEQ ID NO:34; (s) SEQ ID NO:83 and SEQ ID NO:84; (t) SEQ ID NOs:83 and SEQ ID NO:86.

[0033] Suitably, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27; (b) SEQ ID NO:4 and SEQ ID NO:21; (c) SEQ ID NO:8 and SEQ ID NO:21; (d) SEQ ID NO:8 and SEQ ID NO:34; (e) SEQ ID NO:8 and SEQ ID NO:84 ; (f) SEQ ID NO:8 and SEQ ID NO:86; (g) SEQ ID NO:83 and SEQ ID NO:34; (h) SEQ ID NO:83 and SEQ ID NO:84; (i) SEQ ID NO:83 and SEQ ID NO:86.

[0034] Suitably, the method further comprises purifying the target nucleic acid from other components in the sample before step (1).

[0035] Suitably, 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 (i) is from about 15 to 21 contiguous nucleotides contained in the sequence of SEQ ID NO:78; or (ii) is from about 21 to about 30 contiguous nucleotides comprising the sequence of SEQ ID NO:78; or (iii) the sequence consists of SEQ ID NO:44.

[0036] Suitably, the capture probe oligomer sequence comprises or consists of SEQ ID NO:43.

[0037] Suitably, the detecting step (3) comprises contacting said *in vitro* nucleic acid amplification reaction with a detection probe oligomer 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 *Babesia* species in said sample.

[0038] Suitably, the detection probe oligomer comprises a target-hybridizing sequence that is from about 14 to about 40 nucleotides in length and is configured to specifically hybridize to a target sequence comprising or consisting of SEQ ID NO:59, the RNA equivalent of SEQ ID NO:59, the complement of SEQ ID NO:59, the RNA equivalent of the complement of SEQ ID NO:59, SEQ ID NO:65, the DNA equivalent of SEQ ID NO:65, the complement of SEQ ID NO:65, or the DNA equivalent of the complement of SEQ ID NO:65.

[0039] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:59 and includes at least the sequence of SEQ ID NO:42, 92, 94, or 99.

[0040] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:41 and includes at least the sequence of SEQ ID NO:38 or SEQ ID NO:60.

[0041] Suitably, the detection probe oligomer comprises a nucleotide sequence that is from 16 to 25 contiguous nucleotides in length and specifically hybridizes to SEQ ID NO:65, or the DNA equivalent thereof; or specifically hybridizes to the complement of SEQ ID NO:65, or the DNA equivalent thereof.

[0042] Suitably, the detection probe oligomer sequence further comprises a nucleotide sequence containing SEQ ID NO:59 or SEQ ID NO:60.

[0043] Suitably, the detection probe oligomer further comprises a nucleotide sequence consisting of SEQ ID NO:37, 38, 39, 42, or 99.

[0044] Suitably, the detection probe target-hybridizing sequence consists of the sequence selected from the group consisting of: SEQ ID NOs:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 and 99.

[0045] Suitably, the detection probe oligomer further comprises a 2' methoxy modification on at least one of a nucleotide residue member of the nucleotide sequence.

[0046] Suitably, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:11 and SEQ ID NO:39 or SEQ ID NO:37; (b) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38 or SEQ ID NO:39; (c) SEQ ID NO:4 and SEQ ID NO:13 and SEQ ID NO:39 or SEQ ID NO:37; (d) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17 and SEQ ID NO:39; (e)

SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19 and SEQ ID NO:39 or SEQ ID NO:37; (f) SEQ ID NO:4 and SEQ ID NO:20 and SEQ ID NO:39 or SEQ ID NO:37; (g) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37; (h) SEQ ID NO:4 and SEQ ID NO:27 and SEQ ID NO:39 or SEQ ID NO:38; (i) SEQ ID NO:4 and SEQ ID NO:28 and SEQ ID NO:39; (j) SEQ ID NO:4 and SEQ ID NO:29 and SEQ ID NO:39 or SEQ ID NO:37; (k) SEQ ID NO:4 and SEQ ID NO:31 and SEQ ID NO:39; (l) SEQ ID NO:6 and SEQ ID NO:11 and SEQ ID NO:37; (m) SEQ ID NO:6 and SEQ ID NO:13 and SEQ ID NO:37; (n) SEQ ID NO:6 and SEQ ID NO:21 and SEQ ID NO:37; (o) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37 or SEQ ID NO:42; (p) SEQ ID NO:8 and SEQ ID NO:27 and SEQ ID NO:39; (q) SEQ ID NO:8 and SEQ ID NO:32 and SEQ ID NO:37 or SEQ ID NO:42; (r) SEQ ID NO:8 and SEQ ID NO:33 and SEQ ID NO:37 or SEQ ID NO:42; (s) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (t) SEQ ID NO:8 and SEQ ID NO:35 and SEQ ID NO:37 or SEQ ID NO:42; or (u) SEQ ID NO:8 and SEQ ID NO:36 and SEQ ID NO:37 or SEQ ID NO:42; (v) SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (w) SEQ ID NO:8, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; (x) SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; (y) SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (z) SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or (aa) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[0047] Suitably, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38; (b)SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39; (c)SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:37 or SEQ ID NO:39; or (d) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (e) SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (f) SEQ ID NO:8, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; (g) SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; (h) SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (i) SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or (j) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[0048] Suitably, the detection probe comprises a label. Suitably, the label is a chemiluminescent label or a fluorescent label. Suitably, the detecting step (3) occurs during

the amplifying step (2). Suitably, the detection probe comprises a fluorescent label and a quencher. Suitably, the detection probe is selected from the group consisting of a molecular torch, a molecular beacon, and a TaqMan detection probe. Suitably, the detection probe further comprises a non-target-hybridizing sequence. Suitably, the detection probe is a molecular torch or a molecular beacon.

[0049] Suitably, the amplification reaction at step (2) is an isothermal amplification reaction. Suitably, the amplification reaction is a transcription-mediated amplification (TMA) reaction. Suitably, the amplification reaction is a real-time amplification reaction.

[0050] Suitably, the sample is a clinical sample. Suitably, the sample is a blood sample. Suitably, the sample is a lysed blood cell sample.

[0051] Suitably, the lysed blood cell sample is a lysed red blood cell sample.

[0052] In a further aspect, there is described a combination of at least two oligomers for determining the presence or absence of *Babesia* in a sample, said oligomer combination comprising first and second amplification oligomers for amplifying a target region of *Babesia* target nucleic acid, wherein (a) the first amplification oligomer comprises a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101; (iii) comprises or consists of SEQ ID NO:8; (iv) comprises or consists of SEQ ID NO:83; wherein the first amplification oligomer is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the first target-hybridizing sequence;_and (b) the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and (i) is contained in SEQ ID NO:68 and comprises SEQ ID NO:85; or (ii) is contained in SEQ ID NO:70 and comprises SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, or SEQ ID NO:51; or (iii) comprises or consists of SEQ ID NO:84.

[0053] Suitably, the first amplification comprises or consists of the sequence selected from the group consisting of: SEQ ID NOs:2 and 4 and 6 and 8 and 83, suitably, wherein the first amplification comprises or consists of the sequence selected from the group consisting of: SEQ ID NOs:2 and 4 and 8 and 83.

[0054] Suitably, the second amplification oligomer comprises or consists of the sequence selected from the group consisting of: SEQ ID NOs:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35, 36, 84, and 86.

[0055] Suitably, the second amplification oligomer sequence comprises or consists of SEQ ID NO:21 or SEQ ID NO:27 or SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86.

[0056] Suitably, the first amplification oligomer is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the first target-hybridizing sequence. Suitably, the promoter sequence is a T7 promoter sequence. Suitably, the T7 promoter sequence comprises or consists of SEQ ID NO:58.

[0057] Suitably, the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 5 and 7 and 82, suitably, wherein the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 7 and 82.

[0058] Suitably, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 or 6 and SEQ ID NO:11; (b) SEQ ID NO:4 or 6 and SEQ ID NO:13; (c) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17; (d) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19; (e) SEQ ID NO:4 and SEQ ID NO:20; (f) SEQ ID NO:4 or 6 or 8 and SEQ ID NO:21; (g) SEQ ID NO:2 or 4 or 8 and SEQ ID NO:27; (h) SEQ ID NO:4 and SEQ ID NO:28; (i) SEQ ID NO:4 and SEQ ID NO:29; (j) SEQ ID NO:4 and SEQ ID NO:31; (k) SEQ ID NO:8 and SEQ ID NO:32; (l)SEQ ID NO:8 and SEQ ID NO:33; (m) SEQ ID NO:8 and SEQ ID NO:34; (n) SEQ ID NO:8 and SEQ ID NO:35; (o) SEQ ID NO:8 and SEQ ID NO:36; (p) SEQ ID NO:8 and SEQ ID NO:84; (q) SEQ ID NO:8 and SEQ ID NO:86; (r) SEQ ID NO:83 and SEQ ID NO:34; (s) SEQ ID NO:83 and SEQ ID NO:84; or (t) SEQ ID NO:83 and SEQ ID NO:86.

[0059] Suitably, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27; (b) SEQ ID NO:4 and SEQ ID NO:21; (c) SEQ ID NO:8 and SEQ ID NO:21; (d) SEQ ID NO:8 and SEQ ID NO:34; (e) SEQ ID NO:8 and SEQ ID NO:84; (f) SEQ ID NO:8 and SEQ ID NO:86; (g) SEQ ID NO:83 and SEQ ID NO:34; (h) SEQ ID NO:83 and SEQ ID NO:84; or (i) SEQ ID NO:83 and SEQ ID NO:86.

[0060] Suitably, the combination further comprises at least one capture probe oligomer.

[0061] Suitably, the at least one capture probe oligomer comprises a target-hybridizing sequence covalently attached to a sequence or moiety that binds to an immobilized probe, wherein said target-hybridizing sequence (i) is from about 15 to about 21 contiguous

nucleotides contained in the sequence of SEQ ID NO:78, or (ii) is about 21 to 30 contiguous nucleotides comprising the sequence of SEQ ID NO:78; or (iii) the sequence consists of SEQ ID NO:44.

[0062] Suitably, the capture probe oligomer sequence comprises or consists of SEQ ID NO:43.

[0063] Suitably, the combination further comprises a detection probe oligomer.

[0064] Suitably, the detection probe oligomer comprises a target-hybridizing sequence that is from about 14 to about 40 nucleotides in length and is configured to specifically hybridize to a target sequence contained in SEQ ID NO:59, the RNA equivalent of SEQ ID NO:59, the complement of SEQ ID NO:59, the RNA equivalent of the complement of SEQ ID NO:59, SEQ ID NO:65, the DNA equivalent of SEQ ID NO:65, the complement of SEQ ID NO:65, or the DNA equivalent of the complement of SEQ ID NO:65.

[0065] Suitably, the detection probe target-hybridizing sequence contains the sequence of SEQ ID NO:59 and includes at least the sequence of SEQ ID NO:42, 92, 94, or 99.

[0066] Suitably, the detection probe target-hybridizing sequence contains the sequence of SEQ ID NO:60 and includes at least the sequence of SEQ ID NO:38 or SEQ ID NO:39.

[0067] Suitably, the detection probe target hybridising sequence consists of the sequence selected from the group consisting of: SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[0068] Suitably, the detection probe oligomer comprises a nucleotide sequence that is from 16 to 25 contiguous nucleotides in length and specifically hybridizes to SEQ ID NO:65, or the DNA equivalent thereof; or specifically hybridizes to the complement of SEQ ID NO:65, or the DNA equivalent thereof.

[0069] Suitably, the detection probe oligomer sequence further comprises a nucleotide sequence containing SEQ ID NO:59 or SEQ ID NO:60.

[0070] Suitably, the detection probe oligomer further comprises a nucleotide sequence consisting of SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[0071] Suitably, the detection probe oligomer further comprises a 2' methoxy modification on at least one of a nucleotide residue member of the nucleotide sequence.

[0072] Suitably, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:11 and SEQ ID NO:39 or SEQ ID NO:37; (b) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38 or SEQ ID NO:39; (c) SEQ ID NO:4 and SEQ ID NO:13 and SEQ ID NO:39 or SEQ ID

NO:37; (d) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17 and SEQ ID NO:39; (e) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19 and SEQ ID NO:39 or SEQ ID NO:37; (f) SEQ ID NO:4 and SEQ ID NO:20 and SEQ ID NO:39 or SEQ ID NO:37; (g) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37; (h) SEQ ID NO:4 and SEQ ID NO:27 and SEQ ID NO:39 or SEQ ID NO:38; (i) SEQ ID NO:4 and SEQ ID NO:28 and SEQ ID NO:39; (j) SEQ ID NO:4 and SEQ ID NO:29 and SEQ ID NO:39 or SEQ ID NO:37; (k) SEQ ID NO:4 and SEQ ID NO:31 and SEQ ID NO:39; (l) SEQ ID NO:6 and SEQ ID NO:11 and SEQ ID NO:37; (m) SEQ ID NO:6 and SEQ ID NO:13 and SEQ ID NO:37; (n) SEQ ID NO:6 and SEQ ID NO:21 and SEQ ID NO:37; (o) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37 or SEQ ID NO:42; (p) SEQ ID NO:8 and SEQ ID NO:27 and SEQ ID NO:39; (q) SEQ ID NO:8 and SEQ ID NO:32 and SEQ ID NO:37 or SEQ ID NO:42; (r) SEQ ID NO:8 and SEQ ID NO:33 and SEQ ID NO:37 or SEQ ID NO:42; (s) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (t) SEQ ID NO:8 and SEQ ID NO:35 and SEQ ID NO:37 or SEQ ID NO:42; (u) SEQ ID NO:8 and SEQ ID NO:36 and SEQ ID NO:37 or SEQ ID NO:42; (v) SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (w) SEQ ID NO:8, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; (x) SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; (y) SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (z) SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or (aa) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[0073] Suitably, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38; (b) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39; (c) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:37 or SEQ ID NO:39; (d) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (e) SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (f) SEQ ID NO:8, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; (g) SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; (h) SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (i) SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or (j) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[0074] Suitably, the detection probe comprises a label. Suitably, the label is a chemiluminescent label or a fluorescent label. Suitably, the detection probe comprises a fluorescent label and a quencher. Suitably, the detection probe is selected from the group consisting of a molecular torch, a molecular beacon, and a TaqMan detection probe. Suitably, the detection probe further comprises a non-target-hybridizing sequence. Suitably, the detection probe is a molecular torch or a molecular beacon.

[0075] In a further aspect, there is described a detection probe oligomer comprising (a) a target-hybridizing sequence that is (i) SEQ ID NO:37, SEQ ID NO:42, SEQ ID NO:59, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, or SEQ ID NO:98, (ii) the RNA equivalent of (i), (iii) the complement of (i), or (iv) the RNA equivalent of the complement of (i); and (b) a fluorescent or chemiluminescent label.

[0076] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:59 and includes at least the sequence of SEQ ID NO:42, 92, 94 or 99.

[0077] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:65 and includes at least the sequence of SEQ ID NO:59 or SEQ ID NO:94.

[0078] Suitably, the detection probe target hybridising sequence consists of the sequence selected from the group consisting of: SEQ ID NOs:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 and 99.

[0079] Suitably, the detection probe oligomer further comprises a 2' methoxy modification on at least one of a nucleotide residue member of the nucleotide sequence.

[0080] Suitably, the detection probe comprises a label. Suitably, the label is a chemiluminescent label or a fluorescent label. Suitably, the detection probe comprises a fluorescent label and a quencher. Suitably, the detection probe is selected from the group consisting of a molecular torch, a molecular beacon, and a TaqMan detection probe.

[0081] Suitably, the detection probe further comprises a non-target-hybridizing sequence.

[0082] Suitably, the detection probe is a molecular torch or a molecular beacon.

[0083] In a further aspect, there is described a capture probe oligomer for specifically isolating *Babesia* species nucleic acid from a sample, said 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 from about 15 to about 30 contiguous nucleotides contained in the sequence of SEQ ID NO:78.

[0084] Suitably, the capture probe oligomer sequence comprises or consists of SEQ ID NO:43.

[0085] In a further aspect, there is described a kit comprising the combination of at least two oligomers according to any of claims 78 to 107.

[0086] In a further aspect, there is described a reaction mixture comprising the combination of at least two oligomers according to any of claims 78 to 107.

[0087] In a further aspect, there is described the use of the combination of at least two oligomers according to any of claims 78 to 107 for specifically amplifying *Babesia* species nucleic acid in a sample.

[0088] In a further aspect, there is described the use of the detection probe oligomer according to any of claims 108 to 121 for specifically detecting *Babesia* species nucleic acid in a sample.

[0089] In a further aspect, there is described the use of the capture probe oligomer according to claim 112 or 123 for specifically capturing *Babesia* species nucleic acid from a sample.

[0090] Suitably, *Babesia microti* and/or *Babesia divergens* and/or *Babesia duncani* and/or *Babesia venatorum* are detected.

[0090A] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0090B] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

DEFINITIONS

[0091] 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 tothem unless specified otherwise.

[0092] The terms "a," "an," and "the" include plural referents, unless the context clearly indicates otherwise.

[0093] "Sample" includes any specimen that may contain, or is suspected of containing, *Babesia* nucleic acid or components thereof, such as nucleic acids or fragments of *Babesia* 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 *Babesia* 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 *Babesia* 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. 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.

[0094] "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 WO95/32305), 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 aza-pyrimidines, 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 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 although nucleic acids may be purified from natural sources using routine techniques.

[0095] 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.

[0096] 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).

[0097] 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.

[0098] 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).

[0099] 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.

[00100] 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).

[00101] 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.

[00102] 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.

[00103] 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.

[00104] "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 *Babesia*. It is understood that other reasons exist for configuring a target-hybridizing sequence to have less than 100% complementarity to a target nucleic acid.

[00105] The term "targets a sequence," as used herein in reference to a region of *Babesia 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 *Babesia 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 *Babesia 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.

[00106] 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.

[00107] 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 *Babesia 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 *Babesia sp.* target nucleic acid. The oligonucleotide is designed to function as a component of an assay for detection of *Babesia sp.* from a sample, and therefore is designed to target *Babesia 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.

[00108] The term "fragment," as used herein in reference to a *Babesia sp.* targeted nucleic acid, refers to a piece of contiguous nucleic acid.

[00109] The term "region," as used herein, refers to a portion of a nucleic acid wherein said 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.

[00110] 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).

[00111] 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.

[00112] 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 – such as a first amplification oligomer as described herein – 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).

[00113] 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.

[00114] 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.

[00115] "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.

[00116] "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 HSV 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.

[00117] As used herein, the term "real-time TMA" refers to single -primer transcription-mediated amplification ("TMA") of target nucleic acid that is monitored by real-time detection means.

[00118] 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.

[00119] "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.

[00120] 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.

[00121] 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 labelling 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 labelling 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).

[00122] 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 target closing domains may also function as target binding domains. Thus, target closing sequences can include, target binding sequences, non-target binding sequences, and combinations thereof.

[00123] "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. 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., 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., Aio to A40), or of about 14 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 T0-4A1040 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.

[00124] 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

[00125] The present disclosure is generally directed to methods and compositions for determining the presence or absence of the protozoan parasite *Babesia sp.* in a sample – such as a blood sample. Suitably, the methods and compositions described herein are able to detect the presence of *Babesia microti* and/or *Babesia divergens* and/or *Babesia duncani* and/or *Babesia venatorum.* In some embodiments, the present disclosure provides methods and compositions for diagnosing Babesiosis in a subject. In other, non-mutually exclusive embodiments, the present disclosure provides methods for the detection of *Babesia sp.* in a sample, where the method includes performing amplification-based detection of a target nucleic from *Babesia sp.* The present disclosure further provides compositions (including reaction mixtures) and kits comprising a combination of oligomers for detecting *Babesia sp.* – including *Babesia microti* and/or *Babesia divergens* and/or *Babesia duncani* and/or *Babesia venatorum* - in a sample. The oligomer combination generally includes at least two amplification oligomers for detecting *Babesia sp.* – including *Babesia microti* and/or

Babesia divergens and/or *Babesia duncani* and/or *Babesia venatorum* - in a sample, and may further include one or more additional oligomers as described herein for performing amplification-based detection of *Babesia sp.* – including *Babesia microti* and/or *Babesia divergens* and/or *Babesia duncani* and/or *Babesia venatorum* - such as, for example, a capture probe and/or a detection probe.

[00126] The methods for diagnosing Babesiosis *sp.* generally include detecting the presence or absence of *Babesia sp.* in a sample from a subject. The sample may be suspected of being infected with or containing *Babesia sp.* The subject may be suspected of being infected with *Babesia sp.* or having Babesiosis. In particular, an assay is performed for the specific detection in the sample of *Babesia sp.* nucleic acid. Based on the results from the detection assay, a status of either positive or negative is assigned for the *Babesia sp.*. The presence or absence of Babesiosis in the subject can be determined based on the *Babesia sp.* status.

[00127] While *Babesia sp.* nucleic acid may be detected using any suitable method, it is preferred that these protozoan parasites are detected using a nucleic-acid-based detection assay. Nucleic-acid-based detection assays generally utilize oligonucleotides that specifically hybridize to a target nucleic acid of *Babesia sp.* with minimal cross-reactivity to other nucleic acids suspected of being in a sample. Accordingly, oligonucleotides for nucleic-acid-based detection of *Babesia sp.* will have minimal cross-reactivity to other nucleic acids including, for example, *P. falciparum.*

[00128] A positive signal from a nucleic-acid-based detection assay in accordance with the present disclosure is indicative of the presence of one or more of *Babesia microti*, *Babesia divergens*, *Babesia duncani* and/or *Babesia venatorum* in a sample.

[00129] In some embodiments of a method comprising the use of a nucleic-acid-base detection assay – such as an amplification-based assay - is used to detect *Babesia sp.*. Such methods generally include amplifying a target sequence within a target nucleic acid utilizing an *in vitro* nucleic acid amplification reaction and detecting the amplified product by, for example, specifically hybridizing the amplified product with a nucleic acid detection probe that provides a signal to indicate the presence of a target in the sample. The amplification step includes contacting the sample with two or more amplification oligomers specific for a target sequence in a target nucleic acid to produce an amplified product if the target nucleic acid is present in the sample. Amplification synthesizes additional copies of the target sequence or its complement such as, e.g., by using at least one nucleic acid polymerase to extend the sequence from an amplification oligomer (a primer) using a template strand. One

embodiment for detecting the amplified product uses a hybridizing step that includes contacting the amplified product with at least one probe specific for a sequence amplified by the selected amplification oligomers, e.g., a sequence contained in the target sequence flanked by a pair of selected amplification oligomers. 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.

[00130] For example, some amplification methods that use TMA amplification include the following steps. Briefly, the target nucleic acid that contains the sequence to be amplified is provided as single stranded nucleic acid (e.g., ssRNA or ssDNA). Those skilled in the art will appreciate that conventional melting of double stranded nucleic acid (e.g., dsDNA) may be used to provide single-stranded target nucleic acids. A promoter 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.

[00131] 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 configure 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.

[00132] In one aspect, there is provided a method for specifically detecting *Babesia* species nucleic acid in a sample, which comprises the use of at least two amplification oligomers comprising (a) a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or 57; or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ

ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101; (iv) comprises or consists of SEQ ID NO:8; (v) comprises or consists of SEQ ID NO:83 and (b) a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and (i) is contained in SEQ ID NO:68 and comprises SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, or SEQ ID NO:85; or (ii) is contained in SEQ ID NO:67 and comprises SEQ ID NO:45 or SEQ ID NO:52; or (iii) is contained in SEQ ID NO:70 and comprises SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or SEQ ID NO:51; or (iv) comprises or consists of SEQ ID NO:84. In certain embodiments comprising an amplification-based detection assay, a combination of at least two amplification oligomers is therefore utilized for the detection of a *Babesia sp.* nucleic acid.

[00133] Suitably, the first amplification oligomer comprises or consists of a sequence selected from the group consisting of: SEQ ID NOs:2 and 4 and 6 and 8 and 83, more suitably, wherein the first amplification oligomer comprises or consists of the sequence selected from the group consisting of: SEQ ID NOs:2 and 4 and 8 and 83.

[00134] The first amplification oligomer of the combination may be a promoter primer or promoter provider further comprising a promoter sequence located 5' to the first target-hybridizing sequence. Suitably, the promoter sequence is a T7 promoter sequence which optionally comprises or consists of SEQ ID NO:58. According to this embodiment, the first amplification oligomer may comprise or consist of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 5 and 7 and 82, suitably, wherein the first amplification oligomer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:1 and 3 and 7 and 82.

[00135] Suitably, the second amplification oligomer comprises or consists of a sequence selected from the group consisting of: SEQ ID NOs:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, and 86. More suitably, the second amplification oligomer sequence comprises or consists of SEQ ID NO:21 or SEQ ID NO:27 or SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86.

[00136] In one embodiment, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 or 6 and SEQ ID NO:11; (b) SEQ ID NO:4 or 6 and SEQ ID NO:13; (c) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17; (d) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19; (e) SEQ ID NO:4 and SEQ ID NO:20; (f) SEQ ID NO:4 or 6 or 8 and SEQ ID NO:21; (g) SEQ ID

NO:2 or 4 or 8 and SEQ ID NO:27; (h) SEQ ID NO:4 and SEQ ID NO:28; (i) SEQ ID NO:4 and SEQ ID NO:29; (j) SEQ ID NO:4 and SEQ ID NO:31; (k) SEQ ID NO:8 and SEQ ID NO:32; (l) SEQ ID NO:8 and SEQ ID NO:33; (m) SEQ ID NO:8 and SEQ ID NO:34; (n) SEQ ID NO:8 and SEQ ID NO:35; (o) SEQ ID NO:8 and SEQ ID NO:36; (p) SEQ ID NO:8 and SEQ ID NO:84; (q) SEQ ID NO:8 and SEQ ID NO:86; (r) SEQ ID NO:83 and SEQ ID NO:34; (s) SEQ ID NO:83 and SEQ ID NO:84; or (t) SEQ ID NO:83 and SEQ ID NO:86.

[00137] In another embodiment, the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27; (b) SEQ ID NO:4 and SEQ ID NO:21; (c) SEQ ID NO:8 and SEQ ID NO:21; (d) SEQ ID NO:8 and SEQ ID NO:34; (e) SEQ ID NO:8 and SEQ ID NO:84; (f) SEQ ID NO:8 and SEQ ID NO:86; (g) SEQ ID NO:83 and SEQ ID NO:34; (h) SEQ ID NO:83 and SEQ ID NO:84; or (i) SEQ ID NO:83 and SEQ ID NO:86.

[00138] In embodiments where the combination further includes one or more detection probe oligomers, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively may comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:11 and SEQ ID NO:39 or SEQ ID NO:37; (b) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38 or SEQ ID NO:39; (c) SEQ ID NO:4 and SEQ ID NO:13 and SEQ ID NO:39 or SEQ ID NO:37; (d) SEQ ID NO:4 and SEQ ID NO:16 or SEQ ID NO:17 and SEQ ID NO:39; (e) SEQ ID NO:4 and SEQ ID NO:18 or SEQ ID NO:19 and SEQ ID NO:39 or SEQ ID NO:37; (f) SEQ ID NO:4 and SEQ ID NO:20 and SEQ ID NO:39 or SEQ ID NO:37; (g) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37; (h) SEQ ID NO:4 and SEQ ID NO:27 and SEQ ID NO:39 or SEQ ID NO:38; (i) SEQ ID NO:4 and SEQ ID NO:28 and SEQ ID NO:39; (j) SEQ ID NO:4 and SEQ ID NO:29 and SEQ ID NO:39 or SEQ ID NO:37; (k) SEQ ID NO:4 and SEQ ID NO:31 and SEQ ID NO:39; (l) SEQ ID NO:6 and SEQ ID NO:11 and SEQ ID NO:37; (m) SEQ ID NO:6 and SEQ ID NO:13 and SEQ ID NO:37; (n) SEQ ID NO:6 and SEQ ID NO:21 and SEQ ID NO:37; (o) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:39 or SEQ ID NO:37 or SEQ ID NO:42; (p) SEQ ID NO:8 and SEQ ID NO:27 and SEQ ID NO:39; (q) SEQ ID NO:8 and SEQ ID NO:32 and SEQ ID NO:37 or SEQ ID NO:42; (r) SEQ ID NO:8 and SEQ ID NO:33 and SEQ ID NO:37 or SEQ ID NO:42; (s) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (t) SEQ ID NO:8 and SEQ ID NO:35 and SEQ ID NO:37 or SEQ ID NO:42; (u) SEQ ID NO:8 and SEQ ID NO:36 and SEQ ID NO:37 or SEQ ID NO:42; (v) SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (w) SEQ ID NO:8, and SEQ ID NO:86, and

SEQ ID NOs:91, 92 and/or 93; (x) SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; (y) SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (z) SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or (aa) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00139] In other embodiments where the combination further include one or more detection probe oligomers, the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively may comprise or consist of the nucleotide sequences of: (a) SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38; (b) SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39; (c) SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:37 or SEQ ID NO:39; (d) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37 or SEQ ID NO:42; (e) SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (f) SEQ ID NO:8, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; (g) SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; (h) SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; (i) SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or (j) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00140] As will be appreciated, the present disclosure contemplates the use of various combinations of first and second amplification oligomers, including: a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:52; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101,

and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:53; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:54; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:55; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:85; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second

amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and comprises SEQ ID NO:45; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and comprises SEQ ID NO:52; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:46; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:47; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second

amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:48; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:49; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:50; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:51; and a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is

contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising or consisting of SEQ ID NO:84.

[00141] Further combinations of first and second amplification oligomers, include: a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:52; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:53; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:54; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and

comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:55; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:85; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and comprises SEQ ID NO:45; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and comprises SEQ ID NO:52; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and

comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:46; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:47; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:48; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:49; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and

comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:50; a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:51; and a first amplification oligomer comprising a first target-hybridizing sequence (i) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:57 or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101, and a second amplification oligomer comprising or consisting of SEQ ID NO:84.

[00142] Further combinations of first and second amplification oligomers, include: a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:52; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:53; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:54; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:55; a first amplification oligomer comprising or consisting of SEQ

ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:85; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and comprises SEQ ID NO:45; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and comprises SEQ ID NO:52; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:46; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:47; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:48; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:49; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:50; a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:70 and comprises SEQ ID NO:51; and a first amplification oligomer comprising or consisting of SEQ ID NO:8 or 83 and a second amplification oligomer comprising or consisting of SEQ ID NO:84.

[00143] The present disclosure also contemplates the use of other combinations of first and second amplification oligomers, including amplification oligomers in which: the first

target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:13; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:16; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:17; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:18; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:19; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:20; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:21; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:27; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:28; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:29; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:31; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:32; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:33; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:34; the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:35; and the first target-hybridizing sequence comprises or consists of SEQ ID NO:2 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:36.

[00144] The present disclosure also contemplates the use of other combinations of first and second amplification oligomers, including amplification oligomers in which: the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:13; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence

comprises or consists of SEQ ID NO:16; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:17; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:18; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:19; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:20; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:21; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:27; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:28; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:29; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:31; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:32; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:33; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:34; the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:35; and the first target-hybridizing sequence comprises or consists of SEQ ID NO:4 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:36.

[00145] The present disclosure also contemplates the use of other combinations of first and second amplification oligomers, including amplification oligomers in which: the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:13; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:16; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:17; the first target-hybridizing sequence comprises or consists of SEQ ID

NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:18; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:19; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:20; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:21; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:27; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:28; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:29; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:31; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:32; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:33; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:34; the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:35; and the first target-hybridizing sequence comprises or consists of SEQ ID NO:6 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:36.

[00146] The present disclosure also contemplates the use of other combinations of first and second amplification oligomers, including amplification oligomers in which: the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:13; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:16; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:17; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:18; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:19; the first target-

hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:20; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:21; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:27; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:28; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:29; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:31; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:32; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:33; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:34; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:35; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:36; the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:84; and the first target-hybridizing sequence comprises or consists of SEQ ID NO:8 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:86.

[00147] The present disclosure also contemplates the use of other combinations of first and second amplification oligomers, including amplification oligomers in which: the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:13; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:16; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:17; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:18; the first target-hybridizing sequence comprises or consists of SEQ ID

NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:19; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:20; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:21; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:27; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:28; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:29; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:31; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:32; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:33; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:34; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:35; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:36; the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:84; and the first target-hybridizing sequence comprises or consists of SEQ ID NO:83 and the second target-hybridizing sequence comprises or consists of SEQ ID NO:86.

[00148] The present disclosure also contemplates the use of other combinations of first and second amplification oligomers, wherein the first and second target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: SEQ ID NO:2 and SEQ ID NO:11; SEQ ID NO:6 and SEQ ID NO:11; SEQ ID NO:4 and SEQ ID NO:13; SEQ ID NO:6 and SEQ ID NO:13; SEQ ID NO:4 and SEQ ID NO:16; SEQ ID NO:4 and SEQ ID NO:17; SEQ ID NO:4 and SEQ ID NO:18; SEQ ID NO:4 and SEQ ID NO:19; SEQ ID NO:4 and SEQ ID NO:20; SEQ ID NO:4 and SEQ ID NO:21; SEQ ID NO:6 and SEQ ID NO:21; SEQ ID NO:8 and SEQ ID NO:21; SEQ ID NO:2 and SEQ ID NO:27; SEQ ID NO:4 and SEQ ID NO:27; SEQ ID NO:8 and SEQ ID NO:27; SEQ ID NO:4 and SEQ ID

NO:28; SEQ ID NO:4 and SEQ ID NO:29; SEQ ID NO:4 and SEQ ID NO:31; SEQ ID NO:8 and SEQ ID NO:32; SEQ ID NO:8 and SEQ ID NO:33; SEQ ID NO:8 and SEQ ID NO:34; SEQ ID NO:8 and SEQ ID NO:84; SEQ ID NO:8 and SEQ ID NO:86; SEQ ID NO:8 and SEQ ID NO:35; SEQ ID NO:8 and SEQ ID NO:36; SEQ ID NO:83 and SEQ ID NO:32; SEQ ID NO:83 and SEQ ID NO:33; SEQ ID NO:83 and SEQ ID NO:34; SEQ ID NO:83 and SEQ ID NO:84; SEQ ID NO:83 and SEQ ID NO:86; SEQ ID NO:83 and SEQ ID NO:35; or SEQ ID NO:83 and SEQ ID NO:36.

[00149] The present disclosure also contemplates the use of combinations of a first amplification oligomer, a second amplification oligomer and a detection probe in which the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of: SEQ ID NO:2 and SEQ ID NO:11 and SEQ ID NO:39; SEQ ID NO:2 and SEQ ID NO:11 and SEQ ID NO:37; SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:38; SEQ ID NO:2 and SEQ ID NO:27 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:13 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:13 and SEQ ID NO:37; SEQ ID NO:4 and SEQ ID NO:16 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:17 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:18 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:19 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:18 and SEQ ID NO:37; SEQ ID NO:4 and SEQ ID NO:19 and SEQ ID NO:37; SEQ ID NO:4 and SEQ ID NO:20 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:20 and SEQ ID NO:37; SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:21 and SEQ ID NO:37; SEQ ID NO:4 and SEQ ID NO:27 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:27 and SEQ ID NO:38; SEQ ID NO:4 and SEQ ID NO:28 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:29 and SEQ ID NO:39; SEQ ID NO:4 and SEQ ID NO:29 and SEQ ID NO:37; SEQ ID NO:4 and SEQ ID NO:31 and SEQ ID NO:39; SEQ ID NO:6 and SEQ ID NO:11 and SEQ ID NO:37; SEQ ID NO:6 and SEQ ID NO:13 and SEQ ID NO:37; SEQ ID NO:6 and SEQ ID NO:21 and SEQ ID NO:37; SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:39; SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:37; SEQ ID NO:8 and SEQ ID NO:21 and SEQ ID NO:42; SEQ ID NO:8 and SEQ ID NO:27 and SEQ ID NO:39; SEQ ID NO:8 and SEQ ID NO:32 and SEQ ID NO:37; SEQ ID NO:8 and SEQ ID NO:32 and SEQ ID NO:42; SEQ ID NO:8 and SEQ ID NO:33 and SEQ ID NO:37; SEQ ID NO:8 and SEQ ID NO:33 and SEQ ID NO:42; SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:37; SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:42; SEQ ID NO:8 and SEQ ID NO:35 and SEQ ID NO:37; SEQ ID NO:8 and SEQ ID NO:35 and SEQ ID NO:42;

SEQ ID NO:8 and SEQ ID NO:36 and SEQ ID NO:37; or SEQ ID NO:8 and SEQ ID NO:36 and SEQ ID NO:42; SEQ ID NO:8, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; SEQ ID NO:8, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; SEQ ID NO:83, and SEQ ID NO:34, and SEQ ID NOs:91, 92 and/or 93; SEQ ID NOs:83, and SEQ ID NO:84, and SEQ ID NOs:91, 92 and/or 93; SEQ ID NOs:83, and SEQ ID NO:86, and SEQ ID NOs:91, 92 and/or 93; or SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00150] In a further aspect, there is described a method for specifically detecting *Babesia* species nucleic acid in a sample, said method comprising: (1) contacting a sample, said sample suspected of containing *Babesia* species nucleic acid, with at least two oligomers for amplifying a target region of a *Babesia* species target nucleic acid, wherein two of said at least two amplification oligomers are selected from the group consisting of: (a) a first amplification oligomer and a second amplification oligomer, wherein the first amplification oligomer comprises a first target-hybridizing sequence (i) that is from 15 to 33 contiguous nucleobases in length, is contained in SEQ ID NO:66 and contains SEQ ID NO:56 or SEQ ID NO:57, or (ii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101; or (iii) that is from about 15 to about 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101; (iv) comprises/consists of SEQ ID NO:8 or 83; or (b) a first amplification oligomer and a second amplification oligomer, wherein the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is (i) contained in SEQ ID NO:68 and contains SEQ ID NO:52, SEQ ID NO:53 SEQ ID NO:54, SEQ ID NO:55, or SEQ ID NO:85, or (ii) is contained in SEQ ID NO:67 and contains SEQ ID NO:45 or SEQ ID NO:69, or (iii) is contained in SEQ ID NO:70 and contains SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or SEQ ID NO:51; or (iv) comprises or consists of SEQ ID NO:84; (2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Babesia* target nucleic acid present in said sample is used as a template for generating an amplification product, wherein said amplification product has a length of from 180 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof; and (3) detecting the presence or absence of the amplification product, thereby indicating the presence or absence of *Babesia* species target nucleic acid in said sample.

[00151] In those embodiments in which the amplification product has a length of from 180 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof

it is also contemplated that the amplification product has a length of from 180 to 210 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof. It is also contemplated that the amplification product has a length of from 180 to 200 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof. It is also contemplated that the amplification product has a length of from 180 to 190 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof. It is also contemplated that the amplification product has a length of from 190 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof. It is also contemplated that the amplification product has a length of from 200 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof. It is also contemplated that the amplification product has a length of from 210 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof.

[00152] Further combinations of first and second amplification oligomers therefore also include those in which the first amplification oligomer comprises a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in SEQ ID NO:66 and contains SEQ ID NO:56; or comprises a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in SEQ ID NO:66 and contains SEQ ID NO:57; or comprises a first target hybridizing sequence that is from 15 to 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:96 and contains SEQ ID NO:101; or comprises a first target-hybridizing sequence from 15 to 33 contiguous nucleotides in length, is contained in the sequence of SEQ ID NO:97 and contains SEQ ID NO:101; or comprises or consists of SEQ ID NO:8.

[00153] Further combinations of first and second amplification oligomers therefore also include those in which the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:68 and contains SEQ ID NO:52, or contains SEQ ID NO:53 or contains SEQ ID NO:54 or contains SEQ ID NO:55 or contains SEQ ID NO:85.

[00154] Further combinations of first and second amplification oligomers therefore also include those in which the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and is contained in SEQ ID NO:67 and contains SEQ ID NO:45 or contains SEQ ID NO:69.

[00155] Further combinations of first and second amplification oligomers therefore also include those in which the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and

is contained in SEQ ID NO:70 and contains SEQ ID NO:46 or contains SEQ ID NO:47 or contains SEQ ID NO:48 or contains SEQ ID NO:49 or contains SEQ ID NO:50 or contains SEQ ID NO:51.

[00156] Further combinations of first and second amplification oligomers therefore also include those in which the second amplification oligomer comprises a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length and contains SEQ ID NO:84.

[00157] In some embodiments, combinations of certain first and second amplification oligomers are preferred.

[00158] One preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in SEQ ID NO:66 and comprises SEQ ID NO:56 or SEQ ID NO:57 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:67 and comprises SEQ ID NO:45. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:1 or comprising or consisting of the target-hybridizing sequence set forth in SEQ ID NO:2 and a second amplification oligomer comprising or consisting of the target-hybridizing sequence set forth in SEQ ID NO:27. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:38.

[00159] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:66 and comprises SEQ ID NO:56 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:70 and comprises SEQ ID NO:49 or SEQ ID NO:50, or SEQ ID NO:51. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:3 or the target-hybridising sequence set forth in SEQ ID NO:4 and a second amplification oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:21. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:39.

[00160] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:68 and comprises SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, or SEQ ID NO:85. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or 82 or the target-hybridising sequence set forth in SEQ ID NO:8 or 83 and a second amplification oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, or 86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00161] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:70 and comprises SEQ ID NO:49 or SEQ ID NO:50, or SEQ ID NO:51. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or 82 or the target-hybridising sequence set forth in SEQ ID NO:8 or 83 and a second amplification oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, or 86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00162] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence comprising or consisting of SEQ ID NO:84. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or 82 or the target-hybridising sequence set forth in SEQ ID NO:8 or 83 and a second amplification

oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, or 86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00163] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:68 and comprises SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, or SEQ ID NO:85. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or 82 or the target-hybridising sequence set forth in SEQ ID NO:8 or 83 and a second amplification oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, or 86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00164] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:70 and comprises SEQ ID NO:49 or SEQ ID NO:50, or SEQ ID NO:51. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or 82 or the target-hybridising sequence set forth in SEQ ID NO:8 or 83 and a second amplification oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, or 86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00165] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence comprising or consisting of SEQ ID NO:84. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or 82 or the target-hybridising sequence set forth in SEQ ID NO:8 or 83 and a second amplification oligomer comprising or consisting of the target-hybridising sequence set forth in SEQ ID NO:13, 16, 17, 18, 19, 20, 21, 27, 28, 29, 31, 32, 33, 34, 35,36, 84, or 86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00166] Another preferred combination is a first amplification oligomer in which the target-hybridizing sequence comprises or consists of SEQ ID NO:8 or SEQ ID NO:83 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:70 and comprises SEQ ID NO:49 or SEQ ID NO:50, or SEQ ID NO:51. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:82 or SEQ ID NO:83 and a second amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:21. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00167] Another preferred combination is a first amplification oligomer in which the target-hybridizing sequence comprises or consists of SEQ ID NO:8 or SEQ ID NO:83 and a second amplification oligomer comprising a second target-hybridizing sequence that is contained in SEQ ID NO:70 and comprises SEQ ID NO:46 or SEQ ID NO:47 or SEQ ID NO:48 or SEQ ID NO:49 or SEQ ID NO:50 or SEQ ID NO:51. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:82 or SEQ ID NO:83 and a second amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86. According to this embodiment, these combinations

of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00168] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:96 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:85. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:82 or SEQ ID NO:83 and a second amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00169] Another preferred combination is a first amplification oligomer comprising a first target-hybridizing sequence that is from 15 to 33 contiguous nucleobases in length, is contained in the sequence of SEQ ID NO:97 and comprises SEQ ID NO:101 and a second amplification oligomer comprising a second target-hybridizing sequence that is from about 15 to about 33 contiguous nucleotides in length, and is contained in SEQ ID NO:68 and comprises SEQ ID NO:85. A more preferred combination is a first amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:82 or SEQ ID NO:83 and a second amplification oligomer comprising or consisting of the sequence set forth in SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86. According to this embodiment, these combinations of first and second amplification oligomers can be used in combination with a detection probe, suitably, a detection probe in which the target hybridising sequence comprises or consists of the sequence set forth in SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00170] 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 nucleic acid detection probes that are configured to specifically hybridize to a sequence in the amplified product and detecting the presence of the probe:product complex, or by using a complex of probes that may amplify the detectable signal associated with the amplified products (e.g., US Patent Nos. 5,424,413; 5,451,503; and 5,849,481). Directly or indirectly labeled probes that specifically associate with the amplified product provide a detectable signal that indicates the presence of the target nucleic acid in the sample.

[00171] Detection probes (where labelled) 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 the method for diagnosing BV, 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).

[00172] 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).

[00173] In certain embodiments comprising an amplification-based detection assay targeting *Babesia sp.*, the method utilizes one or more detection probes that specifically hybridizes to a *Babesia sp.* amplification product. In particular variations, a *Babesia sp.* -specific detection probe oligomer comprises a target-hybridizing sequence that is from about 14 to about 40 nucleotides in length and is configured to specifically hybridize to a target sequence contained in SEQ ID NO:59, the RNA equivalent of SEQ ID NO:59, the complement of SEQ ID NO:59, the RNA equivalent of the complement of SEQ ID NO:59, SEQ ID NO:65, the DNA equivalent of SEQ ID NO:65, the complement of SEQ ID NO:65, or the DNA equivalent of the complement of SEQ ID NO:65.

[00174] Suitably, the detection probe target-hybridizing sequence contains the sequence of SEQ ID NO:59 and includes at least the sequence of SEQ ID NO:37, 42, or 99.

[00175] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:65 and includes at least the sequence of SEQ ID NO:59, 94, or 99.

[00176] Suitably, the detection probe oligomer comprises a nucleotide sequence that is from 16 to 25 contiguous nucleotides in length and specifically hybridizes to SEQ ID NO:65, or the DNA equivalent thereof; or specifically hybridizes to the complement of SEQ ID NO:65, or the DNA equivalent thereof.

[00177] Suitably, the detection probe oligomer sequence further comprises a nucleotide sequence comprising or consisting of SEQ ID NO:59, 94, or 99.

[00178] Suitably, the detection probe target hybridising sequence consists of the sequence selected from the group consisting of: SEQ ID NOs:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00179] Suitably, the detection probe oligomer further comprises a 2' methoxy modification on at least one of a nucleotide residue member of the nucleotide sequence.

[00180] In some embodiments of a method comprising the use of a nucleic-acid-base detection assay, a non-amplification-based assay is used to detect *Babesia 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.

[00181] In certain embodiments comprising a hybridization assay, a detection probe is utilized for the detection of a *Babesia sp.*. In such embodiments, a detection probe oligomer for detecting *Babesia sp.* comprises a target-hybridizing sequence that is from about 14 to about 40 nucleotides in length and is configured to specifically hybridize to a target sequence contained in SEQ ID NO:59, the RNA equivalent of SEQ ID NO:59, the complement of SEQ ID NO:59, the RNA equivalent of the complement of SEQ ID NO:59, SEQ ID NO:65, the DNA equivalent of SEQ ID NO:65, the complement of SEQ ID NO:65, or the DNA equivalent of the complement of SEQ ID NO:65.

[00182] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:59 and includes at least the sequence of SEQ ID NO:42, 92, 94, or 99.

[00183] Suitably, the detection probe target-hybridizing sequence contains the sequence of SEQ ID NO:59 and includes at least the sequence of SEQ ID NO:37, 42, or 99.

[00184] Suitably, the detection probe target-hybridizing sequence is contained in the sequence of SEQ ID NO:65 and includes at least the sequence of SEQ ID NO:59, 94 or 99.

[00185] Suitably, the detection probe oligomer comprises a nucleotide sequence that is from 16 to 25 contiguous nucleotides in length and specifically hybridizes to SEQ ID NO:65, or the DNA equivalent thereof; or specifically hybridizes to the complement of SEQ ID NO:65, or the DNA equivalent thereof.

[00186] Suitably, the detection probe oligomer sequence further comprises a nucleotide sequence containing SEQ ID NO:59, 94, or 99.

[00187] Suitably, the detection probe target hybridising sequence consists of the sequence selected from the group consisting of: SEQ ID NO:37, 38, 39, 40, 41, 42, 59, 60, 91, 92, 93, 94, 98 or 99.

[00188] Suitably, the detection probe oligomer further comprises a 2' methoxy modification on at least one of a nucleotide residue member of the nucleotide sequence.

[00189] In some embodiments, a non- amplification-based assay for detection of *Babesia 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).

[00190] 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 et al, 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.

[00191] In certain variations, a cleavage-based assay detects an RNA target nucleic acid of *Babesia 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 *Babesia 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 polymerase-deficient 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).

[00192] In some embodiments, a cleavage-based assay detects an RNA target nucleic acid of *Babesia 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.

[00193] In certain embodiments utilizing a nucleic-acid-based detection assay, the method further includes purifying the *Babesia 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 *Babesia sp.* nucleic acid and other sample components.

[00194] In some embodiments, a target nucleic acid of *Babesia 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., WO 2008/016988. In some specific variations comprising target-hybridizing sequence(s) configured to specifically hybridize to a *Babesia sp.* target nucleic acid, a *Babesia*-specific capture probe comprises a target-hybridizing sequence that (i) is from about 15 to about 21 contiguous nucleotides contained in the sequence of SEQ ID NO:78, or (ii) is about 21 to 30 contiguous nucleotides comprising the sequence of SEQ ID NO:78; or (iii) the sequence consists of SEQ ID NO:44. 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.

[00195] 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 *Babesia sp.* target nucleic acid, a *Babesia*-specific capture probe comprises or consists of a the nucleotide sequence of SEQ ID NO:43.

[00196] 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.

[00197] In some embodiments of a method for diagnosing Babesiosis, where detection of *Babesia sp.* indicates Babesiosis in a subject, the method further includes treating Babesiosis in the subject. Treatment regimens for Babesiosis are generally known in the art and include, for example, administration of anti-parasitic medications or red blood cell exchange transfusion as an adjunct therapy. In certain variations, the subject has not been previously diagnosed with Babesiosis. In other embodiments, the subject has been previously diagnosed with Babesiosis and is undergoing treatment for Babesiosis at the time a diagnostic method of the present disclosure is performed. Such variations are particularly useful for monitoring treatment of Babesiosis in a subject. For example, if the method indicates that Babesiosis is still present in the subject, then the subject may continue treatment. In some embodiments, the same treatment regime (i.e., the same treatment that the subject is undergoing at the time the present diagnostic method is performed) is re-administered to the subject. Alternatively, the continued presence of Babesiosis in the subject undergoing treatment may indicate that a change in the ongoing treatment is needed, and a different treatment regime (e.g., a different medication, or an increased dosage and/or frequency of a drug) is administered to the subject.

[00198] In accordance with the present disclosure, detecting the presence or absence of *Babesia 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 (e.g., a method for diagnosing Babesiosis) 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.

[00199] 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 *Babesia sp.* target nucleic acid may or may not be present. A kit comprising an oligomer combination for amplification of *Babesia 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).

[00200] The compositions, methods, reaction mixtures, systems, kits and the like for detection of *Babesia* nucleic acids are further illustrated by the following non-limiting examples.

EXAMPLES

[00201] "Sample 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 sample 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 sample 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 sample 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 sample transport solutions may function equally well.

[00202] "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-MAG^TM MG-CM particles, GE Healthcare Lifesciences) with dT_{14} oligomers covalently bound. Other formulations of Target Capture Reagent may function equally as well.

[00203] "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.

[00204] "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.

[00205] "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.

[00206] "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.

[00207] "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.

[00208] "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: Initial Oligo Screening

Objective:

[00209] Non-T7 and T7 primers and probes were screened using the manual Procleix Enhanced Semi-automated System (eSAS) for transcription mediated amplification (TMA) and hybridization protection assays (HPA) in order to identify primer-probe combinations with the ability to amplify and specifically detect specifically species of *Babesia* – including *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum*. The assay does not discriminate between these *Babesia* species. Reactive results indicate that a sample is positive for *Babesia*.

Materials Methods:

[00210] Initial primer screening was performed using TMA on the manual Procleix system using in-vitro transcripts (IVT) for *Babesia microti* (SEQ ID NO:61), *Babesia divergens* (SEQ ID NO:62), and *Babesia duncani* (SEQ ID NO:63). Reactions for this testing began at the amplification step. An assay rack consisted of 10 rows of Ten-tube units (TTUs). 75 microliters of Amplification Reagent and 10 picomoles each of one T7 promoter provider oligonucleotide from Tables 1-3 and one non-T7 primer oligonucleotide from Tables 1-3 were added to the appropriate tubes on the rack such that each combination of amplification oligomers were tested with two replicates each of *Plasmodium falciparum* IVT (SEQ ID NO:64) at 10,000 copies per reaction and *B. divergens*, *B. duncani*, and *B. microti* IVT at 15 copies per reaction. *P. falciparum* 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 *Babesia*. To achieve the target copies per reaction, 10µL of *P. falciparum* IVT at 1e6 c/mL diluted in a buffer was spiked into the appropriate tubes, and 10µL of *Babesia* species IVT at 1,500 c/mL 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, 200µL of oil was added to each tube and then the rack was covered with sealing cards and vortexed for a minimum of 20 seconds.

[00211] 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 for 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 Diagnostics 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.

[00212] 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 1 acridinium-ester (AE) labeled probe (Tables 1-3) added at a total desired concentration of 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.

[00213] The rack was removed from the water bath, the sealing cards removed, and 250µL of commercially available Procleix Ultrio Plus selection reagent (Grifols Diagnostics 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.

[00214] 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 Diagnostics Solutions, Inc.) and the results were exported for analysis of the signal in Relative Light Units (RLU).

[00215] Primers screened in Group 1a and Group 1b (**Table 1**) were each of SEQ ID NO:3 and SEQ ID NO:5 promoter provider oligomers paired with each of SEQ ID NOs:12 to 16, 18 and 19 to 21 using both detection probe SEQ ID NO:37 and 39. Each rack used system SEQ ID NO:1, 11 and 37 as a control.

Table 1. Primers screened in Group 1a and Group 1b.

SEQ ID NO	Sequence 5' - 3'
1	aatttaatacgactcactatagggagattcacctctgacagttaaatacgaa
3	aatttaatacgactcactatagggagaacagttaaatacgaatgcccccaa
5	aatttaatacgactcactatagggagattcacctctgacagttaaatac
12	actacagcatggaataatga
11	cttgaatactacagcatgga
13	cttgaatactacagcatggaataa
14	acttcagcatggaataatga

SEQ ID NO	Sequence 5' - 3'
15	cttgaatacttcagcatgga
16	actncagcatggaataatga (n is inosine in this example)
18	cttgaatactncagcatgga (n is inosine in this example)
20	actttgagaaaactagagtg
21	agaaaactagagtgtttcaa
39	aguaaugguuaauaggagca
37	ugaaguaggacuuugguucu

[00216] Primers Screened in Group 2 (**Table 2**) were SEQ ID NO:3 promoter provider paired with each of SEQ ID NOs:22-31, 75 and 76 primers using SEQ ID NO:39 detection probe. Each rack used system SEQ ID NO:3, SEQ ID NO:21 and SEQ ID NO:39 as a control.

Table 2. Primers screened in Group 2.

SEQ ID NO:	Sequence 5' - 3'
3	aatttaatacgactcactatagggagaacagttaaatacgaatgcccccaa
21	agaaaactagagtgtttcaa
24	ggttctattttgttggtt
25	tggttctattttgttgg
23	aggactttggttctattttg
75	gaagtaggactttggttctattt
76	atgaagtaggactttggttct
22	ataatgaagtaggactttgg
26	ggaataatgaagtaggacttt
27	atggaataatgaagtaggac
28	atggaataatgaagtagg
29	gcatggaataatgaagtag
30	tacagcatggaataatgaag
31	tactacagcatggaataatg
39	aguaaugguuaauaggagca

[00217] Primers screened in Group 3 (**Table 3**) were SEQ ID NO:1 promoter provider paired with each of SEQ ID NO:21, 27 and 29 primers and SEQ ID NO:38, 40 and 41 detection probes; SEQ ID NO:3 promoter provider paired with each of SEQ ID NO:21, 27

and 29 primers and SEQ ID NO:38 and 40 detection probes; and each of SEQ ID NO:3, 7 and 9 promoter provider paired with SEQ ID NO:21 primer and SEQ ID NO:39 detection probe.

Table 3. Primers screened in Group 3.

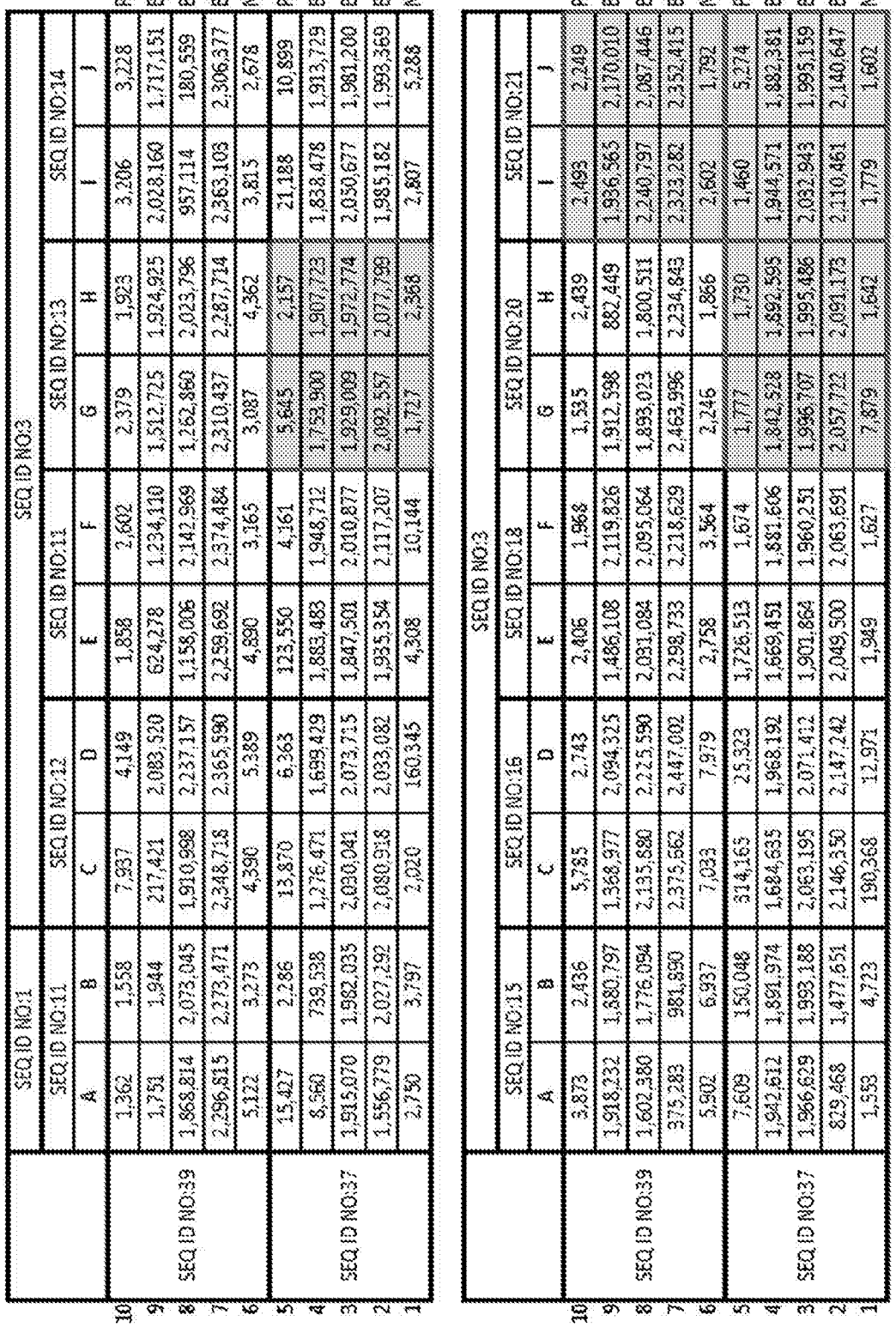
SEQ ID NO:	Sequence 5' - 3'
3	aatttaatacgactcactatagggagaacagttaaatacgaatgcccccaa
1	aatttaatacgactcactatagggagattcacctctgacagttaaatacgaa
7	aatttaatacgactcactatagggagagctttcgcagtagttcgtctttaacaaatc
9	aatttaatacgactcactatagggagactttcgcagtagttcgtctttaac
21	agaaaactagagtgtttcaa
27	atggaataatgaagtaggac
29	gcatggaataatgaagtag
39	aguaaugguuaauaggagca
38	uaaugguuaauaggagcaguug
40	ggacuuugguucuauuuuguugg
41	aaugguuaauaggagcaguuggggg

Results:

[00218] Ideal candidates from screening were expected to be reactive for *Babesia* species IVTs (*B. microti*, *B. divergens* and *B. duncani*) with analyte signals consistently at 1,000,000 RLU or higher for all replicates and negative for *P. falciparum* IVT and negative samples with analyte signals below 10,000 RLU. Some candidates with RLUs near 1,000,000 RLU for *Babesia* and below 30,000 RLU for *P. falciparum* and negatives were also given consideration.

[00219] For the primers and detection probes screened in Group 1a, not all primer-probe combinations consistently amplified and detected all species and some combinations resulted in high analyte signals for negative specimens (*P. falciparum* and Negative). Analyte RLU results for this group are listed in **Table 4**. Preferred candidates in this group were SEQ ID NO:3 promoter provider paired with SEQ ID NO:13, 20 or 21 primers and using SEQ ID NO:37 detection probe; and SEQ ID NO:3 promoter provider paired with SEQ ID NO:21 primer and SEQ ID NO:39 detection probe.

Table 4. Analyte RLU Results for Group 1a.

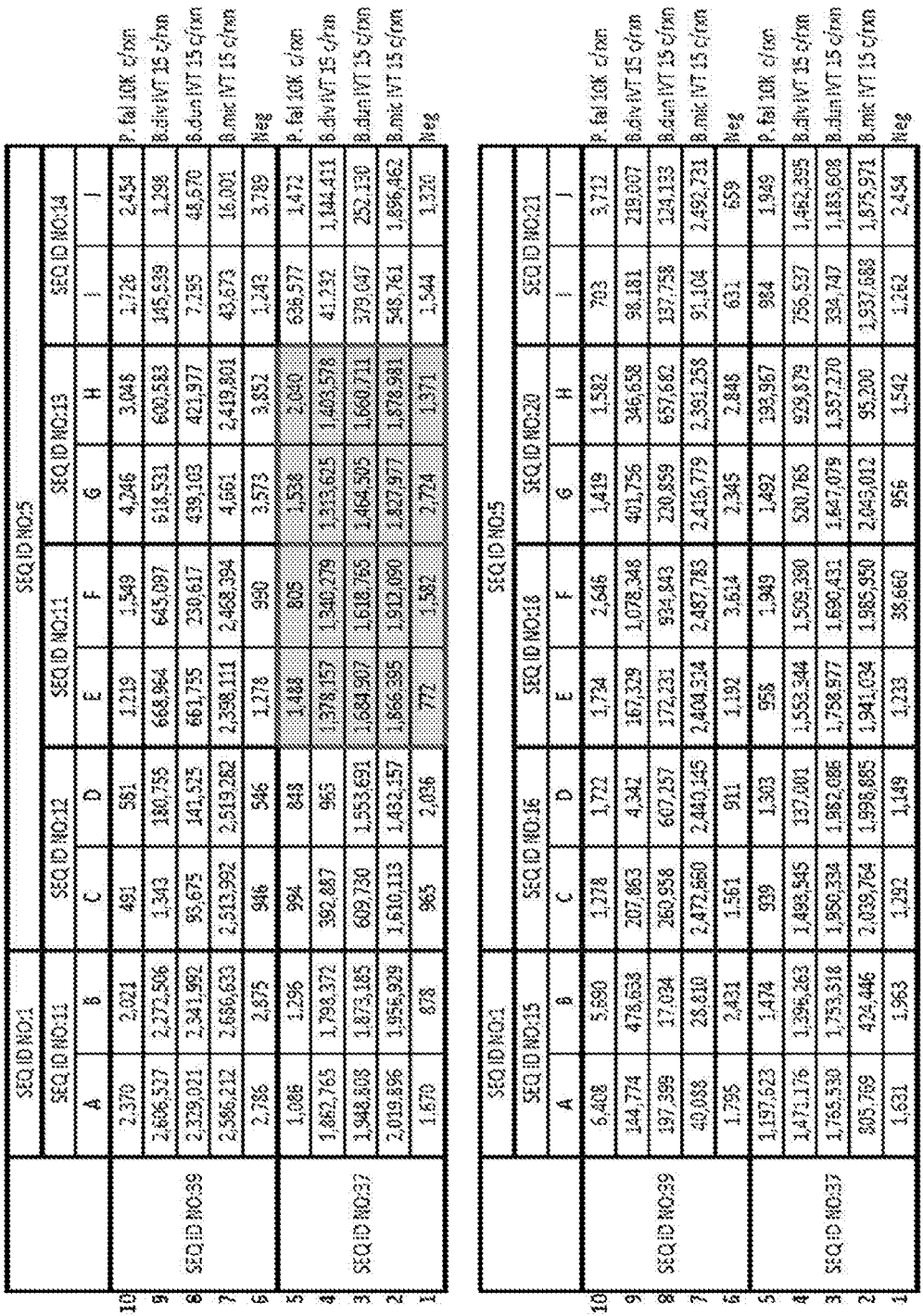


	SEQ ID NO:1		SEQ ID NO:3										
	SEQ ID NO:11		SEQ ID NO:12		SEQ ID NO:11		SEQ ID NO:13			SEQ ID NO:14			
	A	B	C	D	E	F	G	H	I	J	
SEQ ID NO:39	1,362	1,558	7,937	4,149	1,858	2,602	2,379	1,923	3,206	3,228	P. fal 10K c/rxn
	1,753	1,944	217,421	2,083,320	624,278	1,234,110	1,512,725	1,924,925	2,028,160	1,717,151	B.div IVT 15 c/rxn
	1,868,814	2,073,045	1,910,998	2,237,157	1,158,006	2,142,969	1,262,860	2,023,796	957,114	180,599	B.dun IVT 15 c/rxn
	2,296,815	2,273,471	2,348,718	2,365,590	2,259,692	2,374,484	2,210,437	2,287,714	2,263,103	2,306,377	B.mic IVT 15 c/rxn
	5,122	3,273	4,390	5,389	4,890	3,165	3,087	4,362	3,815	2,678	Neg
SEQ ID NO:37	15,427	2,286	13,870	6,263	123,550	4,161	5,645	2,157	21,188	10,899	P. fal 10K c/rxn
	8,360	739,538	1,276,471	1,699,429	1,883,483	1,948,712	1,753,900	1,907,723	1,838,478	1,913,729	B.div IVT 15 c/rxn
	1,915,070	1,982,035	2,030,041	2,073,715	1,847,501	2,010,877	1,929,009	1,972,774	2,050,677	1,981,200	B.dun IVT 15 c/rxn
	1,556,779	2,027,292	2,080,918	2,033,082	1,935,354	2,117,207	2,092,557	2,077,789	1,985,182	1,993,369	B.mic IVT 15 c/rxn
	2,750	3,797	2,020	160,345	4,308	10,144	1,727	2,368	2,807	5,288	Neg

	SEQ ID NO:3										
	SEQ ID NO:15		SEQ ID NO:16		SEQ ID NO:18		SEQ ID NO:20		SEQ ID NO:21		
	A	B	C	D	E	F	G	H	I	J	
SEQ ID NO:39	3,873	2,436	5,735	2,743	2,406	1,968	1,535	2,439	2,493	2,249	P. fal 10K c/rxn
	1,918,232	1,680,797	1,368,977	2,094,325	1,486,108	2,119,826	1,912,598	882,449	1,936,565	2,170,010	B.div IVT 15 c/rxn
	1,602,380	1,776,094	2,135,880	2,225,590	2,031,084	2,095,064	1,893,023	1,800,511	2,240,797	2,087,446	B.dun IVT 15 c/rxn
	375,283	981,890	2,375,562	2,447,002	2,298,733	2,218,629	2,463,996	2,234,843	2,323,282	2,352,415	B.mic IVT 15 c/rxn
	5,902	6,937	7,033	7,979	2,758	3,564	2,246	1,866	2,602	1,792	Neg
SEQ ID NO:37	7,609	150,048	314,165	25,323	1,726,513	1,674	1,777	1,730	1,460	5,274	P. fal 10K c/rxn
	1,942,812	1,891,974	1,684,635	1,968,192	1,869,451	1,881,606	1,842,528	1,892,595	1,944,571	1,882,381	B.div IVT 15 c/rxn
	1,966,629	1,993,188	2,063,195	2,071,412	1,901,864	1,960,251	1,996,107	1,995,486	2,032,943	1,995,159	B.dun IVT 15 c/rxn
	829,468	1,477,651	2,146,350	2,147,242	2,049,500	2,063,691	2,057,722	2,091,173	2,110,461	2,140,647	B.mic IVT 15 c/rxn
	1,353	4,723	190,368	12,971	1,949	1,627	7,879	1,642	1,779	1,602	Neg

[00220] For the primers and detection probes screened in Group 1b, not all primer-probe combinations consistently amplified and detected all species and some combinations resulted in high analyte signals for *P. falciparum*. Analyte RLU results for this group are listed in **Table 5**. Preferred candidates in this group were SEQ ID NO:5 promoter provider paired with SEQ ID NO:11 or 13 primers and using the SEQ ID NO:37 detection probe.

Table 5. Analyte RLU Results for Group 1b.

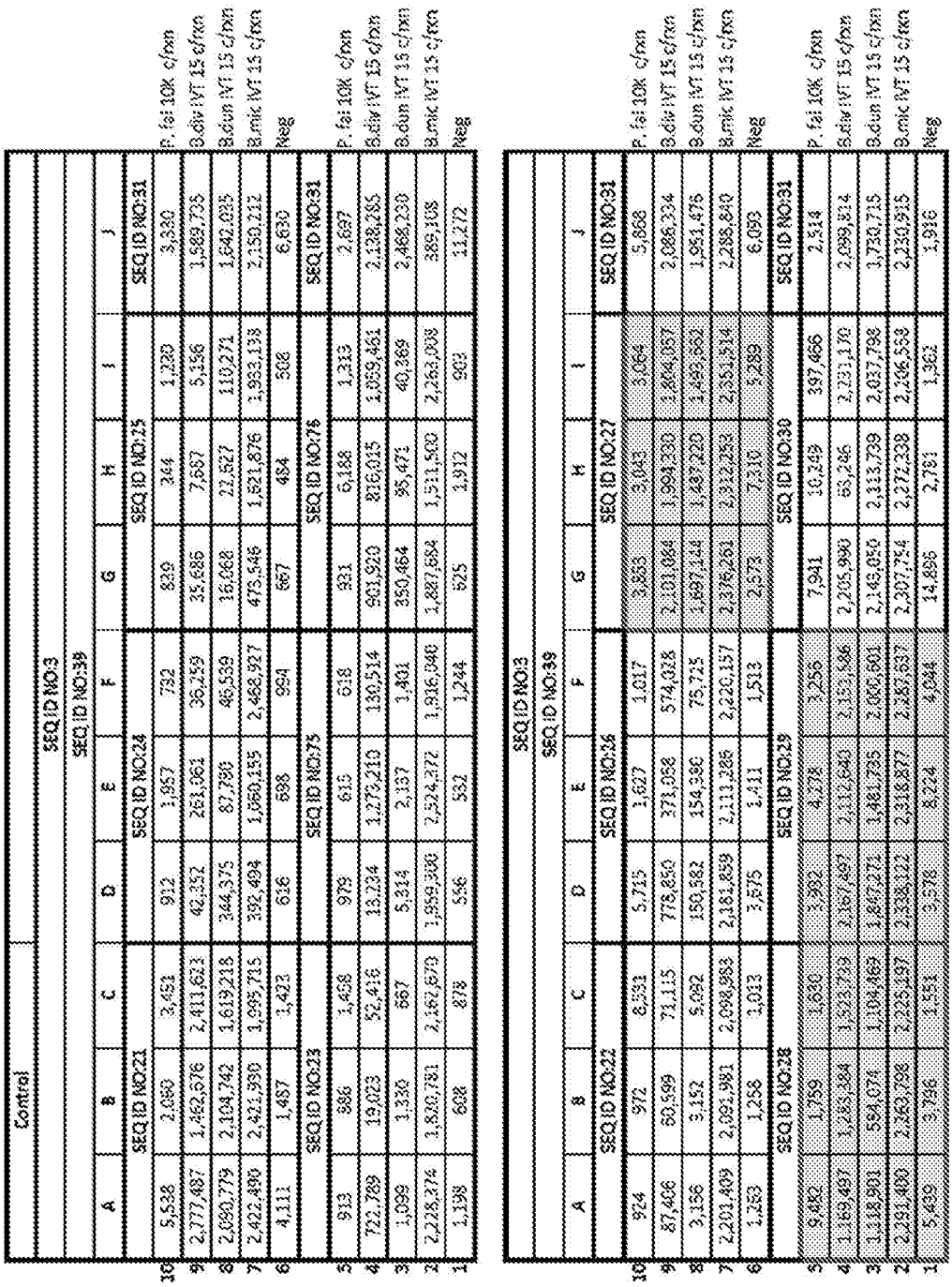


		SEQ ID NO:2		SEQ ID NO:5									
		SEQ ID NO:11		SEQ ID NO:12		SEQ ID NO:11		SEQ ID NO:13		SEQ ID NO:14			
		A	B	C	D	E	F	G	H	I	J		
SEQ ID NO:39	10	2,370	2,021	491	581	1,219	1,549	4,246	3,048	1,726	2,454	P. fal 10K c/rxn	
	9	2,606,537	2,272,506	1,343	180,755	668,964	645,097	618,531	600,583	145,539	1,298	B.div IVT 15 c/rxn	
	8	2,339,021	2,341,992	93,675	142,525	661,755	230,617	439,103	421,977	7,285	48,670	B.dun IVT 15 c/rxn	
	7	2,586,212	2,636,633	2,513,992	2,519,282	2,398,111	2,468,394	4,661	2,419,801	43,673	16,001	B.mic IVT 15 c/rxn	
	6	2,786	2,875	946	946	1,278	950	3,573	3,852	1,242	3,789	Neg	
SEQ ID NO:37	5	1,086	1,296	994	848	1,488	805	1,538	2,040	638,577	1,472	P. fal 10K c/rxn	
	4	1,862,765	1,798,372	392,887	965	1,378,157	1,240,779	1,213,625	1,403,578	41,732	1,144,411	B.div IVT 15 c/rxn	
	3	1,948,808	1,873,185	609,730	1,553,691	1,684,907	1,618,765	1,464,505	1,660,711	379,047	252,130	B.dun IVT 15 c/rxn	
	2	2,018,896	1,956,929	1,610,113	1,432,157	1,866,395	1,912,860	1,827,977	1,878,981	548,761	1,896,462	B.mic IVT 15 c/rxn	
	1	1,670	878	965	2,036	772	1,582	2,724	1,371	1,944	1,328	Neg	

		SEQ ID NO:1		SEQ ID NO:5									
		SEQ ID NO:15		SEQ ID NO:16		SEQ ID NO:18		SEQ ID NO:20		SEQ ID NO:21			
		A	B	C	D	E	F	G	H	I	J		
SEQ ID NO:39	10	6,408	5,890	1,278	1,722	1,724	2,646	1,419	1,582	793	3,712	P. fal 10K c/rxn	
	9	124,774	478,633	207,863	4,342	167,329	1,078,348	401,756	346,658	98,181	219,007	B.div IVT 15 c/rxn	
	8	197,399	17,034	260,958	607,157	172,231	934,843	230,859	657,682	137,758	124,133	B.dun IVT 15 c/rxn	
	7	40,038	28,810	2,472,860	2,440,145	2,404,314	2,487,783	2,416,779	2,391,253	91,104	2,492,731	B.mic IVT 15 c/rxn	
	6	1,795	2,421	1,561	911	1,192	3,614	2,345	2,848	631	659	Neg	
SEQ ID NO:37	5	1,157,623	1,474	939	1,303	958	1,949	1,492	192,967	984	1,949	P. fal 10K c/rxn	
	4	1,470,176	1,396,263	1,483,845	137,801	1,553,344	1,509,390	520,765	929,579	756,537	1,462,295	B.div IVT 15 c/rxn	
	3	1,766,330	1,753,318	1,956,334	1,982,086	1,758,977	1,690,431	1,647,079	1,357,270	334,747	1,183,608	B.dun IVT 15 c/rxn	
	2	805,769	424,446	2,039,764	1,898,885	1,941,034	1,985,950	2,049,012	95,200	1,937,683	1,875,971	B.mic IVT 15 c/rxn	
	1	1,631	1,963	1,292	1,149	1,233	38,660	956	1,542	1,262	2,454	Neg	

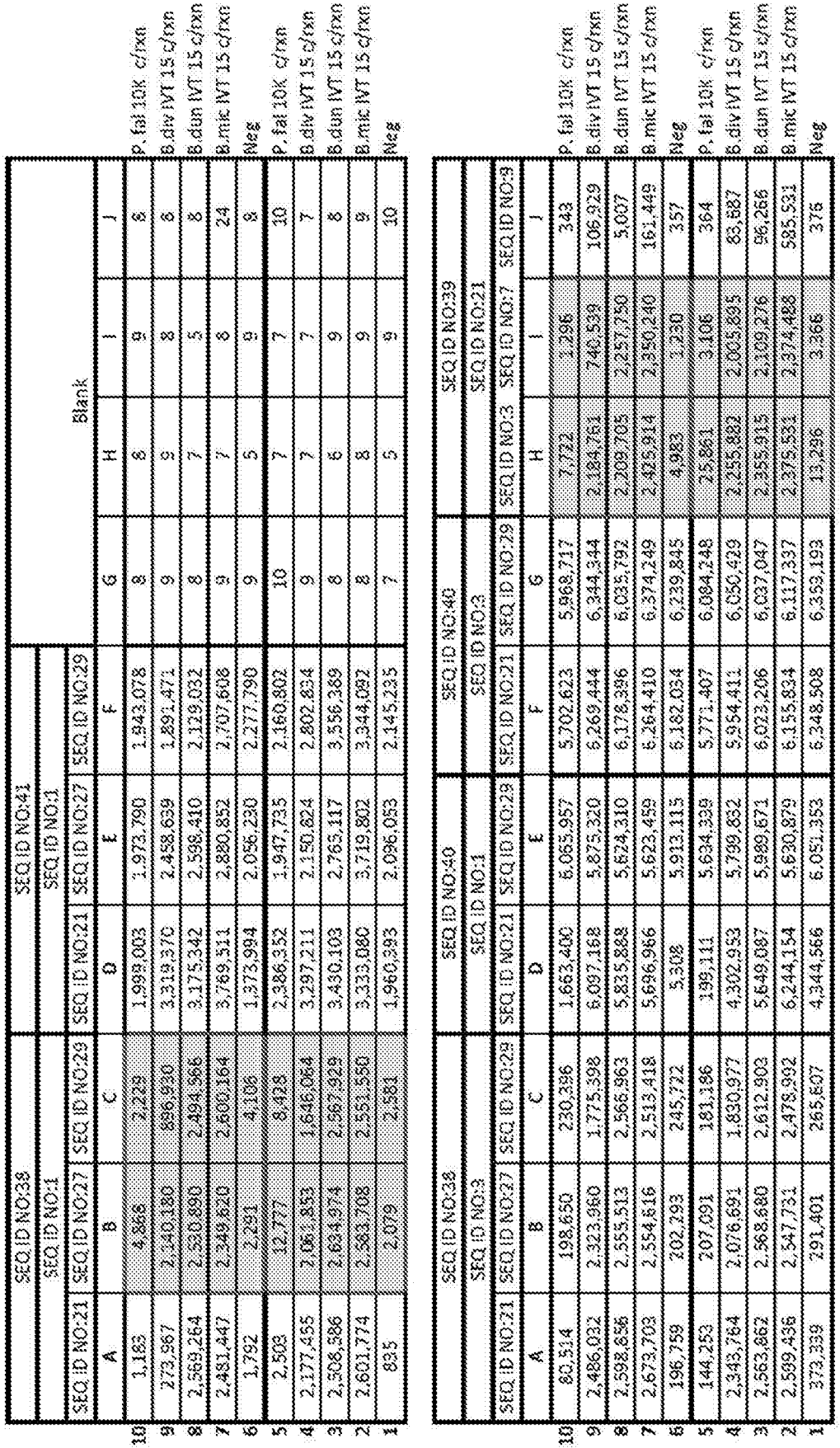
[00221] For the primers and detection probes screened in Group 2, not all primer-probe combinations consistently amplified and detected all species and some combinations resulted in high analyte signals for P. falciparum. Analyte RLU results for this group are listed in **Table 6**. Preferred candidates in this group were SEQ ID NO:3 promoter provider paired with SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29 primers and SEQ ID NO:39 detection probe.

Table 6. Analyte RLU Results for Group 2.



[00222] Analyte RLU results for the primers and detection probes screened in Group 3 are listed in **Table 7.** Primers paired with detection probes SEQ ID NO:40 and SEQ ID NO:41 yielded high analyte signals for *P. falciparum*, negative samples and *Babesia* positive samples indicating poor design of the probe. Thus, SEQ ID NO:40 and SEQ ID NO:41 and were not included in additional examples. Combinations of SEQ ID NO:3 promoter provider with detection probe SEQ ID NO:38 (regardless of the primer used in this example) yielded high analyte signal in negative samples indicating a probable primer-probe interaction, and thus these oligomers were not included in additional examples. Candidates considered for use with additional detection probes were SEQ ID NO:1 promoter provider with SEQ ID NO:27 or 29 primers and SEQ ID NO:38 detection probe. For new T7 promoter provider screened in this group, SEQ ID NO:3 and 7 paired with SEQ ID NO:21 primer and SEQ ID NO:39 detection probe were considered. SEQ ID NO:9 promoter provider, SEQ ID NO:21 primer and SEQ ID NO:39 detection probe showed no amplification and detection of *Babesia* targets.

Table 7. Analyte RLU Results for Group 3.



	SEQ ID NO:38 / SEQ ID NO:1			SEQ ID NO:41 / SEQ ID NO:1			Blank			
	SEQ ID NO:21	SEQ ID NO:27	SEQ ID NO:29	SEQ ID NO:21	SEQ ID NO:27	SEQ ID NO:29				
	A	B	C	D	E	F	G	H	I	J
10	1,183	4,866	2,229	1,999,003	1,973,790	1,943,078	8	8	9	8	P. fal 10k c/rxn
9	273,967	2,140,180	836,920	3,319,370	2,458,639	1,891,471	9	9	8	8	B.div IVT 15 c/rxn
8	2,569,264	2,520,890	2,494,366	3,175,342	2,598,410	2,129,032	8	7	5	8	B.dun IVT 15 c/rxn
7	2,481,447	2,349,620	2,600,164	3,769,511	2,880,852	2,707,608	9	7	8	24	B.mic IVT 15 c/rxn
6	1,792	2,291	4,106	1,373,994	2,056,220	2,277,790	9	5	9	8	Neg
5	2,503	12,777	8,428	2,386,352	1,947,735	2,160,802	10	7	7	10	P. fal 10k c/rxn
4	2,177,455	2,061,853	1,646,064	3,297,211	2,150,824	2,802,834	9	7	7	7	B.div IVT 15 c/rxn
3	2,508,386	2,624,974	2,567,929	3,430,103	2,763,117	3,556,389	8	6	9	8	B.dun IVT 15 c/rxn
2	2,601,774	2,563,708	2,551,550	3,333,080	3,719,802	3,344,092	8	8	9	9	B.mic IVT 15 c/rxn
1	835	2,079	2,581	1,960,393	2,096,053	2,145,235	7	5	9	10	Neg

	SEQ ID NO:38 / SEQ ID NO:3			SEQ ID NO:40 / SEQ ID NO:1			SEQ ID NO:40 / SEQ ID NO:3			SEQ ID NO:39 / SEQ ID NO:21		
	SEQ ID NO:21	SEQ ID NO:27	SEQ ID NO:29	SEQ ID NO:21	SEQ ID NO:27	SEQ ID NO:29	SEQ ID NO:21	SEQ ID NO:27	SEQ ID NO:29	SEQ ID NO:3	SEQ ID NO:7	SEQ ID NO:9
	A	B	C	D	E	F	G	H	I	J		
10	80,514	198,650	230,356	1,663,400	6,065,957	5,702,623	5,968,717	7,722	1,296	343	P. fal 10k c/rxn
9	2,486,032	2,323,960	1,775,398	6,097,168	5,875,320	6,269,444	6,344,344	2,184,761	740,538	106,929	B.div IVT 15 c/rxn
8	2,598,856	2,555,513	2,566,963	5,835,888	5,624,310	6,178,396	6,035,792	2,209,705	2,257,750	5,007	B.dun IVT 15 c/rxn
7	2,673,703	2,554,616	2,513,418	5,696,966	5,623,459	6,264,410	6,374,249	2,425,914	2,350,240	161,449	B.mic IVT 15 c/rxn
6	196,759	202,393	245,722	5,308	5,913,115	6,182,034	6,239,845	4,923	1,230	357	Neg
5	144,253	207,091	181,186	199,111	5,634,339	5,771,407	6,084,248	25,861	3,106	364	P. fal 10k c/rxn
4	2,343,764	2,076,691	1,830,977	4,302,953	5,799,632	5,954,411	6,050,429	2,155,882	2,005,895	83,687	B.div IVT 15 c/rxn
3	2,563,862	2,568,680	2,612,903	5,649,087	5,999,671	6,023,206	6,037,047	2,355,915	2,109,276	96,266	B.dun IVT 15 c/rxn
2	2,599,436	2,547,731	2,478,992	6,244,154	5,630,879	6,155,834	6,117,337	2,375,532	2,374,488	585,531	B.mic IVT 15 c/rxn
1	373,339	291,401	265,607	4,344,566	6,051,353	6,348,508	6,359,193	13,236	3,366	376	Neg

Conclusions:

[00223] The results of this initial screening demonstrated that not all primers and probes reliably amplify and detect all *Babesia* species. Some candidates were not specific to *Babesia*, showing some amplification of P. falciparum, while other candidates demonstrated false positives, false negatives and/or primer-probe interactions. Initial screening of amplification and detection systems identified several candidates that show sensitive and specific detection of *B. microti, B. divergens and B. duncani*. These candidates do not cross react with *P. falciparum* further demonstrating specificity of the systems. The sensitive and specific combinations from this example were considered for further senisitivy and specificity evaluation.

EXAMPLE 2: Secondary Oligo Screening for the *Babesia* Assay

Objective:

[00224] Candidate amplification combinations identified in Example 1 for the *Babesia* Assay using the manual Procleix Enhanced Semi-automated System (eSAS) were screened on the fully automated Procleix Panther System to determine the best candidates in terms of specificity and sensitivity using *Babesia* Species in-vitro transcripts (IVT).

Materials and Methods:

[00225] Candidate amplification systems were tested on the automated Procleix Panther system (Grifols Diagnostics Solutions, Inc.). Combinations of amplification and detection oligomers tested are listed in **Table 8**. Sequences for each oligo are listed in **Table 9**. A total of 8 conditions were screened. Conditions screened were tested against 45 replicates of a negative panel and 6 replicates each of diluted *Babesia* in-vitro transcripts (IVT) for *B. microti, B. divergens and B. duncani* (SEQ ID NOs:61, 62, & 63, respectively) at 30 c/ml. An assay calibrator comprising a *B. microti* 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 $\geq$ 1 are considered reactive, while those $<$ 1 are non-reactive. Assay Reagents used included the following: a Target Capture Reagent (TCR) comprising of a single Target Capture Oligo (TCO) added at a concentration of 5 pmoles per reaction; an Amplification Reagent comprising one T7 promoter provider and one non-T7 primer each added at a concentration

of 10 pmoles per reaction; a Probe reagent consisting of 1 acridinium-ester (AE) labeled probe added at a total concentration of 5e6 Relative Light Units (RLU) per reaction to a solution of ; Enzyme Reagent; and Selection Reagent.

[00226] A second round of oligo screening was performed to follow up on candidate systems identified in Example 1. Conditions tested in this group are listed in **Table 10**. Sequences for each oligo are listed in **Table 11**. A total of 6 additional conditions were screened. Conditions screened were tested against 8 replicates of a negative sample and 7 replicates of diluted *Babesia* in-vitro transcripts (IVT) for each of *B. microti, B. divergens* and *B. duncani* at 100, 30, and 10 c/ml. A cross reactivity panel consisting of 8 replicates *P. falciparum* IVT at 1e6 c/mL was also tested to determine if the system is specific to *Babesia* in the presence of *Plasmodium*. An assay calibrator made from a *B. microti* IVT panel at 500 c/mL.

Table 8. Conditions 1-8 Tested on the Panther System

Condition #	Target Capture Oligo	T7 Primer	Non-T7 Primer	Probe
1	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:11	SEQ ID NO:37
2	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:27	SEQ ID NO:39
3	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:27	SEQ ID NO:38
4	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:13	SEQ ID NO:37
5	SEQ ID NO:43	SEQ ID NO:3	SEQ ID NO:21	SEQ ID NO:37
6	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:21	SEQ ID NO:37
7	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:27	SEQ ID NO:39
8	SEQ ID NO:43	SEQ ID NO:5	SEQ ID NO:21	SEQ ID NO:37

Table 9. Sequences for Oligos listed in Table 8.

Reagent	Short Name	Sequence 5' - 3'
TCR	SEQ ID NO:43	uaggccaauacccuaccgucctttaaaaaaaaaaaaaaaaaaaaaaaaaaaa
Reagent	**Short Name**	**Sequence 5' - 3'**
Amplification	SEQ ID NO:1	aatttaatacgactcactatagggagattcacctctgacagttaaatacgaa
	SEQ ID NO:3	aatttaatacgactcactatagggagaacagttaaatacgaatgcccccaa
	SEQ ID NO:5	aatttaatacgactcactatagggagattcacctctgacagttaaatac
	SEQ ID NO:7	aatttaatacgactcactatagggagagctttcgcagtagttcgtctttaacaaatc
	SEQ ID NO:13	cttgaatactacagcatggaataa

Reagent	Short Name	Sequence 5' - 3'
	SEQ ID NO:21	agaaaactagagtgtttcaa
	SEQ ID NO:27	atggaataatgaagtaggac
Reagent	**Short Name**	**Sequence 5' - 3'**
Probe	SEQ ID NO:37	ugaaguaggacuuugguucu
	SEQ ID NO:38	uaaugguuaauaggagcaguug
	SEQ ID NO:39	aguaaugguuaauaggagca

Table 10. Conditions 9-14 tested on the Panther System

Condition	Target Capture Oligo	T7 Primer	Non T7 Primer	Probe
9	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:21	SEQ ID NOs:37 + 42
10	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:32	SEQ ID NOs:37 + 42
11	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:33	SEQ ID NOs:37 + 42
12	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:34	SEQ ID NOs:37 + 42
13	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:35	SEQ ID NOs:37 + 42
14	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:36	SEQ ID NOs:37 + 42

Table 11. Sequences for Oligos listed in Table 10.

Reagent	Short Name	Sequence 5' - 3'
TCR	SEQ ID NO:43	uaggccaauacccuaccgucctttaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
Reagent	**Short Name**	**Sequence 5' - 3'**
Amplification	SEQ ID NO:7	aatttaatacgactcactatagggagagctttcgcagtagttcgtctttaacaaatc
	SEQ ID NO:21	agaaaactagagtgtttcaa
	SEQ ID NO:32	agaaaactagagtgtttca
	SEQ ID NO:33	agaaaattagagtgtttcaa
	SEQ ID NO:34	gagaaaactagagtgtttcaa
	SEQ ID NO:35	gagaaaattagagtgtttcaa
	SEQ ID NO:36	tgagaaaactagagtgtttc
Reagent	**Short Name**	**Sequence 5' - 3'**
Probe	SEQ ID NO:37	ugaaguaggacuuugguucu
	SEQ ID NO:42	aguaggacuuugguucu

Results:

[00227] An analyte signal cutoff of 100,000 RLU was used to determine reactivity for specimens screened for Conditions 1-8 in **Table 12**. Samples above this cutoff were considered reactive. Conditions 1 and 4 yielded a false positive rate in negative specimens of 22% and 13% respectively indicating a primer interaction with the probe. Condition 7 was unable to detect *B. divergens* and *B. duncani* IVT at 30 c/mL at 100% reactivity. Due to the high rate of false positives seen in this example using the SEQ ID NO:1 promoter provider, additional screening was performed on alternative candidates.

Table 12. Results for Conditions 1-8

Condition #	Sample Description	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
1	*B.microti* Calibrator	3	3	100	2,895,211	95,178
	Negative Buffer	45	10	22	83,538	169,164
	B.microti 30c/mL	6	6	100	2,785,208	192,438
	B.duncani 30 c/mL	6	6	100	2,704,843	52,325
	B.divergens 30 c/mL	5	5	100	2,679,799	131,010
2	*B.microti* Calibrator	3	3	100	2,164,104	85,510
	Negative Buffer	45	0	0	1,419	881
	B.microti 30c/mL	5	5	100	2,184,976	107,021
	B.duncani 30 c/mL	5	5	100	1,842,832	79,094
	B.divergens 30 c/mL	5	5	100	1,710,700	484,713
3	*B.microti* Calibrator	3	3	100	5,202,778	356,597
	Negative Buffer	45	0	0%	5,048	2,938
	B.microti 30c/mL	6	6	100	5,282,576	336,909
	B.duncani 30 c/mL	6	6	100	4,987,308	396,702
	B.divergens 30 c/mL	6	6	100	4,898,995	261,124
4	*B.microti* Calibrator	3	3	100	2,847,426	127,963
	Negative Buffer	45	6	13	62,163	212,258
	B.microti 30c/mL	6	6	100	2,920,118	186,525
	B.duncani 30 c/mL	6	6	100	2,637,399	213,549
	B.divergens 30 c/mL	6	6	100	2,555,922	322,889
5	*B.microti* Calibrator	3	3	100	2,614,010	43,029
	Negative Buffer	45	0	0	1,064	604

Condition #	Sample Description	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
	B.microti 30c/mL	6	6	100	2,562,482	60,848
	B.duncani 30 c/mL	6	6	100	2,317,458	47,231
	B.divergens 30 c/mL	6	6	100	2,329,932	33,382
6	*B.microti* Calibrator	3	3	100	2,849,900	59,411
	Negative Buffer	45	0	0	1,007	548
	B.microti 30c/mL	6	6	100	2,779,409	77,390
	B.duncani 30 c/mL	6	6	100	2,472,381	110,379
	B.divergens 30 c/mL	6	6	100	2,608,790	176,482
7	*B.microti* Calibrator	3	3	100	5,706,224	96,503
	Negative Buffer	45	0	0	3,062	1,006
	B.microti 30c/mL	6	6	100	5,513,204	219,801
	B.duncani 30 c/mL	6	2	33	124,483	155,936
	B.divergens 30 c/mL	6	3	50	301,854	494,564
8	*B.microti* Calibrator	3	3	100	2,963,935	70,693
	Negative Buffer	45	0	0	932	645
	B.microti 30c/mL	6	6	100	2,977,421	189,225
	B.duncani 30 c/mL	6	6	100	2,678,642	179,586
	B.divergens 30 c/mL	6	6	100	2,517,233	223,392

[00228] In the previous round of screening, several candidates showed good sensitivity and specificity for *Babesia* species IVT. Condition 6 had good sensitivity and specificity with strong analyte signal for the *Babesia* IVT specimens tested and low analyte signal for negative specimens. Additional screening was performed on the Panther system using the SEQ ID NO:7 promoter provider paired with a number of non-T7 primers to determine the performance of these oligomer combinations. Condition 12 had the best performance of the conditions screened in this round. Condition 12 had best performance in terms of percent reactivity for all levels tested for all *Babesia* species IVTs and strongest RLU signal for all levels. Condition 12 also had the lowest analyte signal for negative specimens.

Table 13. Results for Conditions 9-14.

Condition #	Sample Description	Level	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
9	Negative Calibrator	0	3	0	0	3,064	2,870
	B.microti Calibrator	500 c/mL	3	3	100	2,634,036	20,467
	B. microti IVT	100 c/mL	7	7	100	1,782,835	124,480
		30 c/mL	7	6	86	1,478,196	136,864
		10 c/mL	7	5	71	1,504,124	110,673
	B. duncani IVT	100 c/mL	7	7	100	1,615,981	207,184
		30 c/mL	7	7	100	1,046,502	282,167
		10 c/mL	7	6	86	817,242	310,430
	B. divergens IVT	100 c/mL	7	7	100	1,107,329	280,864
		30 c/mL	7	7	100	780,602	285,167
		10 c/mL	7	3	43	312,826	199,281
	Negative IC Buffer	0	8	0	0	921	1,069
	P. falciparum IVT	1.00e6 c/mL	8	0	0	1,546	1,944
10	Negative Calibrator	0	3	0	0	2,083	1,176
	B.microti Calibrator	500 c/mL	3	3	100	2,612,367	134,872
	B. microti IVT	100 c/mL	7	7	100	1,841,300	261,033
		30 c/mL	7	6	86	1,329,659	395,229
		10 c/mL	7	4	57	708,829	275,561
	B. duncani IVT	100 c/mL	7	7	100	1,475,991	196,296
		30 c/mL	7	7	100	717,189	386,202
		10 c/mL	7	4	57	471,736	348,996
	B. divergens IVT	100 c/mL	7	7	100	910,674	330,110
		30 c/mL	7	6	86	565,166	200,817
		10 c/mL	7	3	43	158,352	37,831
	Negative IC Buffer	0	8	0	0	2,802	2,381
	P. falciparum IVT	1.00e6 c/mL	8	0	0	3,459	3,274
11	Negative Calibrator	0	3	0	0	1,687	1,471
	B.microti Calibrator	500 c/mL	3	3	100	2,476,171	150,100
	B. microti IVT	100 c/mL	7	7	100	1,887,931	90,174
		30 c/mL	7	7	100	1,042,715	590,288

Condition #	Sample Description	Level	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
		10 c/mL	7	2	29	961,097	598,572
	B. duncani IVT	100 c/mL	7	7	100	1,402,621	146,419
		30 c/mL	7	7	100	580,415	252,668
		10 c/mL	7	4	57	252,969	139,120
	B. divergens IVT	100 c/mL	7	7	100	903,456	261,139
		30 c/mL	7	7	100	484,118	271,417
		10 c/mL	7	6	86	283,109	129,194
	Negative IC Buffer	0	8	0	0	2,334	1,997
	P. falciparum IVT	1.00e6 c/mL	8	0	0	2,035	1,681
12	Negative Calibrator	0	3	0	0	370	640
	B.microti Calibrator	500 c/mL	3	3	100	2,742,289	194,078
	B. microti IVT	100 c/mL	7	7	100	2,092,032	386,586
		30 c/mL	7	7	100	1,568,851	376,918
		10 c/mL	7	5	71	1,334,103	542,119
	B. duncani IVT	100 c/mL	7	7	100	1,867,880	208,460
		30 c/mL	7	7	100	1,264,832	533,439
		10 c/mL	7	7	100	881,072	287,187
	B. divergens IVT	100 c/mL	7	7	100	1,456,923	228,759
		30 c/mL	7	7	100	1,013,056	162,035
		10 c/mL	7	6	86	941,635	247,989
	Negative IC Buffer	0	8	0	0	391	944
	P. falciparum IVT	1.00e6 c/mL	8	0	0	479	744
13	Negative Calibrator	0	3	0	0	18	31
	B.microti Calibrator	500 c/mL	3	3	100	2,618,344	183,612
	B. microti IVT	100 c/mL	7	7	100	1,689,812	234,944
		30 c/mL	7	7	100	1,295,755	316,505
		10 c/mL	7	5	71	494,319	480,634
	B. duncani IVT	100 c/mL	7	5	71	644,015	376,799
		30 c/mL	7	7	100	1,026,959	141,543
		10 c/mL	7	7	100	1,808,125	209,910
	B. divergens IVT	100 c/mL	7	7	100	1,129,487	153,036

Condition #	Sample Description	Level	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
		30 c/mL	7	7	100	670,066	221,406
		10 c/mL	7	6	86	270,616	143,462
	Negative IC Buffer	0	8	0	0	400	644
	P. falciparum IVT	1.00e6 c/mL	8	0	0	1,237	1,803
14	Negative Calibrator	0	3	0	0	673	843
	B.microti Calibrator	500 c/mL	3	3	100	2,656,960	89,493
	B. microti IVT	100 c/mL	7	7	100	2,364,847	143,184
		30 c/mL	7	6	86	2,051,745	116,278
		10 c/mL	7	5	71	1,450,039	626,015
	B. duncani IVT	100 c/mL	7	4	57	1,136,704	353,951
		30 c/mL	7	7	100	1,839,238	336,415
		10 c/mL	7	7	100	2,315,286	141,813
	B. divergens IVT	100 c/mL	7	7	100	1,650,341	270,259
		30 c/mL	7	7	100	1,068,065	468,460
		10 c/mL	7	3	43	1,459,374	758,660
	Negative IC Buffer	0	8	0	0	574	689
	P. falciparum IVT	1.00e6 c/mL	8	0	0	941	1,394

Conclusions:

[00229] Several candidate systems identified on the manual eSAS system yielded good specificity and sensitivity with *Babesia* species IVTs for the *Babesia* Assay during secondary screening on the fully automated Panther system. Others combinations showed poor sensitivity (Condition 7) or specificity (Condition 1 and 4) and were not advanced to additional screening. A second round of screening on the Panther system identified a new system (Condition 12) with sensitivity in lower dilutions of *Babesia* species IVTs superior to other conditions tested and good specificity for the *Babesia* assay.

EXAMPLE 3: Clinical Sample Screening

Objective:

[00230] Candidate amplification systems previously identified for the *Babesia* Assay on the manual Procleix Enhanced Semi-automated System (eSAS) were screened on the fully automated Procleix Panther System to determine the best candidates in terms of specificity and sensitivity using a *Babesia* clinical sample. Primers screened in this example are the same as Example 2. This experiment served as an additional test to determine the best performing candidates.

Materials and Methods:

[00231] Candidate amplification systems were tested on the automated Procleix Panther system. Combinations tested are listed in **Table 14**. Sequences for each oligo are listed in **Table 15**. A total of 8 conditions were screened. Conditions screened were tested using 9 replicates each of a lysed negative whole blood and diluted *Babesia*-infected clinical sample. The *Babesia* clinical sample consisted of a PCR Positive *Babesia*-infected human red blood cell (RBC) sample. The sample was received with an estimated parasite per mL value. Based upon the estimated parasite per mL value, the clinical sample was diluted in normal negative human whole blood to an estimated 30, 10, and 3 parasites per mL. The diluted *Babesia*-infected whole blood was lysed at a 1 to 6 ratio or 0.8 mL of whole blood in 4.8mL of lysis solution Aptima Urine Transport Medium (commercially available). The negative panel was lysed according to the same procedure. Assay Reagents used included the following: a Target Capture Reagent (TCR) comprising of a single Target Capture Oligo (TCO) added at a concentration of 5 pmoles per reaction; an Amplification Reagent comprising one T7 promoter provider and one non-T7 primer each added at a concentration of 10 pmoles per reaction; a Probe reagent consisting of 1 acridinium-ester (AE) labeled probe added at a total concentration of 5e6 Relative Light Units (RLU) per reaction to a solution of ; Enzyme Reagent; and Selection Reagent.

[00232] Conditions tested in this group are listed in **Table 16**. Sequences for each oligo are listed in **Table 17**. A total of 6 additional conditions were screened. Conditions were tested using 5 replicates of each of a *Babesia*-negative whole blood sample and a diluted *Babesia*-infected whole blood clinical sample. Prior to testing the negative and positive samples, the parasite concentration of the *Babesia*-infected clinical sample was estimated

by using a calibration curve. The calibration curve was generated using lysates from serial dilutions of a known standard (a quantified *Babesia*-positive whole blood specimen). The clinical samples and the serial dilutions of the known standard were tested using a real-time assay. The Ttimes from each of the dilutions of the known standard were plotted against the parasites/mL to generate an equation. The equation was then used to convert Ttime to parasites/mL when the clinical sample was run using the same assay. The clinical sample was then diluted in normal negative human whole blood to an estimated 30 and 10 parasites per mL. The diluted clinical sample was lysed at a ratio of 1mL whole blood to 3mL lysis buffer. An assay calibrator comprising a *B. microti* IVT panel at 500 c/mL was also used. Similar to the screening described in the above examples, a single TCO was added to TCR at 5 pmoles per reaction, One T7 promoter provider and one non-T7 primer were added to Amplification Reagent at 10 pmoles per reaction each. The Probe reagent in this round of screening consisted of SEQ ID NO:37 detection probe oligomer at 2.5e6 RLU per reaction and SEQ ID NO:42 detection probe oligomer at 5e6 RLU per reaction.

Table 14. Conditions 1-8 Tested on Panther

Condition #	Target Capture Oligo	T7 Primer	Non-T7 Primer	Probe
1	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:11	SEQ ID NO:37
2	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:27	SEQ ID NO:39
3	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:27	SEQ ID NO:38
4	SEQ ID NO:43	SEQ ID NO:1	SEQ ID NO:13	SEQ ID NO:37
5	SEQ ID NO:43	SEQ ID NO:3	SEQ ID NO:21	SEQ ID NO:37
6	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:21	SEQ ID NO:37
7	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:27	SEQ ID NO:39
8	SEQ ID NO:43	SEQ ID NO:5	SEQ ID NO:21	SEQ ID NO:37

Table 15. Sequences for Oligos listed in Table 14.

Reagent	Short Name	Sequence 5' - 3'
TCR	SEQ ID NO:43	uaggccaauacccuaccgucctttaaaaaaaaaaaaaaaaaa aaaaaaaaaaa
Reagent	**Short Name**	**Sequence 5' - 3'**
Amplification	SEQ ID NO:1	aatttaatacgactcactatagggagattcacctctgacagttaa atacgaa
	SEQ ID NO:3	aatttaatacgactcactatagggagaacagttaaatacgaatg cccccaa
	SEQ ID NO:5	aatttaatacgactcactatagggagattcacctctgacagttaa atac
	SEQ ID NO:7	aatttaatacgactcactatagggagagctttcgcagtagttcgt ctttaacaaatc
	SEQ ID NO:13	cttgaatactacagcatggaataa
	SEQ ID NO:21	agaaaactagagtgtttcaa
	SEQ ID NO:27	atggaataatgaagtaggac
Reagent	**Short Name**	**Sequence 5' - 3'**
Probe	SEQ ID NO:37	ugaaguaggacuuugguucu
	SEQ ID NO:38	uaaugguuaauaggagcaguug
	SEQ ID NO:39	aguaaugguuaauaggagca

Table 16. Conditions 9-14 tested on Panther

Condition #	Target Capture Oligo	T7 Primer	Non-T7 Primer	Probe
9	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:21	SEQ ID NOs:37+ 42
10	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:32	SEQ ID NOs:37+ 42
11	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:33	SEQ ID NOs:37+ 42
12	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:34	SEQ ID NOs:37+ 42
13	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:35	SEQ ID NOs:37+ 42
14	SEQ ID NO:43	SEQ ID NO:7	SEQ ID NO:36	SEQ ID NOs:37+ 42

Table 17. Sequences for Oligos listed in Table 16.

Reagent	SEQ ID NO:	Sequence 5' - 3'
TCR	SEQ ID NO:43	uaggccaauacccuaccgucctttaaaaaaaaaaaaaaaaaaaaaaaaaaaa
Reagent	**SEQ ID NO:**	**Sequence 5' - 3'**
Amplification	SEQ ID NO:7	aatttaatacgactcactatagggagagctttcgcagtagttcgtctttaacaaatc
	SEQ ID NO:21	agaaaactagagtgtttcaa
	SEQ ID NO:32	agaaaactagagtgtttca
	SEQ ID NO:33	agaaaattagagtgtttcaa
	SEQ ID NO:34	gagaaaactagagtgtttcaa
	SEQ ID NO:35	gagaaaattagagtgtttcaa
	SEQ ID NO:36	tgagaaaactagagtgtttc
Reagent	**SEQ ID NO:**	**Sequence 5' - 3'**
Probe	SEQ ID NO:37	ugaaguaggacuuugguucu
	SEQ ID NO:42	aguaggacuuugguucu

Results:

[00233] An analyte signal cutoff of 100,000 RLU was used to determine reactivity for specimens screened for Conditions 1-8 in **Table 18**. Samples above this cutoff were considered reactive. Conditions 1 and 4 yielded a false positive in lysed negative whole blood specimens at a rate of 11% and 33%, respectively. Conditions 2, 5 and 8 detected *Babesia* at less than 100% in lysed *Babesia*-infected clinical samples at 10 and 3 parasites per mL. Due to the high rate of false positives with SEQ ID NO:1 promoter provider and the high number of lower performing candidates, additional screening was performed to identify alternative candidates.

Table 18. Average Analyte Results for Conditions 1-9 testing lysed Clinical Sample Dilutions on the Panther System.

Condition #	Sample Description	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
1	*B.microti* Calibrator	3	3	100%	2,895,211	95,178
	Babesia ARC 0 p/mL	9	1	11%	43,072	111,361
	Babesia ARC 3 p/mL	9	9	100%	2,881,463	51,926
	Babesia ARC 10 p/mL	9	9	100%	2,947,197	42,332
	Babesia ARC 30 p/mL	9	9	100%	2,921,132	65,204
2	*B.microti* Calibrator	3	3	100%	2,164,104	85,510
	Babesia ARC 0 p/mL	9	0	0%	655	63
	Babesia ARC 3 p/mL	9	6	67%	1,263,656	996,750
	Babesia ARC 10 p/mL	9	9	100%	2,134,186	27,346
	Babesia ARC 30 p/mL	9	9	100%	2,167,935	51,972
3	*B.microti* Calibrator	3	3	100%	5,202,778	356,597
	Babesia ARC 0 p/mL	9	0	0%	1,460	140
	Babesia ARC 3 p/mL	9	9	100%	5,018,781	930,364
	Babesia ARC 10 p/mL	9	9	100%	5,282,309	286,096
	Babesia ARC 30 p/mL	9	9	100%	5,347,545	183,066
4	*B.microti* Calibrator	3	3	100%	2,847,426	127,963
	Babesia ARC 0 p/mL	9	3	33%	248,507	581,559
	Babesia ARC 3 p/mL	9	9	100%	2,970,739	88,830
	Babesia ARC 10 p/mL	9	9	100%	3,007,234	76,308
	Babesia ARC 30 p/mL	9	9	100%	3,034,392	82,795
5	B.microti Calibrator	3	3	100%	2,614,010	43,029
	Babesia ARC 0 p/mL	9	0	0%	1,103	519
	Babesia ARC 3 p/mL	9	7	78%	1,714,505	1,012,100
	Babesia ARC 10 p/mL	9	6	67%	1,372,599	1,250,728
	Babesia ARC 30 p/mL	9	9	100%	2,455,769	54,600
6	*B.microti* Calibrator	3	3	100%	2,849,900	59,411
	Babesia ARC 0 p/mL	9	0	0%	765	72
	Babesia ARC 3 p/mL	9	9	100%	2,784,938	77,517
	Babesia ARC 10 p/mL	9	9	100%	2,741,662	63,328
	Babesia ARC 30 p/mL	9	9	100%	2,734,625	46,780
7	*B.microti* Calibrator	3	3	100%	5,706,224	96,503
	Babesia ARC 0 p/mL	9	0	0%	3,383	1,376

Condition #	Sample Description	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
	Babesia ARC 3 p/mL	9	9	100%	5,192,042	511,510
	Babesia ARC 10 p/mL	9	9	100%	5,240,227	135,033
	Babesia ARC 30 p/mL	9	9	100%	5,425,945	228,652
8	*B.microti* Calibrator	3	3	100%	2,963,935	70,693
	Babesia ARC 0 p/mL	9	0	0%	1,163	596
	Babesia ARC 3 p/mL	9	5	56%	1,708,479	1,623,206
	Babesia ARC 10 p/mL	9	9	100%	3,060,059	187,482
	Babesia ARC 30 p/mL	9	9	100%	3,112,370	83,507

[00234] In the previous round of screening, only a few candidates showed good sensitivity and specificity for lysed *Babesia*-infected clinical samples. Condition 6 had good specificity and sensitivity detecting the lysed clinical sample down to 3 parasites per mL at 100% reactivity and with strong analyte signal for the positive specimens tested and low analyte signal for negative specimens. Additional screening was performed on the Panther system using SEQ ID NO:7 promoter provider paired with a number of additional non-T7 primers. Conditions in this round of screening were again tested using lysed dilutions of a clinical sample. All conditions except Condition 11 detected the 30 and 10 p/mL lysates at 100% reactivity. None of conditions yielded any false positives in negative specimens.

Table 19. Average Analyte Results for Conditions 9-14 testing lysed Wadsworth Center (WC) sample dilutions on Panther.

Condition #	Sample Description	Level	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
9	Negative Calibrator	0	3	0	0	3,064	2,870
	B.microti Calibrator	500 c/mL	3	3	100	2,634,036	20,467
	Babesia WC	30 p/mL	5	5	100	2,335,851	100,942
		10 p/mL	5	5	100	1,970,484	170,746
	Negative	0	5	0	0	1,229	1,061

Condition #	Sample Description	Level	Valid	Reactive	% Reactive	Average Analyte RLU	StdDev Analyte RLU
10	Negative Calibrator	0	3	0	0	2,083	1,176
	B.microti Calibrator	500 c/mL	3	3	100	2,612,367	134,872
	Babesia WC	30 p/mL	5	5	100	1,700,198	40,527
		10 p/mL	5	5	100	1,639,511	112,124
	Negative	0	5	0	0	879	899
11	Negative Calibrator	0	3	0	0	1,687	1,471
	B.microti Calibrator	500 c/mL	3	3	100	2,476,171	150,100
	Babesia WC	30 p/mL	5	5	100	2,083,998	176,018
		10 p/mL	5	3	60	833,006	790,769
	Negative	0	5	0	0	2,237	1,256
12	Negative Calibrator	0	3	0	0	370	640
	B.microti Calibrator	500 c/mL	3	3	100	2,742,289	194,078
	Babesia WC	30 p/mL	5	5	100	2,220,041	241,385
		10 p/mL	5	5	100	2,098,119	175,399
	Negative	0	5	0	0	970	648
13	Negative Calibrator	0	3	0	0	18	31
	B.microti Calibrator	500 c/mL	3	3	100	2,618,344	183,612
	Babesia WC	30 p/mL	5	5	100	1,861,428	170,146
		10 p/mL	5	5	100	2,032,573	300,759
	Negative	0	5	0	0	405	871
14	Negative Calibrator	0	3	0	0	673	843
	B.microti Calibrator	500 c/mL	3	3	100	2,656,960	89,493
	Babesia WC	30 p/mL	5	5	100	2,523,855	87,845
		10 p/mL	5	5	100	2,376,263	191,838
	Negative	0	5	0	0	2,381	4,735

Conclusion:

[00235] Considering results for secondary screening with *Babesia* species IVTs in Example 2 and screening with clinical sample in this example, Conditions 3 and 6 were the best candidates in terms of specificity and sensitivity. Condition 12 had the best performance of the conditions screened in Conditions 9-14. Condition 12 had best performance in terms of percent reactivity for all levels tested and consistent RLU signal for all levels tested for

Babesia species IVTs and lysed clinical sample dilutions on the Panther system. Additionally, Condition 12 had the lowest analyte signal for negative specimens.

[00236] From the foregoing, it will be appreciated that, although specific embodiments have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of this explicit disclosure . Accordingly, the invention is not limited by the explicit disclosure. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entireties for all purposes.

EXAMPLE 4: *Babesia* IVTs and *Babesia* Positive Whole Blood Sample Screening

Objective:

[00237] Candidate amplification systems were screened on the Procleix Panther System to determine the oligo candidates in terms of specificity and sensitivity using IVTs and a contrived *Babesia* clinical sample.

Materials and Methods:

[00238] Candidate amplification systems were tested on the automated Procleix Panther system. Combinations tested are listed in **Table 20**. Sequences for each oligo are listed in **Table 21**. A total of 8 conditions were screened. Conditions were screened using 16 replicates of panel members consisting of in-vitro transcript diluted in buffer for *B. microti*, *B. divergens*, *B. duncani*, and *B. venatorum* (SEQ ID NOs:61, 62, 63, & 100) at either 30 c/mL or 10 c/mL. Conditions were also screened against 12 replicates each of a contrived clinical sample. The contrived clinical sample comprised a mixture of *Babesia*-negative human whole blood and *Babesia microti*-infected hamster whole blood (acquired from the American Red Cross (ARC)). In short, *Babesia microti* infected hamster blood having a predetermined parasitemia value was diluted with *Babesia*-negative human whole blood to yield an estimated 4 parasites/mL (p/mL) of an infected blood mixture. The infected blood mixture was then lysed at a 1 to 3 ratio (here using 0.9 mL of whole blood in 2.7 mL of an aqueous solution of 100 mM TRIS, 30 mM magnesium chloride, and 6% (v/v) LLS, at pH 7.5). The lysed infected blood mixture is referred to herein as a hamster blood lysate. In some test conditions hamster blood lysate was further diluted in negative lysate to a level equating to 0.01 parasites/mL. A negative lysate was prepared according to the same

procedure. An assay positive calibrator consisted of a *B. microti* in-vitro transcript (IVT) (SEQ ID NO:61) panel at 500 c/mL. Negative calibrator and negative buffered specimens contained only buffer. Assay Reagents used consisted of: Target Capture Reagent (TCR) comprising of a single Target Capture Oligo (TCO) added at a concentration of approximately 5 pmoles per reaction; Amplification Reagent comprising of T7 and NT7 primers added at a concentration of approximately 5 pmoles each per reaction; Probe reagent consisting of acridinium-ester (AE) labeled probes added at a total concentration of approximately 1e6 Relative Light Units (RLU) per reaction. Commercially available Procleix Ultrio Plus Enzyme and Selection reagents were used.

Table 20. Conditions 1-8 Tested on Panther

Condition #	Oligo Combination
1	SEQ ID NO:89, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:34, SEQ ID NO:86, SEQ ID NO:91, SEQ ID NO:42, SEQ ID NO:98
2	SEQ ID NO:87, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:34, SEQ ID NO:86, SEQ ID NO:91, SEQ ID NO:42, SEQ ID NO:98
3	SEQ ID NO:43, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:34, SEQ ID NO:86, SEQ ID NO:91, SEQ ID NO:42, SEQ ID NO:98
4	SEQ ID NO:43, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:91, SEQ ID NO:42, SEQ ID NO:98
5	SEQ ID NO:43, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:86, SEQ ID NO:91, SEQ ID NO:42, SEQ ID NO:98
6	SEQ ID NO:43, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:34, SEQ ID NO:86, SEQ ID NO:91
7	SEQ ID NO:43, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:34, SEQ ID NO:86, SEQ ID NO:92
8	SEQ ID NO:43, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:7, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:34, SEQ ID NO:86, SEQ ID NO:93

Table 21. Sequences for Oligos listed in Table 20.

Seq #	Reagent	Class	Sequences (5'-3')
SEQ ID NO:43	TCR	Capture Oligo	uaggccaauacccuaccgucctttaaaaaaaaaaaaaaaaaaaaaaaaaaaa
SEQ ID NO:87		Capture Oligo	aaagacuuugauuucucucaaggtttaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
SEQ ID NO:89		Capture Oligo	caagaaagagcuaucaaucugucaaucctttaaaaaaaaaaaaaaaaaaaaaaaaaaa
SEQ ID NO:7	Amplification	T7 Primer	aatttaatacgactcactatagggagagctttcgcagtagttcgtctttaacaaatc
SEQ ID NO:82		T7 Primer	aatttaatacgactcactatagggagaggcaaatgctttcgcagtagttigtcttta aca
SEQ ID NO:84		Non-T7 primer	gcggtaattccagctccaatag
SEQ ID NO:34		Non-T7 primer	gagaaaactagagtgtttcaa
SEQ ID NO:86		Non-T7 primer	cttgaatacticagca
SEQ ID NO:91	Probe	Probe	aguaggacuuugguuct
SEQ ID NO:92		Probe	aguaggacuuugguuc
SEQ ID NO:93		Probe	aguaggacuauugguuc
SEQ ID NO:42		Probe	aguaggacuuugguucu
SEQ ID NO:98		Probe	aguaggacuauugguucu

Results:

[00239] An analyte signal cutoff calculated was calculated using negative and positive calibrators and the Panther Software. Test results yielding a signal to cutoff ratio of greater than or equal to 1.0 were considered reactive. All conditions tested detected *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum* as well as the hamster blood lysate panel members to some degree (**Table 22**). All conditions detected all species at 100% reactivity at the 30c/mL level except Condition 5 in which 15 out of 16 replicates were detected. For conditions 1-5 all hamster blood lysate panels at 4 p/mL were 100% reactive except Condition 2 in which 8 out of 12 replicates were detected. For Conditions 6-8, which tested hamster blood lysates at 0.01 p/mL, results show 14/16, 15/16, and 14/16 positive. There were no false positive in negative reactions.

Table 22. Average Analyte Results for Conditions 1-8 testing.

Condition #	Sample Description	c/mL	Valid	Reactive	% Reactive	Analyte RLUs	SD Analyte RLUs	Analyte S/CO	SD Analyte S/CO
1	Negative Calibrator	0	3	0	0.0	0	0	0.00	0.00
	Positive Calibrator	500	3	3	100.0	1,310,257	45,626	33.33	1.16
	B. microti	30 c/mL	16	16	100.0	691,469	302,377	17.59	7.69
		10 c/mL	16	15	93.8	648,137	282,342	16.49	7.18
	B. duncani	30 c/mL	16	16	100.0	1,100,887	309,481	28.01	7.87
		10 c/mL	16	16	100.0	973,540	246,634	24.77	6.27
	B. divergens	30 c/mL	16	16	100.0	1,099,778	294,789	27.98	7.50
		10 c/mL	16	16	100.0	959,134	335,825	24.40	8.54
	B. venatorum	30 c/mL	16	16	100.0	1,052,533	280,903	26.78	7.15
		10 c/mL	16	16	100.0	902,987	309,501	22.97	7.87
	Negative Buffer	0 c/mL	16	0	0.0	0	0	0.00	0.00
	Hamster Blood Lysate	4	12	12	100.0	1,307,118	28,853	33.25	0.73
	Hamster Blood Lysate	0	12	0	0.0	4	15	0.00	0.00
2	Negative Calibrator	0	3	0	0.0	14	24	0.00	0.00
	Positive Calibrator	500	3	3	100.0	1,360,358	88,289	33.32	2.16
	B. microti	30 c/mL	16	16	100.0	990,774	405,235	24.27	9.93
		10 c/mL	16	13	81.3	674,210	358,804	16.52	8.79
	B. duncani	30 c/mL	16	16	100.0	1,261,270	234,066	30.90	5.73
		10 c/mL	16	16	100.0	1,010,323	412,156	24.75	10.10
	B. divergens	30 c/mL	16	16	100.0	1,136,882	281,770	27.85	6.90
		10 c/mL	16	16	100.0	1,138,086	288,726	27.88	7.07
	B. venatorum	30 c/mL	16	16	100.0	1,095,242	357,885	26.83	8.77
		10 c/mL	16	16	100.0	979,611	405,233	24.00	9.93
	Negative Buffer	0 c/mL	16	0	0.0	0	0	0.00	0.00
	Hamster Blood Lysate	4	12	8	66.7	1,148,331	589,637	28.13	14.44

Condition #	Sample Description	c/mL	Valid	Reactive	% Reactive	Analyte RLUs	SD Analyte RLUs	Analyte S/CO	SD Analyte S/CO
	Hamster Blood Lysate	0	12	0	0.0	220	763	0.01	0.02
3	Negative Calibrator	0	3	0	0.0	0	0	0.00	0.00
	Positive Calibrator	500	3	3	100.0	1,247,077	16,587	33.33	0.44
	B. microti	30 c/mL	16	16	100.0	996,284	147,903	26.63	3.95
		10 c/mL	16	14	87.5	733,412	225,774	19.60	6.03
	B. duncani	30 c/mL	16	16	100.0	1,154,290	46,421	30.85	1.24
		10 c/mL	16	16	100.0	1,051,223	93,259	28.10	2.49
	B. divergens	30 c/mL	16	16	100.0	1,160,454	42,968	31.02	1.15
		10 c/mL	16	16	100.0	1,067,057	104,387	28.52	2.79
	B. venatorum	30 c/mL	16	16	100.0	1,120,866	52,999	29.96	1.42
		10 c/mL	16	16	100.0	988,682	127,271	26.43	3.40
	Negative Buffer	0 c/mL	16	0	0.0	0	0	0.00	0.00
	Hamster Blood Lysate	4	12	12	100.0	1,153,646	24,699	30.84	0.66
	Hamster Blood Lysate	0	12	0	0.0	0	0	0.00	0.00
4	Negative Calibrator	0	3	0	0.0	0	0	0.00	0.00
	Positive Calibrator	500	3	3	100.0	689,988	39,272	33.33	1.90
	B. microti	30 c/mL	16	16	100.0	236,235	167,806	11.41	8.11
		10 c/mL	16	11	68.8	142,663	122,889	6.89	5.94
	B. duncani	30 c/mL	16	16	100.0	331,217	55,106	16.00	2.66
		10 c/mL	16	16	100.0	174,519	88,419	8.43	4.27
	B. divergens	30 c/mL	16	16	100.0	589,500	20,005	28.48	0.97
		10 c/mL	16	16	100.0	482,888	106,825	23.33	5.16
	B. venatorum	30 c/mL	16	16	100.0	560,636	66,993	27.09	3.24
		10 c/mL	16	16	100.0	406,685	129,080	19.65	6.24
	Negative Buffer	0 c/mL	16	0	0.0	0	0	0.00	0.00

Condition #	Sample Description	c/mL	Valid	Reactive	% Reactive	Analyte RLUs	SD Analyte RLUs	Analyte S/CO	SD Analyte S/CO
	Hamster Blood Lysate	4	12	12	100.0	870,137	62,684	42.04	3.03
	Hamster Blood Lysate	0	12	0	0.0	0	0	0.00	0.00
5	Negative Calibrator	0	3	0	0.0	0	0	0.00	0.00
	Positive Calibrator	500	3	3	100.0	1,041,315	125,263	33.33	4.01
	B. microti	30 c/mL	16	15	93.8	306,912	218,362	9.82	6.99
		10 c/mL	16	11	68.8	243,155	159,289	7.78	5.10
	B. duncani	30 c/mL	16	16	100.0	748,753	281,035	23.97	9.00
		10 c/mL	16	16	100.0	751,922	286,380	24.07	9.17
	B. divergens	30 c/mL	16	16	100.0	807,915	258,741	25.86	8.28
		10 c/mL	16	16	100.0	643,482	256,352	20.60	8.21
	B. venatorum	30 c/mL	16	16	100.0	792,659	264,484	25.37	8.47
		10 c/mL	16	16	100.0	747,633	243,096	23.93	7.78
	Negative Buffer	0 c/mL	16	0	0.0	0	0	0.00	0.00
	Hamster Blood Lysate	4	12	12	100.0	1,410,648	33,678	45.16	1.08
	Hamster Blood Lysate	0	12	0	0.0	84	210	0.00	0.01
6	Negative Calibrator	0 c/mL	3	0	0.0	0	0	0.00	0.00
	Positive Calibrator	500 c/mL	3	3	100.0	524,314	5,119	33.33	0.33
	B. microti	30 c/mL	16	16	100.0	507,560	25,719	32.26	1.64
	B. divergens	30 c/mL	16	16	100.0	471,886	64,616	30.00	4.11
	B. duncani	30 c/mL	16	16	100.0	502,786	46,565	31.96	2.96
	B. venatorum	30 c/mL	16	16	100.0	495,758	19,503	31.51	1.24
	Hamster Blood Lysate	0.01 p/mL	16	14	87.5	337,759	102,178	24.48	7.41
	Negative Buffer	0 c/mL	16	0	0.0	145	387	0.00	0.00

Condition #	Sample Description	c/mL	Valid	Reactive	% Reactive	Analyte RLUs	SD Analyte RLUs	Analyte S/CO	SD Analyte S/CO
7	Negative Calibrator	0 c/mL	3	0	0.0	254	439	0.01	0.02
	Positive Calibrator	500 c/mL	3	3	100.0	668,273	11,200	32.91	0.55
	B. microti	30 c/mL	16	16	100.0	654,530	36,463	32.24	1.80
	B. divergens	30 c/mL	16	16	100.0	652,634	44,688	32.14	2.20
	B. duncani	30 c/mL	16	16	100.0	628,767	49,861	30.97	2.46
	B. venatorum	30 c/mL	16	16	100.0	620,345	63,099	30.55	3.11
	Hamster Blood Lysate	0.01 p/mL	16	15	93.8	397,026	182,905	23.35	10.76
	Negative Buffer	0 c/mL	16	0	0.0	91	151	0.00	0.00
8	Negative Calibrator	0 c/mL	3	0	0.0	280	486	0.02	0.03
	Positive Calibrator	500 c/mL	3	3	100.0	521,485	14,518	32.74	0.91
	B. microti	30 c/mL	16	16	100.0	460,267	52,434	28.90	3.29
	B. divergens	30 c/mL	16	16	100.0	423,530	61,700	26.59	3.87
	B. duncani	30 c/mL	16	16	100.0	428,292	43,574	26.89	2.74
	B. venatorum	30 c/mL	16	16	100.0	415,273	64,983	26.07	4.08
	Hamster Blood Lysate	0.01 p/mL	16	14	87.5	198,996	127,811	13.96	8.97
	Negative Buffer	0 c/mL	16	0	0.0	817	885	0.00	0.00

Conclusion:

[00240] Oligo combinations tested herein exemplify specific and sensitive capture, amplification, and detection of *Babesia* species including *B. microti*, *B. divergens*, *B. duncani*, and *B. ventorum* and demonstrated detection of *Babesia microti* parasite in whole blood.

SEQUENCES

SEQ ID NO.	Sequence (5' to 3')	Comments
1	aatttaatacgactcactatagggagattcacctctgacagttaaatacgaa	T7 promoter primer
2	ttcacctctgacagttaaatacgaa	SEQ ID NO:1 without promoter sequence
3	aatttaatacgactcactatagggagaacagttaaatacgaatgcccccaa	T7 promoter primer
4	acagttaaatacgaatgcccccaa	SEQ ID NO:3 without promoter sequence
5	aatttaatacgactcactatagggagattcacctctgacagttaaatac	T7 promoter primer
6	ttcacctctgacagttaaatac	SEQ ID NO:5 without promoter sequence
7	aatttaatacgactcactatagggagagctttcgcagtagttcgtctttaacaaatc	T7 promoter primer
8	gctttcgcagtagttcgtctttaacaaatc	SEQ ID NO:7 without promoter sequence
9	aatttaatacgactcactatagggagactttcgcagtagttcgtctttaac	T7 promoter primer
10	ctttcgcagtagttcgtctttaac	SEQ ID NO:9 without promoter sequence
11	cttgaatactacagcatgga	Non-T7 primer
12	actacagcatggaataatga	Non-T7 primer
13	cttgaatactacagcatggaataa	Non-T7 primer
14	acttcagcatggaataatga	Non-T7 primer
15	cttgaatacttcagcatgga	Non-T7 primer
16	actncagcatggaataatga	Non-T7 primer, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
17	actwcagcatggaataatga	Non-T7 primer

SEQ ID NO.	Sequence (5' to 3')	Comments
18	cttgaatactncagcatgga	Non-T7 primer, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
19	cttgaatactwcagcatgga	Non-T7 primer
20	actttgagaaaactagagtg	Non-T7 primer
21	agaaaactagagtgtttcaa	Non-T7 primer
22	ataatgaagtaggactttgg	Non-T7 primer
23	aggactttggttctattttg	Non-T7 primer
24	ggttctattttgttggtt	Non-T7 primer
25	tggttctattttgttgg	Non-T7 primer
26	ggaataatgaagtaggacttt	Non-T7 primer
27	atggaataatgaagtaggac	Non-T7 primer
28	atggaataatgaagtagg	Non-T7 primer
29	gcatggaataatgaagtag	Non-T7 primer
30	tacagcatggaataatgaag	Non-T7 primer
31	tactacagcatggaataatg	Non-T7 primer
32	agaaaactagagtgtttca	Non-T7 primer
33	agaaaattagagtgtttcaa	Non-T7 primer
34	gagaaaactagagtgtttcaa	Non-T7 primer
35	gagaaaattagagtgtttcaa	Non-T7 primer
36	tgagaaaactagagtgtttc	Non-T7 primer
37	ugaaguaggacuuugguucu	Probe
38	uaaugguuaauaggagcaguug	Probe
39	aguaaugguuaauaggagca	Probe
40	ggacuuugguucuauuuuguugg	Probe
41	aaugguuaauaggagcaguuggggg	Probe
42	aguaggacuuugguucu	Probe
43	uaggccaauacccuaccgucctttaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	Target capture
44	uaggccaauacccuaccgucc	SEQ ID NO:43, without capture tail sequence

SEQ ID NO.	Sequence (5' to 3')	Comments
45	atggaataatgaagtag	Same sequence in SEQ ID NOs:27, 28, 29 and 31
46	agaaaactagagtg	Same sequence in SEQ ID NOs:20, 21, 32, 34 and 36
47	agaaaattagagtg	Same sequence in SEQ ID NOs:33 and 35.
48	agaaaaytagagtg	Substitutes y for c in SEQ ID NO:46 and y for t in SEQ ID NO:47, wherein "y" means c or t/u (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
49	agaaaactagagtgtttc	Same sequence in SEQ ID NOs:19, 20, 21, 32, 34 and 36.
50	agaaaattagagtgtttc	Same sequence in SEQ ID NOs:33 and 35.
51	agaaaaytagagtgtttc	Substitutes y for c in SEQ ID NO:49) and y for t in SEQ ID NO:50, wherein "y" means c or t/u (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
52	tactacagcatggaataa	Same sequence in SEQ ID NO:13 and 31.

SEQ ID NO.	Sequence (5' to 3')	Comments
53	actncagcatgga	Residue "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1), and "other preferably means "i" or inosine
54	actwcagcatgga	
55	actacagcatgga	Same sequence as SEQ ID NO:54, except that "n" is "a".
56	gtatttaactgt	Same sequence in SEQ ID NOs:1, 3 and 5.
57	Gtatttaactgtcagaggtgaa	Same sequence inSEQ ID NOs:1 and 5.
58	aatttaatacgactcactatagggaga	T7 promoter
59	aguaggacuuugguucu	Same sequence in SEQ ID NO:42.
60	uaaugguuaauaggagca	Same sequence in SEQ ID NOs:38 and 39.

SEQ ID NO.	Sequence (5' to 3')	Comments
61	gggcgaauuggguaccgggccccccucgaggucgacgcuuaguauaaagcuuuua uacagcgaaacugcgaauggcucauuaaaacaguuauaguuuauuugauguucgu uuuacauggauaaccgugguaauucuagggcuaauacaugcucgaggcgcguuuu cgcguggcguuuauuagacuuuaaccaacccuucgggguaaucggugauucauaau aaauuagcgaaucgcauggcuuugccggcgauguaucauucaaguuucugaccua ucagcuuuggacgguaggguauuggccuaccggggcgacgacgggugacggggaa uugggguucgauuccggagagggagccugagaaacggcuaccacaucuaaggaag gcagcaggcgcgcaaauuacccaauccugacacagggagguagugacaagaaauaa caauacagggcuuaaagucuuguaauuggaaugaugggaaucuaaacccuuccca gaguaucaauuggagggcaagucuggugccagcagccgcgguaauuccagcucca auagcguauauuaaaguuguugcaguuaagaagcucguaguugaauuucugccuu gucauuaaucucgcuuccgagcguuuuuuuauugacuuggcaucuucuggauuu ggugccuucggguacuauuuuccaggauuuacuuugagaaaacuagaguguuuca aacaggcauucgccuugaauacuacagcauggaauaaugaaguaggacuuugguu cuauuuuguugguuauugagccagaguaaugguuaauaggagcaguuggggca uucguauuuaacugucagaggugaaauucuuagauuuguuaaagacgaacuacug cgaaagcauuugccaaggauguuuucauuaaucaagaacgaaaguuaggggaucg aagacgaucagauaccgucguaguccuaaccauaaacuaugccgacuagagauugg aggucgucaguuuaaacgacuccuucagcaccuugagagaaaucaaagucuuugg guucuggggggaguauggucgcaagucugaaacuuaaaggaauugacggaagggc accaccaggcguggagccugcggcuuaauuugacucaacacgggaaaccucaccag guccagacauagaggaggauugacagauugauagcucuuucuugaugaauu	*B. microti* IVT Sequence

SEQ ID NO.	Sequence (5' to 3')	Comments
62	gggcgaauuggguaccgggccccccucgaggucgacgguaucgauaagcuugau aucgaauuccugcagcccgggggauccaaccugguugauccugccaguagucaua ugcuugucuuaaagauuaagccaugcaugucuaaguacaaacuuuuuacggugaa acugcgaauggcucauuacaacaguuauaguuucuuugguauucguuuuccaugg auaaccgugcuaauuguagggcuaauacaaguucgaggccuuuuggcggcguuua uuaguucuaaaaccaucccuuuuggguuuucggugauucauaauaaaacuugcgaau cgcaauuuuuugcgauggaccauucaaguuucugacccaucagcuugacgguagg guauuggccuaccgaggcagcaacgggguaacggggaauuaggguucgauuccgga gagggagccugagaaacggcuaccacauccaaggaaggcagcaggcgcgcaaauua cccaauccugacacagggagguagugacaagaaauaacaauacagggcaauugucu uguaauuggaaugauggugaccuaaaacccucaccagaguaacaauuggagggcaa gucuggugccagcagccgcgguaauuccagcuccaauagcguauauuaaacuugu ugcaguuaaaaagcucguaguugaauuuuugcgugguguuaauauugacuaaug ucgagauugcacuucgcuuuugggauuuaucccuuuuuacuuugagaaaauuaga guguuucaagcagacuuuugucuugaauacuucagcauggaauaauagaguagga cuuugguucuauuuuguugguuugugaaccuuaguaaugguuaauaggaacggu ugggggcauucguauuuaacugucagaggugaaauucuuagauuuguuaaagacg aacuacugcgaaagcauuugccaaggacguuuucauuaaucaagaacgaaaguuag gggaucgaagacgaucagauaccgucguaguccuaaccauaaacuaugccgacuag ggauuggaggucgucauuuuuccgacuccuucagcaccuugagagaaaucaaagu cuuuggguucuggggggaguauggucgcaaggcugaaacuuaaaggaauugacg gaagggcaccaccaggcguggagccugcggcuuaauuugacucaacacggggaaac ucaccagguccagacaauguuaggauugacagauugauagcucuuucuugauucu uuggguggugcggcc	*B. divergens* IVT Sequence

SEQ ID NO.	Sequence (5' to 3')	Comments
63	gggcgaauuggguaccgggccccccucgaggucgacguugauccugccaguagu cauaugcuugucuuaaagauuaagccaugcaugucuaaguauaaacuuuuauaug gugaaacugcgaauggcucauuacaacaguuauaguuuauuugaaagucguuuuu acauggauaaccgugcuaauuguagggcuaauacaugcucgaggccuuggcuccu gucuuggcugcguuuauuagacucgaaaccuucccgcuugcgguacucggugauu cauaauaaauuugcgaaucgcauggcuuuugccggcgaugguucauucaaguuuc ugaccuaucagcuuuggacgguaggguauuggccuaccggggcagcgacgggguaa cggggaauuaggguucgauuccggagagggagccugagaaacggcuaccacaucu aaggaaggcagcaggcgcgcaaauuacccaauacggacaccgugagguagugacaa gaaauaacaauacagggcuuaaagcuuuguaauuggaaugaugggaauccaaaccc cuuccagaguaucaauuggagggcaagucuggugccagcagccgcgguaauucca gcuccaauagcguauauuaaacuuguugcaguuaaaaagcucguaguugaacuuc ugccgcuuggccuuucguucccuuggguuucguucgccugguggcuuaccuc uggcgguguucuccauuugccaguuuuacuuugagaaaauuagaguguuucaa gcaggcuuuugccuugaauacuucagcauggaauaauaaaguaggacuuugguuc uauuuuguuggguuucaggaccaaaguaaugguuaauaggaacaguuggggcau ucguauuuaacugucagaggugaaauucuuagauuuguuaaagacgaacuacugc gaaagcauuugccaaggauguuuucauuaaucaagaacgaaaguuaggggcucga agacgaucagauaccgucguaguccuaacuauaaaacuaugccgacuagagauugga ggucgucauuuuaaacgacuccuucagcaccuugagagaaaucaaagucuuuggg uucuggggggaguauggucgcaaggcugaaacuuaaaggaauugacggaagggca ccaccaggcguggagccugcggcuuaauuugacucaacacggggaaccucaccagg uccagacauaguuaggauugacagauugauagcucgaauu	*B. duncani* IVT Sequence

SEQ ID NO.	Sequence (5' to 3')	Comments
64	gggcgaauuggguaccguagaaacugcgaacggcucauuaaaacaguuauagucu acuugacauuuuuauuauaaggauaacuacggaaaagcuguagcuaaauacuugcu uuauuauccuuugauuuuuuaucuuuggauaaguauuuguuaggccuuauaagaa aaaaguuauuaacuuaaggaauuauaacaaagaaguaacacguaauaaauuuauuu uauuuaguguguaucaaucgaguuucugaccuaucagcuuuugauguuagggua uuggccuaacauggcuaugacggguaacggggaauuagaguucgauuccggagag ggagccugagaaauagcuaccacaucuaaggaaggcagcaggcgcguaaauuaccc aauucuaaaaaagagagguagugacaagaaauaacaaugcaaggccaauuuuuggu uuuguaauuggaauggugggaauuuaaaaccuucccagaguaacaauuggagggc aagucuggugccagcagccgcgguaauuccagcuccaauagcguauauuaaaauu guugcaguuaaaacgcucguaguugaauuucaaagaaucgauauuuuauuguaac uauucuaggggaacuauuuuaggguuuucgcuuuaauacgcuuccucuauuauuau guucuuuaaauaacaaagauucuuuuuaaaaucccccacuuuugcuuuuuugggga auuuguuacuuugaauaaauuagaggugucaaagcaaacaguuaaagcauuuacu guguuugaauacuauagcauggaauaacaaaauugaacaagcuaaaauuuuuugu ucuuuuuucuuauuuuggcuuaguuacgauuaauaggaguagcuuggggacauu cguauucagaugucagaggugaaauucuuagauuuucuggagacgaacaacugcg aaagcauuugucuaaaauacuuccauuaaucaagaacgaaaguuaagggagugaag acgaucagauaccgucguaaucuuaaccauaaacuaugccgacuagguguuggau gaaaguguuaaaaauaaaagucaucuuucuaggugacuuuuagauugcuuccuuc aguaccuuaugagaaaucaaagucuuuggguucuggggcgaguauucgcgcaagc gagaaaguuaaaagaauugacggaagggcaccaccaggcguggagcuugcggcuu aauuugacucaacacggggaaacucacuaguuuaagacaagaguaggauugacaga uuaauagcucuuucuugauuucuuggauggugaugcauggccguuuuuaguucg ugaauaugauuugucuggguuaauuccgauaacgaacgagaucggauccacuaguu cuagagcggcc	*P. falciparum* IVT Sequence
65	ugaaguaggacuuuggguucuauuuuguugguuauugagccagaguaaugguuaa uaggagcaguuggggg	
66	ttcacctctgacagttaaatacgaatgcccccaa	Contains SEQ ID NOs:56 and 57, which the target hybridising sequence can contain

SEQ ID NO.	Sequence (5' to 3')	Comments
67	tactncagcatggaataatgaagtaggactttgg	Contains SEQ ID NOs:45 & 69, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
68	cttgaatactncagcatggaataatga	Contains SEQ ID NOs:53, 54 or 55, which the target hybridising sequence can contain, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
69	atggaataatg	Contained in SEQ ID NO:67
70	actttgagaaaaytagagtgtttcaaa	Contains SEQ ID NOs:46, 47, 48, 49, 50, and 51, wherein "y" means c or t/u (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

71	aacctggttgatcctgccagtagtcatatgcttgtcttaaagattaagccatgcatgtcttagtat aagcttttatacagcgaaactgcgaatggctcattaaaacagttatagtttatttgatgttcgtttt acatggataaccgtggtaattctagggctaatacatgctcgaggcgcgttttcgcgtggcgttt attagactttaaccaaccccttcgggtaatcggtgattcataataaattagcgaatcgcatggctt tgccggcgatgtatcattcaagtttctgacctatcagctttggacggtagggtattggcctacc ggggcgacgacgggtgacggggaattggggttcgattccggagagggagcctgagaaa cggctaccacatctaaggaaggcagcaggcgcgcaaattacccaatcctgacacagggag gtagtgacaagaaataacaatacagggcttaaagtcttgtaattggaatgatgggaatctaaa cccttcccagagtatcaattggagggcaagtctggtgccagcagccgcggtaattccagctc caatagcgtatattaaagttgttgcagttaagaagctcgtagttgaatttctgccttgtcattaatc tcgcttccgagcgttttttattgacttggcatcttctggatttggtgccttcgggtactattttcca ggatttactttgagaaaactagagtgtttcaaacaggcattcgccttgaatactacagcatgga ataatgaagtaggactttggttctattttgttggttattgagccagagtaatggttaataggagca gttgggggcattcgtatttaactgtcagaggtgaaattcttagatttgttaaagacgaactactg cgaaagcatttgccaaggatgttttcattaatcaagaacgaaagttaggggatcgaagacga tcagataccgtcgtagtcctaaccataaactatgccgactagagattggaggtcgtcagttta aacgactccttcagcaccttgagagaaatcaaagtctttgggttctgggggagtatggtcg caagtctgaaacttaaaggaattgacggaagggcaccaccaggcgtggagcctgcggctt aatttgactcaacacgggaaacctcaccaggtccagacatagagaggattgacagattgata gctctttcttgattctatgggtggtggtgcatggccgttcttagttggtggagtgatttgtctggtt aattccgttaacgaacgagaccttaacctgctaaattaggatctgggacaagctttgctgttcc agtatcgcttcttagagggactttgcgttcataaaacgcaaggaagtgtaaggcaataacag gtctgtgatgcccttagatgtcctgggctgcacgcgcgctacactgatgcattcaacgagtttt tccttggccgtcgggtccgggtaatcttacagtatgcatcgtgatggggatagattattgcaat tattaatcttgaacgaggaatgcctagtaggcgcgagtcatcagctcgtgccgactacgtcc ctgccctttgtacacaccgcccgtcgctcctaccgatcgagtgatccggtgaattattcggac caagaaacgtggattcgtccttcgttttttggaaagttttgtgaaccttatcacttaaaggaagg agaagtcgtaacaaggtttccgtaggtgaacctgcggaaggatcattc	GenBank Accession No: AY693840.1 *Babesia* microti isolate Gray 18S ribosomal RNA gene, partial sequence

SEQ ID NO.	Sequence (5' to 3')	Comments
72	aacctggttgatcctgccagtagtcatatgcttgtcttaaagattaagccatgcatgtctaagta caaacttttacggtgaaactgcgaatggctcattacaacagttatagtttctttggtattcgtttt ccatggataaccgtgctaattgtagggctaatacaagttcgaggccttttggcggcgtttatta gttctaaaaccatccctttggttttcggtgattcataataaacttgcgaatcgcaatttttgcgat ggaccattcaagtttctgacccatcagcttgacggtagggtattggcctaccgaggcagcaa cgggtaacggggaattagggttcgattccggagagggagcctgagaaacggctaccacat ccaaggaaggcagcaggcgcgcaaattacccaatcctgacacagggaggtagtgacaag aaataacaatacagggcaattgtcttgtaattggaatgatggtgacctaaaccctcaccagag taacaattggagggcaagtctggtgccagcagccgcggtaattccagctccaatagcgtata ttaaacttgttgcagttaaaaagctcgtagttgaatttttgcgtggtgttaatattgactaatgtcg agattgcacttcgcttttgggatttatccctttttactttgagaaaattagagtgtttcaagcagac ttttgtcttgaatacttcagcatggaataatagagtaggactttggttctattttgttggtttgtgaa ccttagtaatggttaataggaacggttgggggcattcgtatttaactgtcagaggtgaaattctt agatttgttaaagacgaactactgcgaaagcatttgccaaggacgttttcattaatcaagaacg aaagttaggggatcgaagacgatcagataccgtcgtagtcctaaccataaactatgccgact agggattggaggtcgtcatttttccgactccttcagcaccttgagagaaatcaaagtctttggg ttctgggggggagtatggtcgcaaggctgaaacttaaaggaattgacggaagggcaccacc aggcgtggagcctgcggcttaatttgactcaacacggggaaactcaccaggtccagacaat gttaggattgacagattgatagctctttcttgattctttgggtggtggtgcatggccgttcttagtt ggtggagtgatttgtctggttaattccgttaacgaacgagaccttaacctgctaactagtgtcc gtaaaaaggttcgtccgttacggtttgcttcttagagggactttgcggctctaagccgcaagg aagtttaaggcaataacaggtctgtgatgcccttagatgtcctgggctgcacgcgcgctaca ctgatgcattcatcgagttttatcccttcccgaaagggctgggtaatctttagtatgcatcgtga cggggattgatttttgcaattctaaatcatgaacgaggaatgcctagtatgcgcaagtcatcag cttgtgcagattacgtccctgccctttgtacacaccgcccgtcgctcctaccgatcgagtgatc cggtgaattattcggaccgtggccttccgattcgtcggcttggcctagggaagtcttgtgaa ccttatcacttaaaggaaggagaagtcgtaacaaggtttccgtaggtgaacctgcggaagg atcattc	GenBank Accession No: AY789076.1 *Babesia* divergens 18S ribosomal RNA gene, partial sequence

73	ccttggttgatcctgccagtagtcatatgcttgtcttaaggattaagccatgcatgtctaagtata aacttttatatggtgaaactgcgaatggctcattacaacagttatagtttatttgaaagtcgttttt acatggataaccgtgctaattgtagggctaatacatgctcgaggccttggcttctgtcttggct gcgtttattagactcgaaaccttcccgcttgcggtactcggtgattcataataaatttgcgaatc gcatggcttttgccggcgatggttcattcaagtttctgacctatcagctttggacggtagggtat tggcctaccggggcagcgacgggtaacggggaattagggttcgattccggagagggagc ctgagaaacggctaccacatctaaggaaggcagcaggcgcgcaaattacccaatacggac accgtgaggtagtgacaagaaataacaatacagggctttaagctttgtaattggaatgatggg aatccaaaccccttccagagtatcaattggagggcaagtctggtgccagcagccgcggtaa ttccagctccaatagcgtatattaaacttgttgcagttaaaaagctcgtagttgaacttctgccg cttggcctttcgttcccctttgggtttcgttcgcctggtggcttacctctggcggtggttctccat ttgccagttttactttgagaaaattagagtgtttcaagcaggcttttgccttgaatacttcagcat ggaataataaagtaggactttggttctattttgttggtttcaggaccaaagtaatggttaatagg aacagttgggggcattcgtatttaactgtcagaggtgaaattcttagatttgttaaagacgaact actgcgaaagcatttgccaaggatgttttcattaatcaagaacgaaagttaggggctcgaag acgatcagataccgtcgtagtcctaactataaactatgccgactagagattggaggtcgtcat ttaaacgactccttcagcaccttgagagaaatcaaagtctttgggttctgggggagtatggt cgcaaggctgaaacttaaaggaattgacggaagggcaccaccaggcgtggagcctgcgg cttaatttgactcaacacggggaacctcaccaggtccagacatagttaggattgacagattga tagctctttcttgattctatgggtagtggtgcatggccgttcttagttggtggagtgatttgtctgg ttaattccgttaacgaacgagaccttaacctgctaaatagcagctgagaataatctcttgtttca gttttgcttcttagagggactttgcggtcataaatcgcaaggaagtttaaggcaataacaggtc tgtgatgcccttagatgtcctgggctgcacgcgcgctacactgatgcattcatcgagttttatc cttgcccgaaagggtttggtaatctttagtatgcatcgtgatggggattgattattgcaattatta atcatgaacgaggaatgcctagtaggcgcgagtcatcagctcgtgccgactacgtccctgc cctttgtacacaccgcccgtcgctcctaccgatcgagtgatccggtgaattattcggaccgtg acgcttctaattcgttagaaatgtctagggaagttttgtgaaccttatcacttaaaggaaggag aagtcgtaacaaggtttccg	GenBank Accession No: AY027815.1 *Babesia sp.* WA1 isolate CA5 18S ribosomal RNA gene, partial sequence

SEQ ID NO.	Sequence (5' to 3')	Comments
74	tcaaagattaagccatgcaagtgaaagtatatatatatttatatgtagaaactgcgaacggctc attaaaacagttatagtctacttgacatttttattataaggataactacggaaaagctgtagctaa tacttgctttattatcctttgatttttatctttggataagtatttgttaggccttataagaaaaaagtta ttaacttaaggaattataacaaagaagtaacacgtaataaatttatttttatttagtgtgtatcaatc gagtttctgacctatcagcttttgatgttagggtattggcctaacatggctatgacgggtaacg gggaattagagttcgattccggagagggagcctgagaaatagctaccacatctaaggaag gcagcaggcgcgtaaattacccaattctaaaaaagagaggtagtgacaagaaataacaatg caaggccaatttttggtttgtaattggaatggtgggaatttaaaaccttcccagagtaacaatt ggagggcaagtctggtgccagcagccgcggtaattccagctccaatagcgtatattaaaatt gttgcagttaaaacgctcgtagttgaatttcaaagaatcgatattttattgtaactattctagggg aactattttaggttttcgctttaatacgcttcctctattattatgttctttaaataacaaagattcttttt aaaatccccacttttgcttttgcttttttggggaatttgttactttgaataaattagaggtgtcaaag caaacagttaaagcatttactgtgtttgaatactatagcatggaataacaaaattgaacaagct aaaatttttttgttctttttttcttattttggcttagttacgattaataggagtagcttggggacattcgt attcagatgtcagaggtgaaattcttagattttctggagacgaacaactgcgaaagcatttgtc taaaatacttccattaatcaagaacgaaagttaagggagtgaagacgatcagataccgtcgta atcttaaccataaactatgccgactaggtgttggatgaaagtgttaaaaataaaagtcatctttc taggtgacttttagattgcttccttcagtaccttatgagaaatcaaagtctttgggttctggggcg agtattcgcgcaagcgagaaagttaaaagaattgacggaagggcaccaccaggcgtgga gcttgcggcttaatttgactcaacacggggaaactcactagtttaagacaagagtaggattga cagattaatagctctttcttgatttcttggatggtgatgcatggccgtttttagttcgtgaatatgat ttgtctggttaattccgataacgaacgagatcttaacctgctaattagcggcgagtacactatat tcttatttgaaattgaacataggtaactatacatttattcagtaatcaaattaggatatttttattaaa atatccttttccctgttctactaataatttgtttttttactctatttctctcttcttttaagaatgtacttgct tgattgaaaagcttcttagaggaacattgtgtgtctaacacaaggaagtttaaggcaacaaca ggtctgtgatgtccttagatgaactaggctgcacgcgtgctacactgatatatataacgagtttt taaaaat	GenBank Accession No: JQ627151.1 *Plasmodium falciparum* isolate SF3 18S ribosomal RNA gene, partial sequence
75	gaagtaggactttggttctattt	Non-T7 primer
76	atgaagtaggactttggttct	Non-T7 primer

SEQ ID NO.	Sequence (5' to 3')	Comments
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78	gyygccycgguaggccaauacccuaccguccaaagcugaur	
79	gyygccycggtaggccaataccctaccgtccaaagctgatr	

SEQ ID NO.	Sequence (5' to 3')	Comments
80	aatttaatacgactcactatagggagaggcaaatgctttcgcagtagttngtctttaaca	T7 promoter primer, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
81	ggcaaatgctttcgcagtagttngtctttaaca	SEQ ID NO:80 without promoter sequence, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
82	aatttaatacgactcactatagggagaggcaaatgctttcgcagtagttigtctttaaca	T7 promoter primer. "i" means inosine.
83	ggcaaatgctttcgcagtagttigtctttaaca	SEQ ID NO:82 without promoter sequence. "i" means inosine.
84	gcggtaattccagctccaatag	Non-T7 primer
85	cttgaatactncagca	Non-T7 primer, wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
86	cttgaatacticagca	Non-T7 primer. "i" means inosine.
87	aaagacuuugauuucucucaaggtttaaaaaaaaaaaaaaaaaaaaaaaaaaaa	Target capture

SEQ ID NO.	Sequence (5' to 3')	Comments
88	aaagacuuugauuucucucaagg	SEQ ID NO:87 without capture tail sequence
89	caagaaagagcuaucaaucugucaaucctttaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	Target capture
90	caagaaagagcuaucaaucugucaaucc	SEQ ID NO:89 without capture tail sequence
91	aguaggacuuugguuct	Probe
92	aguaggacuuugguuc	Probe
93	aguaggacuauugguuc	Probe
94	aguaggacxuugguuc	Probe, wherein "x" means (i) u; or (ii) ua
95	actttgagaaaaytagagtgtttcaaacaggccatttgccttgaatactncagcatggaataatgaagtaggactttggttctattttgttggtt	"n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
96	ggcaaatgctttcgcagtagttngtctttaacaaatctaagaatttcacctctgacagttaaatacgaatgcccccaa	"n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)
97	ggcaaatgctttcgcagtagttngtctttaacaaatc	"n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

SEQ ID NO.	Sequence (5' to 3')	Comments
98	aguaggacuauugguucu	Probe
99	aguaggacxuugguucu	Probe, wherein "x" means (i) u; or (ii) ua

SEQ ID NO.	Sequence (5' to 3')	Comments
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SEQ ID NO.	Sequence (5' to 3')	Comments
101	ctttcgcagtagttngtctttaac	Wherein "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1).

****

CLAIMS

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

(1) contacting a sample, said sample suspected of containing *Babesia* species nucleic acid, with at least two oligomers for amplifying a target region of a *Babesia* species target nucleic acid, wherein the at least two amplification oligomers comprise:

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

(i) that consists of SEQ ID NO:8; or

(ii) that consists of SEQ ID NO:83;

wherein the first amplification oligomer further comprises a promoter sequence joined to the 5'-end of the first target-hybridizing sequence; and

(b) a second amplification oligomer comprising a second target-hybridizing sequence that consists of SEQ ID NO: 34; SEQ ID NO:84; or SEQ ID NO:86;

(2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Babesia* 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 *Babesia* species target nucleic acid in said sample.

2. The method of claim 1, wherein the first target-hybridizing sequence consists of SEQ ID NO:8; and wherein the second target hybridizing sequence consists of SEQ ID NO:34 or SEQ ID NO:84 or SEQ ID NO:86.

3. The method of any one of claims 1 or 2, wherein the first and second target-hybridizing sequences respectively consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34;

(b) SEQ ID NO:8 and SEQ ID NO:84;

(c) SEQ ID NO:8 and SEQ ID NO:86;

(d) SEQ ID NO:83 and SEQ ID NO:34;

(e) SEQ ID NO:83 and SEQ ID NO:84; or

(f) SEQ ID NO:83 and SEQ ID NO:86.

4. The method of any one of claims 1 to 3, wherein the first and second target-hybridizing sequences respectively consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34;

(b) SEQ ID NO:8 and SEQ ID NO:84;

(c) SEQ ID NO:8 and SEQ ID NO:86;

(d) SEQ ID NO:83 and SEQ ID NO:34;

(e) SEQ ID NO:83 and SEQ ID NO:84; and

(f) SEQ ID NO:83 and SEQ ID NO:86.

5. The method of any one of the preceding claims, wherein the detecting step (3) comprises contacting said *in vitro* nucleic acid amplification reaction with a detection probe oligomer 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 *Babesia* species in said sample.

6. The method of claim 5, wherein the detection probe oligomer comprises a target-hybridizing sequence that consists of the sequence selected from the group consisting of: SEQ ID NOs: 42, 91, 92, 93, 94, and 98

7. The method of claim 5, wherein the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92, or 98;

(b) SEQ ID NO:8 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92, or 98;

(c) SEQ ID NO:8 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, or 98;

(d) SEQ ID NO:83 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92, or 98;

(e) SEQ ID NO:83 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92, or 98;

(f) SEQ ID NO:83 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, or 98; or

(g) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:42, 91, 92, or 98.

8. The method of claim 5, wherein the first and second amplification oligomer targethybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92 and 98;

(b) SEQ ID NO:8 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92 and 98;

(c) SEQ ID NO:8 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, and 98;

(d) SEQ ID NO:83 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92, and 98;

(e) SEQ ID NO:83 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92, and 98;

(f) SEQ ID NO:83 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, and 98; or

(g) SEQ ID NO:8 and 83, SEQ ID NOs:34, 84 and 86, and SEQ ID NOs: 42, 91, 92, and 98.

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

(1) contacting a sample, said sample suspected of containing *Babesia* species nucleic acid, with at least two oligomers for amplifying a target region of a *Babesia* species target nucleic acid, wherein two of said at least two amplification oligomers are selected from the group consisting of:

(a) a first amplification oligomer and a second amplification oligomer, wherein the first amplification oligomer comprises a first target-hybridizing sequence

(i) that consists of SEQ ID NO:8; or

(ii) that consists of SEQ ID NO:83

wherein the first amplification oligomer further comprises a promoter sequence joined to the 5'-end of the first target-hybridizing sequence; and

(b) a first amplification oligomer and a second amplification oligomer, wherein the second amplification oligomer comprises a second target-hybridizing sequence that consists of SEQ ID NO:34; SEQ ID NO:84 or SEQ ID NO:86;

(2) performing an *in vitro* nucleic acid amplification reaction, wherein any *Babesia* target nucleic acid present in said sample is used as a template for generating an amplification product, wherein said amplification product has a length of from 180 to 220 contiguous nucleotides and contains SEQ ID NO:65 or the complement thereof; and

(3) detecting the presence or absence of the amplification product, thereby indicating the presence or absence of *Babesia* species target nucleic acid in said sample.

10. The method of claim 9, wherein the first and second target-hybridizing sequences respectively consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34;

(b) SEQ ID NO:8 and SEQ ID NO:84;

(c) SEQ ID NO:8 and SEQ ID NO:86;

(d) SEQ ID NO:83 and SEQ ID NO:34;

(e) SEQ ID NO:83 and SEQ ID NO:84;

(f) SEQ ID NO:83 and SEQ ID NO:86.

11. The method of claim 9 or claim 10, wherein the first and second amplification oligomer sequences respectively consist of the nucleotide sequences of:

(a) SEQ ID NO:7 and SEQ ID NO:34;

(b) SEQ ID NO:7 and SEQ ID NO:84;

(c) SEQ ID NO:7 and SEQ ID NO:86;

(d) SEQ ID NO:82 and SEQ ID NO:34;

(e) SEQ ID NO:82 and SEQ ID NO:84;

(f) SEQ ID NO:82 and SEQ ID NO:86.

12. The method of any of claims 9 to 11, wherein the detecting step (3) comprises contacting said *in vitro* nucleic acid amplification reaction with a detection probe oligomer 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 *Babesia* species in said sample.

13. The method of claim 12, wherein the detection probe oligomer comprises a target-hybridizing sequence that consists of the sequence selected from the group consisting of: SEQ ID NOs:42, 91, 92, 93, 94 and 98

14. The method of claim 12, wherein the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92, or 98;

(b) SEQ ID NO:8 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92, or 98;

(c) SEQ ID NO:8 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, or 98;

(d) SEQ ID NO:83 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92, or 98;

(e) SEQ ID NO:83 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92, or 98;

(f) SEQ ID NO:83 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, or 98; or

(g) SEQ ID NO:8 or 83, SEQ ID NO:34, 84 or 86, and SEQ ID NO:42, 91, 92 or 98 or

wherein the first and second amplification oligomer target-hybridizing sequences and the detection probe oligomer target-hybridizing sequences respectively comprise or consist of the nucleotide sequences of:

(a) SEQ ID NO:8 and SEQ ID NO:34 and SEQ ID NO:42, 91, 92, and 98;

(b) SEQ ID NO:8 and SEQ ID NO:84 and SEQ ID NO:42, 91, 92, and 98;

(c) SEQ ID NO:8 and SEQ ID NO:86 and SEQ ID NO:42, 91, 92, and 98;

(d) SEQ ID NO:83 and SEQ ID NO:34 and SEQ ID NO: 42, 91, 92, and 98;

(e) SEQ ID NO:83 and SEQ ID NO:84 and SEQ ID NO: 42, 91, 92, and 98;

(f) SEQ ID NO:83 and SEQ ID NO:86 and SEQ ID NO: 42, 91, 92, and 98; or

(g) SEQ ID NO:8 and 83, SEQ ID NOs:34, 84 and 86, and SEQ ID NO: 42, 91, 92, and 98.

15. A combination of at least two oligomers for determining the presence or absence of *Babesia* in a sample, said oligomer combination comprising first and second amplification oligomers for amplifying a target region of *Babesia* target nucleic acid, wherein

(a) the first amplification oligomer comprises a first target-hybridizing sequence

(i) that consists of SEQ ID NO:8; or

(ii) that consists of SEQ ID NO:83;

wherein the first amplification oligomer is a promoter primer or promoter provider further comprising a promoter sequence located 5' to the first target-hybridizing sequence; and

(b) the second amplification oligomer comprises a second target-hybridizing sequence that consists of SEQ ID NO:34; SEQ ID NO:84; or SEQ ID NO: 86.

SEQUENCE LISTING

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BRES, Vanessa
SELF, Deanna
LINNEN, Jeffrey M.

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<223> Synthetic Oligonucleotide

<400> 36
tgagaaaact agagtgtttc 20

<210> 37
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 37
ugaaguagga cuuugguucu 20

<210> 38
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 38
uaaugguuaa uaggagcagu ug 22

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 39
aguaaugguu aauaggagca 20

<210> 40
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 40
ggacuuuggu ucuauuuugu ugg 23

<210> 41
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 41
aaugguuaau aggagcaguu ggggg 25

<210> 42
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 42
aguaggacuu ugguucu 17

<210> 43
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<220>
<221> misc_feature
<222> (22)..(54)
<223> capture sequence

<400> 43
uaggccaaua cccuaccguc ctttaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 54

<210> 44
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 44
uaggccaaua cccuaccguc c 21

<210> 45
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 45
atggaataat gaagtag 17

<210> 46
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 46
agaaaactag agtg 14

<210> 47
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 47
agaaaattag agtg 14

<210> 48
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 48
agaaaaytag agtg 14

<210> 49
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 49
agaaaactag agtgtttc 18

<210> 50
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 50
agaaaattag agtgtttc 18

<210> 51
<211> 18

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 51
agaaaaytag agtgtttc 18

<210> 52
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 52
tactacagca tggaataa 18

<210> 53
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<220>
<221> variation
<222> (4)..(4)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (4)..(4)
<223> n is a, c, g, or t

<400> 53
actncagcat gga 13

<210> 54
<211> 13
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic Oligonucleotide

<400> 54
actwcagcat gga 13

<210> 55
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 55
actacagcat gga 13

<210> 56
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 56
gtatttaact gt 12

<210> 57
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 57
gtatttaact gtcagaggtg aa 22

<210> 58
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 58
aatttaatac gactcactat agggaga 27

<210> 59
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 59
aguaggacuu ugguucu 17

<210> 60
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 60
uaaugguuaa uaggagca 18

<210> 61
<211> 1206
<212> DNA
<213> Artificial Sequence

<220>
<223> B. microti In Vitro Transcript

<400> 61
gggcgaauug gguaccgggc ccccccucga ggucgacgcu uaguauaagc uuuuauacag 60

cgaaacugcg aauggcucau uaaaacaguu auaguuuauu ugauguucgu uuuacaugga 120

uaaccguggu aauucuaggg cuaauacaug cucgaggcgc guuuucgcgu ggcguuuauu 180

agacuuuaac caacccuucg gguaaucggu gauucauaau aaauuagcga aucgcauggc 240

uuugccggcg auguaucauu caaguuucug accuaucagc uuuggacggu aggguauugg 300

ccuaccgggg cgacgacggg ugacggggaa uuggguucg auuccggaga gggagccuga 360

gaaacggcua ccacaucuaa ggaaggcagc aggcgcgcaa auuacccaau ccugacacag 420

ggagguagug acaagaaaua acaauacagg gcuuaaaguc uuguaauugg aaugauggga 480

aucuaaaccc uucccagagu aucaauugga gggcaagucu ggugccagca gccgcgguaa 540

uuccagcucc aauagcguau auuaaaguug uugcaguuaa gaagcucgua guugaauuuc 600

ugccuuguca uuaaucucgc uuccgagcgu uuuuuuauug acuuggcauc uucuggauuu 660

ggugccuucg gguacuauuu uccaggauuu acuuugagaa aacuagagug uuucaaacag 720

gcauucgccu ugaauacuac agcauggaau aaugaaguag gacuuugguu cuauuuuguu 780

gguuauugag ccagaguaau gguuaauagg agcaguuggg ggcauucgua uuuaacuguc 840

agaggugaaa uucuuagauu uguuaaagac gaacuacugc gaaagcauuu gccaaggaug 900

uuuucauuaa ucaagaacga aaguuagggg aucgaagacg aucagauacc gucguagucc 960

uaaccauaaa cuaugccgac uagagauugg aggucgucag uuuaaacgac uccuucagca 1020

ccuugagaga aaucaaaguc uuuggguucu ggggggagua uggucgcaag ucugaaacuu 1080

aaaggaauug acggaagggc accaccaggc guggagccug cggcuuaauu ugacucaaca 1140

cgggaaaccu caccaggucc agacauagag aggauugaca gauugauagc ucuuucuuga 1200

ugaauu 1206

<210> 62
<211> 1282
<212> DNA
<213> Artificial Sequence

<220>
<223> B. divergens In Vitro Transcript

<400> 62
gggcgaauug gguaccgggc ccccccucga ggucgacggu aucgauaagc uugauaucga 60

auuccugcag cccgggggau ccaaccuggu ugauccugcc aguagucaua ugcuugucuu 120

aaagauuaag ccaugcaugu cuaaguacaa acuuuuuacg gugaaacugc gaauggcuca 180

uuacaacagu uauaguuucu uugguauucg uuuuccaugg auaaccgugc uaauuguagg 240

gcuaauacaa guucgaggcc uuuuggcggc guuuauuagu ucuaaaacca ucccuuuugg 300

uuuucgguga uucauaauaa acuugcgaau cgcaauuuuu ugcgauggac cauucaaguu 360

ucugacccau cagcuugacg guaggguauu ggccuaccga ggcagcaacg gguaacgggg 420

aauuaggguu cgauuccgga gagggagccu gagaaacggc uaccacaucc aaggaaggca 480

gcaggcgcgc aaauuaccca auccugacac agggagguag ugacaagaaa uaacaauaca 540

gggcaauugu cuuguaauug gaaugauggu gaccuaaacc cucaccagag uaacaauugg 600

agggcaaguc uggugccagc agccgcggua auuccagcuc caauagcgua uauuaaacuu 660

guugcaguua aaaagcucgu aguugaauuu uugcguggug uuaauauuga cuaaugucga 720

gauugcacuu cgcuuuuggg auuuaucccu uuuuacuuug agaaaauuag aguguuucaa 780

gcagacuuuu gucuugaaua cuucagcaug gaauaauaga guaggacuuu gguucuauuu 840

uguugguuug ugaaccuuag uaaugguuaa uaggaacggu ugggggcauu cguauuuaac 900

ugucagaggu gaaauucuua gauuuguuaa agacgaacua cugcgaaagc auuugccaag 960

gacguuuuca uuaaucaaga acgaaaguua ggggaucgaa gacgaucaga uaccgucgua 1020

guccuaacca uaaacuaugc cgacuaggga uuggaggucg ucauuuuucc gacuccuuca 1080

gcaccuugag agaaaucaaa gucuuugggu ucuggggggа guauggucgc aaggcugaaa 1140

cuuaaaggaa uugacggaag ggcaccacca ggcguggagc cugcggcuua auuugacuca 1200

acacggggaa acucaccagg uccagacaau guuaggauug acagauugau agcucuuucu 1260

ugauucuuug gguggugcgg cc 1282

<210> 63
<211> 1251
<212> DNA
<213> Artificial Sequence

<220>
<223> B. duncani In Vitro Transcript

<400> 63
gggcgaauug gguaccgggc ccccccucga ggucgacguu gauccugcca guagucauau 60

gcuugucuua aagauuaagc caugcauguc uaaguauaaa cuuuuauaug gugaaacugc 120

gaauggcuca uuacaacagu uauaguuuau uugaaagucg uuuuuacaug gauaaccgug 180

cuaauuguag ggcuaauaca ugcucgaggc cuuggcuccu gucuuggcug cguuuauuag 240

acucgaaacc uucccgcuug cgguacucgg ugauucauaa uaaauuugcg aaucgcaugg 300

cuuuugccgg cgaugguuca uucaaguuuc ugaccuauca gcuuuggacg guaggguauu 360

ggccuaccgg ggcagcgacg gguaacgggg aauuaggguu cgauuccgga gagggagccu 420

gagaaacggc uaccacaucu aaggaaggca gcaggcgcgc aaauuaccca auacggacac 480

cgugagguag ugacaagaaa uaacaauaca gggcuuaaag cuuuguaauu ggaaugaugg 540

gaauccaaac cccuuccaga guaucaauug gagggcaagu cuggugccag cagccgcggu 600

aauuccagcu ccaauagcgu auauuaaacu uguugcaguu aaaaagcucg uaguugaacu 660

ucugccgcuu ggccuuucgu uccccuuggg guuucguucg ccugguggcu uaccucuggc 720

ggugguucuc cauuugccag uuuuacuuug agaaaauuag aguguuucaa gcaggcuuuu 780

gccuugaaua cuucagcaug gaauaauaaa guaggacuuu gguucuauuu uguugguuuc 840

aggaccaaag uaaugguuaa uaggaacagu ugggggcauu cguauuuaac ugucagaggu 900

gaaauucuua gauuuguuaa agacgaacua cugcgaaagc auuugccaag gauguuuuca 960

uuaaucaaga acgaaaguua ggggcucgaa gacgaucaga uaccgucgua guccuaacua 1020

uaaacuaugc cgacuagaga uuggaggucg ucauuuuaaa cgacuccuuc agcaccuuga 1080

gagaaaucaa agucuuuggg uucuggggggg aguauggucg caaggcugaa acuuaaagga 1140

auugacggaa gggcaccacc aggcguggag ccugcggcuu aauuugacuc aacacgggga 1200

accucaccag guccagacau aguuaggauu gacagauuga uagcucgaau u 1251

<210> 64
<211> 1387
<212> DNA
<213> Artificial Sequence

<220>
<223> P. falciparum In Vitro Transcript

<400> 64
gggcgaauug gguaccguag aaacugcgaa cggcucauua aaacaguuau agucuacuug 60

acauuuuuau uauaaggaua acuacggaaa agcuguagcu aauacuugcu uuauuauccu 120

uugauuuuua ucuuuggaua aguauuuguu aggccuuaua agaaaaaagu uauuaacuua 180

aggaauuaua acaaagaagu aacacguaau aaauuuauuu uauuuagugu guaucaaucg 240

aguuucugac cuaucagcuu uugauguuag gguauuggcc uaacauggcu augacgggua 300

acggggaauu agaguucgau uccggagagg gagccugaga aauagcuacc acaucuaagg 360

aaggcagcag gcgcguaaau uacccaauuc uaaaaaagag agguagugac aagaaauaac 420

aaugcaaggc caauuuuugg uuuuguaauu ggaauggugg gaauuuaaaa ccuucccaga 480

guaacaauug gagggcaagu cuggugccag cagccgcggu aauuccagcu ccaauagcgu 540

auauuaaaau uguugcaguu aaaacgcucg uaguugaauu ucaaagaauc gauauuuuau 600

uguaacuauu cuaggggaac uauuuuaggu uuucgcuuua auacgcuucc ucuauuauua 660

uguucuuuaa auaacaaaga uucuuuuuaa aauccccacu uuugcuuuuu uggggaauuu 720

guuacuuuga auaaauuaga ggugucaaag caaacaguua aagcauuuac uguguuugaa 780

uacuauagca uggaauaaca aaauugaaca agcuaaaauu uuuuguucuu uuuucuuauu 840

uuggcuuagu uacgauuaau aggaguagcu uggggacauu cguauucaga ugucagaggu 900

gaaauucuua gauuuucugg agacgaacaa cugcgaaagc auuugucuaa aauacuucca 960

uuaaucaaga acgaaaguua agggagugaa gacgaucaga uaccgucgua aucuuaacca 1020

uaaacuaugc cgacuaggug uuggaugaaa guguuaaaaa uaaaagucau cuuucuaggu 1080

gacuuuuaga uugcuuccuu caguaccuua ugagaaauca aagucuuugg guucuggggc 1140

gaguauucgc gcaagcgaga aaguuaaaag aauugacgga agggcaccac caggcgugga 1200

gcuugcggcu uaauuugacu caacacgggg aaacucacua guuuaagaca agaguaggau 1260

ugacagauua auagcucuuu cuugauuucu uggaugguga ugcauggccg uuuuuaguuc 1320

gugaauauga uuugucuggu uaauuccgau aacgaacgag aucggaucca cuaguucuag 1380

agcggcc 1387

<210> 65
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 65
ugaaguagga cuuugguucu auuuuguugg uuauugagcc agaguaaugg uuaauaggag 60

caguuggggg 70

<210> 66
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 66
ttcacctctg acagttaaat acgaatgccc ccaa 34

<210> 67
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<220>
<221> variation
<222> (5)..(5)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (5)..(5)
<223> n is a, c, g, or t

<400> 67
tactncagca tggaataatg aagtaggact ttgg 34

<210> 68
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<220>
<221> variation
<222> (11)..(11)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (11)..(11)
<223> n is a, c, g, or t

<400> 68
cttgaatact ncagcatgga ataatga 27

<210> 69
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 69
atggaataat g 11

<210> 70
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 70
actttgagaa aaytagagtg tttcaaa 27

<210> 71
<211> 1767
<212> DNA
<213> Babesia microti

<300>
<308> AY693840.1
<309> 2004-09-05
<313> (1)..(1767)

<400> 71
aacctggttg atcctgccag tagtcatatg cttgtcttaa agattaagcc atgcatgtct 60

tagtataagc ttttatacag cgaaactgcg aatggctcat taaaacagtt atagtttatt 120

tgatgttcgt tttacatgga taaccgtggt aattctaggg ctaatacatg ctcgaggcgc 180

gttttcgcgt ggcgtttatt agactttaac caacccttcg ggtaatcggt gattcataat 240

aaattagcga atcgcatggc tttgccggcg atgtatcatt caagtttctg acctatcagc 300

tttggacggt agggtattgg cctaccgggg cgacgacggg tgacggggaa ttggggttcg 360

attccggaga gggagcctga gaaacggcta ccacatctaa ggaaggcagc aggcgcgcaa 420

attacccaat cctgacacag ggaggtagtg acaagaaata acaatacagg gcttaaagtc 480

ttgtaattgg aatgatggga atctaaaccc ttcccagagt atcaattgga gggcaagtct 540

ggtgccagca gccgcggtaa ttccagctcc aatagcgtat attaaagttg ttgcagttaa 600

gaagctcgta gttgaatttc tgccttgtca ttaatctcgc ttccgagcgt ttttttattg 660

acttggcatc ttctggattt ggtgccttcg ggtactattt tccaggattt actttgagaa 720

aactagagtg tttcaaacag gcattcgcct tgaatactac agcatggaat aatgaagtag 780

gactttggtt ctattttgtt ggttattgag ccagagtaat ggttaatagg agcagttggg 840

ggcattcgta tttaactgtc agaggtgaaa ttcttagatt tgttaaagac gaactactgc 900

gaaagcattt gccaaggatg ttttcattaa tcaagaacga aagttagggg atcgaagacg 960

atcagatacc gtcgtagtcc taaccataaa ctatgccgac tagagattgg aggtcgtcag 1020

tttaaacgac tccttcagca ccttgagaga aatcaaagtc tttgggttct gggggggagta 1080

tggtcgcaag tctgaaactt aaaggaattg acggaagggc accaccaggc gtggagcctg 1140

cggcttaatt tgactcaaca cgggaaacct caccaggtcc agacatagag aggattgaca 1200

gattgatagc tctttcttga ttctatgggt ggtggtgcat ggccgttctt agttggtgga 1260

gtgatttgtc tggttaattc cgttaacgaa cgagacctta acctgctaaa ttaggatctg 1320

ggacaagctt tgctgttcca gtatcgcttc ttagagggac tttgcgttca taaaacgcaa 1380

ggaagtgtaa ggcaataaca ggtctgtgat gcccttagat gtcctgggct gcacgcgcgc 1440

tacactgatg cattcaacga gtttttcctt ggccgtcggg tccgggtaat cttacagtat 1500

gcatcgtgat ggggatagat tattgcaatt attaatcttg aacgaggaat gcctagtagg 1560

cgcgagtcat cagctcgtgc cgactacgtc cctgcccttt gtacacaccg cccgtcgctc 1620

ctaccgatcg agtgatccgg tgaattattc ggaccaagaa acgtggattc gtccttcgtt 1680

ttttggaaag ttttgtgaac cttatcactt aaaggaagga gaagtcgtaa caaggtttcc 1740

gtaggtgaac ctgcggaagg atcattc 1767

<210> 72
<211> 1728
<212> DNA
<213> Babesia divergens

<300>
<308> AY789076.1
<309> 2004-11-17
<313> (1)..(1728)

<400> 72
aacctggttg atcctgccag tagtcatatg cttgtcttaa agattaagcc atgcatgtct 60

aagtacaaac tttttacggt gaaactgcga atggctcatt acaacagtta tagtttcttt 120

ggtattcgtt ttccatggat aaccgtgcta attgtagggc taatacaagt tcgaggcctt 180

ttggcggcgt ttattagttc taaaaccatc ccttttggtt ttcggtgatt cataataaac 240

ttgcgaatcg caatttttttg cgatggacca ttcaagtttc tgacccatca gcttgacggt 300

agggtattgg cctaccgagg cagcaacggg taacggggaa ttagggttcg attccggaga 360

gggagcctga gaaacggcta ccacatccaa ggaaggcagc aggcgcgcaa attacccaat 420

cctgacacag ggaggtagtg acaagaaata acaatacagg gcaattgtct tgtaattgga 480

atgatggtga cctaaaccct caccagagta acaattggag ggcaagtctg gtgccagcag 540

ccgcggtaat tccagctcca atagcgtata ttaaacttgt tgcagttaaa aagctcgtag 600

ttgaattttt gcgtggtgtt aatattgact aatgtcgaga ttgcacttcg cttttgggat 660

ttatcccttt ttactttgag aaaattagag tgtttcaagc agacttttgt cttgaatact 720

tcagcatgga ataatagagt aggactttgg ttctattttg ttggtttgtg aaccttagta 780

atggttaata ggaacggttg ggggcattcg tatttaactg tcagaggtga aattcttaga 840

tttgttaaag acgaactact gcgaaagcat ttgccaagga cgttttcatt aatcaagaac 900

gaaagttagg ggatcgaaga cgatcagata ccgtcgtagt cctaaccata aactatgccg 960

actagggatt ggaggtcgtc atttttccga ctccttcagc accttgagag aaatcaaagt 1020

ctttgggttc tgggggggagt atggtcgcaa ggctgaaact taaaggaatt gacggaaggg 1080

caccaccagg cgtggagcct gcggcttaat ttgactcaac acggggaaac tcaccaggtc 1140

cagacaatgt taggattgac agattgatag ctctttcttg attctttggg tggtggtgca 1200

tggccgttct tagttggtgg agtgatttgt ctggttaatt ccgttaacga acgagacctt 1260

aacctgctaa ctagtgtccg taaaaaggtt cgtccgttac ggtttgcttc ttagagggac 1320

tttgcggctc taagccgcaa ggaagtttaa ggcaataaca ggtctgtgat gcccttagat 1380

gtcctgggct gcacgcgcgc tacactgatg cattcatcga gttttatccc ttcccgaaag 1440

ggctgggtaa tctttagtat gcatcgtgac ggggattgat ttttgcaatt ctaaatcatg 1500

aacgaggaat gcctagtatg cgcaagtcat cagcttgtgc agattacgtc cctgcccttt 1560

gtacacaccg cccgtcgctc ctaccgatcg agtgatccgg tgaattattc ggaccgtggc 1620

ctttccgatt cgtcggcttg gcctagggaa gtcttgtgaa ccttatcact taaaggaagg 1680

agaagtcgta acaaggtttc cgtaggtgaa cctgcggaag gatcattc 1728

<210> 73
<211> 1742
<212> DNA
<213> Babesia strain WA1

<300>
<308> AY027815.1
<309> 2001-03-02
<313> (1)..(1742)

<400> 73
ccttggttga tcctgccagt agtcatatgc ttgtcttaag gattaagcca tgcatgtcta 60

agtataaact tttatatggt gaaactgcga atggctcatt acaacagtta tagtttattt 120

gaaagtcgtt tttacatgga taaccgtgct aattgtaggg ctaatacatg ctcgaggcct 180

tggcttctgt cttggctgcg tttattagac tcgaaacctt cccgcttgcg gtactcggtg 240

attcataata aatttgcgaa tcgcatggct tttgccggcg atggttcatt caagtttctg 300

acctatcagc tttggacggt agggtattgg cctaccgggg cagcgacggg taacggggaa 360

ttagggttcg attccggaga gggagcctga gaaacggcta ccacatctaa ggaaggcagc 420

aggcgcgcaa attacccaat acggacaccg tgaggtagtg acaagaaata acaatacagg 480

gctttaagct ttgtaattgg aatgatggga atccaaaccc cttccagagt atcaattgga 540

gggcaagtct ggtgccagca gccgcggtaa ttccagctcc aatagcgtat attaaacttg 600

ttgcagttaa aaagctcgta gttgaacttc tgccgcttgg cctttcgttc cccttggggt 660

ttcgttcgcc tggtggctta cctctggcgg tggttctcca tttgccagtt ttactttgag 720

aaaattagag tgtttcaagc aggcttttgc cttgaatact tcagcatgga ataataaagt 780

aggactttgg ttctattttg ttggtttcag gaccaaagta atggttaata ggaacagttg 840

ggggcattcg tatttaactg tcagaggtga aattcttaga tttgttaaag acgaactact 900

gcgaaagcat ttgccaagga tgttttcatt aatcaagaac gaaagttagg ggctcgaaga 960

cgatcagata ccgtcgtagt cctaactata aactatgccg actagagatt ggaggtcgtc 1020

attttaaacg actccttcag caccttgaga gaaatcaaag tctttgggtt ctgggggggag 1080

tatggtcgca aggctgaaac ttaaaggaat tgacggaagg gcaccaccag gcgtggagcc 1140

tgcggcttaa tttgactcaa cacggggaac ctcaccaggt ccagacatag ttaggattga 1200

cagattgata gctctttctt gattctatgg gtagtggtgc atggccgttc ttagttggtg 1260

gagtgatttg tctggttaat tccgttaacg aacgagacct taacctgcta aatagcagct 1320

gagaataatc tcttgtttca gttttgcttc ttagagggac tttgcggtca taaatcgcaa 1380

ggaagtttaa ggcaataaca ggtctgtgat gcccttagat gtcctgggct gcacgcgcgc 1440

tacactgatg cattcatcga gttttatcct tgcccgaaag ggtttggtaa tctttagtat 1500

gcatcgtgat ggggattgat tattgcaatt attaatcatg aacgaggaat gcctagtagg 1560

cgcgagtcat cagctcgtgc cgactacgtc cctgcccttt gtacacaccg cccgtcgctc 1620

ctaccgatcg agtgatccgg tgaattattc ggaccgtgac gcttctaatt cgttagaaat 1680

gtctagggaa gttttgtgaa ccttatcact taaaggaagg agaagtcgta acaaggtttc 1740

cg 1742

<210> 74
<211> 1708
<212> DNA
<213> Plasmodium falciparum

<300>
<308> JQ627151.1
<309> 2012-08-14
<313> (1)..(1708)

<400> 74
tcaaagatta agccatgcaa gtgaaagtat atatatattt tatatgtaga aactgcgaac 60

ggctcattaa aacagttata gtctacttga catttttatt ataaggataa ctacggaaaa 120

gctgtagcta atacttgctt tattatcctt tgatttttat ctttggataa gtatttgtta 180

ggccttataa gaaaaaagtt attaacttaa ggaattataa caaagaagta acacgtaata 240

aatttatttt atttagtgtg tatcaatcga gtttctgacc tatcagcttt tgatgttagg 300

gtattggcct aacatggcta tgacgggtaa cggggaatta gagttcgatt ccggagaggg 360

agcctgagaa atagctacca catctaagga aggcagcagg cgcgtaaatt acccaattct 420

aaaaaagaga ggtagtgaca agaaataaca atgcaaggcc aatttttggt tttgtaattg 480

gaatggtggg aatttaaaac cttcccagag taacaattgg agggcaagtc tggtgccagc 540

agccgcggta attccagctc caatagcgta tattaaaatt gttgcagtta aaacgctcgt 600

agttgaattt caaagaatcg atattttatt gtaactattc taggggaact attttaggtt 660

ttcgctttaa tacgcttcct ctattattat gttctttaaa taacaaagat tctttttaaa 720

atccccactt ttgcttttgc ttttttgggg aatttgttac tttgaataaa ttagaggtgt 780

caaagcaaac agttaaagca tttactgtgt ttgaatacta tagcatggaa taacaaaatt 840

gaacaagcta aaatttttttg ttctttttttc ttattttggc ttagttacga ttaataggag 900

tagcttgggg acattcgtat tcagatgtca gaggtgaaat tcttagattt tctggagacg 960

aacaactgcg aaagcatttg tctaaaatac ttccattaat caagaacgaa agttaaggga 1020

gtgaagacga tcagataccg tcgtaatctt aaccataaac tatgccgact aggtgttgga 1080

tgaaagtgtt aaaaataaaa gtcatctttc taggtgactt ttagattgct tccttcagta 1140

ccttatgaga aatcaaagtc tttgggttct ggggcgagta ttcgcgcaag cgagaaagtt 1200

aaaagaattg acggaagggc accaccaggc gtggagcttg cggcttaatt tgactcaaca 1260

cggggaaact cactagttta agacaagagt aggattgaca gattaatagc tctttcttga 1320

tttcttggat ggtgatgcat ggccgttttt agttcgtgaa tatgatttgt ctggttaatt 1380

ccgataacga acgagatctt aacctgctaa ttagcggcga gtacactata ttcttatttg 1440

aaattgaaca taggtaacta tacatttatt cagtaatcaa attaggatat ttttattaaa 1500

atatccttttt ccctgttcta ctaataattt gtttttttact ctatttctct cttcttttaa 1560

gaatgtactt gcttgattga aaagcttctt agaggaacat tgtgtgtcta acacaaggaa 1620

gtttaaggca acaacaggtc tgtgatgtcc ttagatgaac taggctgcac gcgtgctaca 1680

ctgatatata taacgagttt ttaaaaat 1708

<210> 75
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 75
gaagtaggac tttggttcta ttt 23

<210> 76
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 76
atgaagtagg actttggttc t 21

<210> 77
<211> 1727
<212> DNA
<213> Babesia sp. EU1 venatorum

<300>
<308> AY046575.1
<309> 2003-07-09
<313> (1)..(1727)

<400> 77
aacctggttg atcctgccag tagtcatatg cttgtcttaa agattaagcc atgcatgtct 60

aagtacaaac tttttacggt gaaactgcga atggctcatt acaacagtta tagtttcttt 120

ggtattcgtt ttccatggat aaccgtgcta attgtagggc taatacaagt tcgaggcctt 180

ttggcggcgt ttattagttc tataaccacc cttttggttt tcggtgattc ataataaact 240

cgcgaatcgc aatttattgc gatggaccat tcaagtttct gacccatcag cttgacggta 300

gggtattggc ctaccgaggc agcaacgggt aacggggaat tagggttcga ttccggagag 360

ggagcctgag aaacggctac cacatccaag gaaggcagca ggcgcgcaaa ttacccaatc 420

ctgacacagg gaggtagtga caagaaataa caatacaggg caattgtctt gtaattggaa 480

tgatggtgac ctaaaccctc accagagtaa caattggagg gcaagtctgg tgccagcagc 540

cgcggtaatt ccagctccaa tagcgtatat taaacttgtt gcagttaaaa agctcgtagt 600

tgaatttctg cgttatcgag ttattgactc ttgtctttaa tcgatttcgc ttttgggatt 660

tatccctttt tactttgaga aaattagagt gtttcaagca gacttttgtc ttgaatactt 720

cagcatggaa taatagagta ggactttggt tctattttgt tggtttttga accttagtaa 780

tggttaatag gaacggttgg gggcattcgt atttaactgt cagaggtgaa attcttagat 840

ttgttaaaga cgaactactg cgaaagcatt tgccaaggac gtttccatta atcaagaacg 900

aaagttaggg gatcgaagac gatcagatac cgtcgtagtc ctaaccataa actatgccga 960

ctagggattg gaggtcgtca tttttccgac tccttcagca ccttgagaga aatcaaagtc 1020

tttgggttct gggggggagta tggtcgcaag gctgaaactt aaaggaattg acggaagggc 1080

accaccaggc gtggagcctg cggcttaatt tgactcaaca cggggaaact caccaggtcc 1140

agacaatgtt aggattgaca gattgatagc tctttcttga ttctttgggt ggtggtgcat 1200

ggccgttctt agttggtgga gtgatttgtc tggttaattc cgttaacgaa cgagacctta 1260

acctgctaac tagtacccgt aaaaaggttc gtccgttacg gtttgcttct tagagggact 1320

ttgcggctct aagccgcaag gaagtttaag gcaataacag gtctgtgatg cccttagatg 1380

tcctgggctg cacgcgcgct acactgatgc attcatcgag tttaatcctg tcccgaaagg 1440

gctgggtaat ctttagtatg catcgtgacg gggattgatt tttgcaattc taaatcatga 1500

acgaggaatg cctagtatgc gcaagtcatc agcttgtgca gattacgtcc ctgccctttg 1560

tacacaccgc ccgtcgctcc taccgatcga gtgatccggt gaattattcg gaccgtggct 1620

tttccgattc gtcggttttg cctagggaag tctcgtgaac cttatcactt aaaggaagga 1680

gaagtcgtaa caaggtttcc gtaggtgaac ctgcagaagg atcaagc 1727

<210> 78
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 78
gyygccycgg uaggccaaua cccuaccguc caaagcugau r 41

<210> 79
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<400> 79
gyygccycgg taggccaata ccctaccgtc caaagctgat r 41

<210> 80
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Oligonucleotide

<220>
<221> promoter
<222> (1)..(27)

<220>
<221> variation
<222> (50)..(50)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (50)..(50)
<223> n is a, c, g, or t

<400> 80
aatttaatac gactcactat agggagaggc aaatgctttc gcagtagttn gtctttaaca 60

<210> 81
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>

<221> variation
<222> (23)..(23)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (23)..(23)
<223> n is a, c, g, or t

<400> 81
ggcaaatgct ttcgcagtag ttngtcttta aca 33

<210> 82

<400> 82
000

<210> 83

<400> 83
000

<210> 84
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 84
gcggtaattc cagctccaat ag 22

<210> 85
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> variation
<222> (11)..(11)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>

<221> misc_feature
<222> (11)..(11)
<223> n is a, c, g, or t

<400> 85
cttgaatact ncagca 16

<210> 86

<400> 86
000

<210> 87
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (24)..(56)
<223> capture tail

<400> 87
aaagacuuug auuucucuca aggtttaaaa aaaaaaaaaa aaaaaaaaaa aaaaaa 56

<210> 88
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 88
aaagacuuug auuucucuca agg 23

<210> 89
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (29)..(61)
<223> capture tail

<400> 89
caagaaagag cuaucaaucu gucaaucctt taaaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

a 61

<210> 90
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 90
caagaaagag cuaucaaucu gucaaucc 28

<210> 91
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 91
aguaggacuu ugguuct 17

<210> 92
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 92
aguaggacuu ugguuc 16

<210> 93
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 93
aguaggacua uugguuc 17

<210> 94

<400> 94
000

<210> 95
<211> 95
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> variation
<222> (50)..(50)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (50)..(50)
<223> n is a, c, g, t or u

<400> 95
actttgagaa aaytagagtg tttcaaacag gccatttgcc ttgaatactn cagcatggaa 60

taatgaagta ggactttggt tctattttgt tggtt 95

<210> 96
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> variation
<222> (23)..(23)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (23)..(23)
<223> n is a, c, g, t or u

<400> 96
ggcaaatgct ttcgcagtag ttngtcttta acaaatctaa gaatttcacc tctgacagtt 60

aaatacgaat gcccccaa 78

<210> 97
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> variation
<222> (23)..(23)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (23)..(23)
<223> n is a, c, g, t or u

<400> 97
ggcaaatgct ttcgcagtag ttngtcttta acaaatc 37

<210> 98
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 98
aguaggacua uugguucu 18

<210> 99

<400> 99
000

<210> 100
<211> 1281
<212> RNA
<213> Artificial Sequence

<220>
<223> B. venatorum In Vitro Transcript

<400> 100
gggcgaauug gguaccgggc ccccccucga ggucgacggu aucgauaagc uugauaucga 60

auuccugcag cccgggggau ccaaccuggu ugauccugcc aguagucaua ugcuugucuu 120

aaagauuaag ccaugcaugu cuaaguacaa acuuuuuacg gugaaacugc gaauggcuca 180

uuacaacagu uauaguuucu uugguauucg uuuuccaugg auaaccgugc uaauuguagg 240

gcuaauacaa guucgaggcc uuuuggcggc guuuauuagu ucuauaacca cccuuuuggu 300

uuucggugau ucauaauaaa cucgcgaauc gcaauuuauu gcgauggacc auucaaguuu 360

cugacccauc agcuugacgg uaggguauug gccuaccgag gcagcaacgg guaacgggga 420

auuaggguuc gauuccggag agggagccug agaaacggcu accacaucca aggaaggcag 480

caggcgcgca aauuacccaa uccugacaca gggagguagu gacaagaaau aacaauacag 540

ggcaauuguc uuguaauugg aaugauggug accuaaaccc ucaccagagu aacaauugga 600

gggcaagucu ggugccagca gccgcgguaa uuccagcucc aauagcguau auuaaacuug 660

uugcaguuaa aaagcucgua guugaauuuc ugcguuaucg aguuauugac ucuugucuuu 720

aaucgauuuc gcuuuuggga uuuaucccuu uuuacuuuga gaaaauuaga guguuucaag 780

cagacuuuug ucuugaauac uucagcaugg aauaauagag uaggacuuug guucuauuuu 840

guugguuuuu gaaccuuagu aaugguuaau aggaacgguu gggggcauuc guauuuaacu 900

gucagaggug aaauucuuag auuuguuaaa gacgaacuac ugcgaaagca uuugccaagg 960

acguuuccau uaaucaagaa cgaaaguuag gggaucgaag acgaucagau accgucguag 1020

uccuaaccau aaacuaugcc gacuagggau uggaggucgu cauuuuuccg acuccuucag 1080

caccuugaga gaaaucaaag ucuuuggguu cuggggggag uauggucgca aggcugaaac 1140

uuaaaggaau ugacggaagg gcaccaccag gcguggagcc ugcggcuuaa uuugacucaa 1200

cacggggaaa cucaccaggu ccagacaaug uuaggauuga cagauugaua gcucuuucuu 1260

gauucuuugg guggugcggc c 1281

<210> 101
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> variation
<222> (15)..(15)
<223> "n" means a or g or c or t/u, or unknown, or other (WIPO Standard ST.25 (1998), Appendix 2, Table 1)

<220>
<221> misc_feature
<222> (15)..(15)
<223> n is a, c, g, or t

<400> 101
ctttcgcagt agttngtctt taac 24