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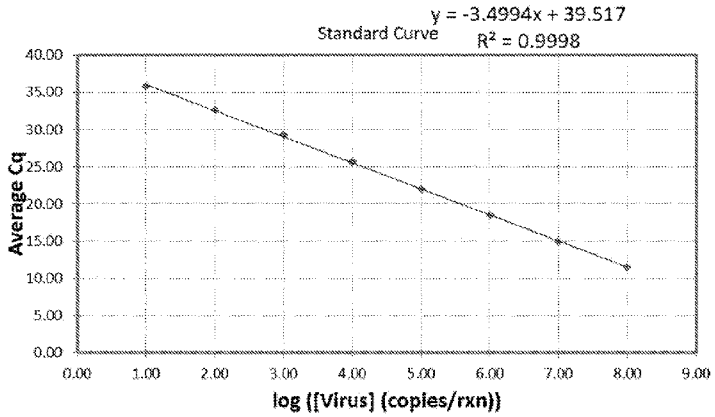

FIG. 1

(57) Abstract: Methods, compositions and kits for detection of one or more of Human Parvovirus B19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), and Hepatitis A Virus (HAV) in sample(s) are disclosed herein.

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VIRAL DETECTION ASSAYS

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 63/356,833, filed June 29, 2022. The entire content of this application is hereby expressly incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 80EM-341706-WO_SequenceListing, created April 26, 2023, which is 32 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

Field

[0003] The present disclosure relates generally to methods and compositions for the detection of viruses in a sample. More specifically, the present disclosure relates to detection of Human Parvovirus 19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), and Hepatitis A Virus (HAV) in sample by nucleic acid-based testing methods.

Description of the Related Art

[0004] Nucleic acid testing for the presence or absence of viruses in a sample are needed for, e.g., diagnostic purposes as well as for detecting viral contamination in compositions for treating disease, e.g., cell therapy. There is still a need for new methods and compositions for determining the presence and amount of viral pathogens in a sample.

SUMMARY

[0005] Disclosed herein include methods for detecting Human Parvovirus B19 (B19) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of B19 genome, wherein the target region comprises the NS1 gene region of B19, and wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; (b) generating amplicons of the target region of B19 from the sample, if the sample comprises B19; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of B19 in the sample. In some embodiments, each primer in the pair of primers comprises the sequence of SEQ ID NO: 1 or SEQ ID NO: 2, or a

sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 3, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3. In some embodiments, the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4 to the sample. In some embodiments, the linear dsDNA comprises the sequence of SEQ ID NO: 4, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 4. In some embodiments, the sample comprises no more than 1×10^8 copies of B19 genome.

[0006] Disclosed herein include methods for detecting Human Herpes Virus 6 (HHV6) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV6 genome, wherein the target region comprises the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6;(b) generating amplicons of the target region of HHV6 from the sample, if the sample comprises HHV6; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV6 in the sample. In some embodiments, each primer in the pair of primers comprises the sequence of SEQ ID NO: 5 or SEQ ID NO: 6, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 7. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 7, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 7. In some embodiments, the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 8. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 8 to the sample. In some embodiments, the linear dsDNA comprises the sequence of SEQ ID NO: 8, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 8. In some embodiments, the sample comprises no more than 1×10^8 copies of HHV6 genome.

[0007] Disclosed herein include methods for detecting Human Herpes Virus 7 (HHV7) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV7 genome, wherein the target region comprises the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; (b) generating amplicons of the target region of HHV7 from the sample, if the sample comprises HHV7; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV7 in the sample. In some embodiments, each primer in the pair of primers comprises the sequence of SEQ ID NO: 9 or SEQ ID NO: 10, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9 or SEQ ID NO: 10. In some embodiments, determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 11. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 11, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 11. In some embodiments, the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12 to the sample. In some embodiments, the linear dsDNA comprises the sequence of SEQ ID NO: 12, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 12. In some embodiments, the sample comprises no more than 1×10^6 copies of HHV7 genome.

[0008] Disclosed herein include methods for detecting Human Herpes Virus 8 (HHV8) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV8 genome, wherein the target region comprises the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; (b) generating amplicons of the target region of HHV8 from the sample, if the sample comprises HHV8; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV8 in the sample. In some embodiments, each primer in the pair of primers comprises the sequence of SEQ ID NO: 13 or SEQ ID NO: 14, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13 or SEQ ID NO: 14. In some embodiments, determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 15. In some

embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 15, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 15. In some embodiments, the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16 to the sample. In some embodiments, the linear dsDNA comprises the sequence of SEQ ID NO: 16, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 16. In some embodiments, the sample comprises no more than 1×10^6 copies of HHV8 genome.

[0009] Disclosed herein include methods for detecting Epstein Barr Virus (EBV) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of EBV genome, wherein the target region comprises the NA1 gene of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; (b) generating amplicons of the target region of EBV from the sample, if the sample comprises EBV; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of EBV in the sample. In some embodiments, each primer in the pair of primers comprises the sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18. In some embodiments, determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 19. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 19, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 19. In some embodiments, the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20 to the sample. In some embodiments, the linear dsDNA comprises the sequence of SEQ ID NO: 20, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 20. In some embodiments, the sample comprises no more than 1×10^6 copies of EBV genome.

[0010] Disclosed herein include methods for detecting Hepatitis A Virus (HAV) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HAV genome, wherein the target region is the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence

that exhibits at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22; (b) generating amplicons of the target region of HAV from the sample, if the sample comprises HAV; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HAV in the sample. In some embodiments, each primer in the pair of primers comprises the sequence of SEQ ID NO: 21 or SEQ ID NO: 22, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21 or SEQ ID NO: 22. In some embodiments, determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 23. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 23, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 23. In some embodiments, the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24 to the sample. In some embodiments, the linear dsDNA comprises the sequence of SEQ ID NO: 24, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 24. In some embodiments, the sample comprises no more than 1×10^6 copies of HAV genome.

[0011] Disclosed herein include methods for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV viruses in a sample. In some embodiments, the method comprises: a) contacting the sample with a plurality of pairs of primers, wherein the plurality of pairs of primers comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers

comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22; b) generating amplicons of the NS1 gene region of B19, amplicons of the U31 gene region of HHV6, amplicons of the U57 gene region of HHV7, amplicons of the BSLF1 gene region of HHV8, amplicons of the NA1 gene region of EBV, amplicons of the 5'-UTR region of HAV, or any combination thereof, if the sample comprises one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV; and c) determining the presence or amount of one or more amplicons as an indication of the presence of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in the sample.

[0012] In some embodiments, the pair of primers capable of hybridizing to the NS1 gene region of B19 comprises (a) a primer having the sequence of SEQ ID NO: 1 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1 and (b) a primer having the sequence of SEQ ID NO: 2 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 2; the pair of primers capable of hybridizing to the U31 gene region of HHV6 comprises (a) a primer having the sequence of SEQ ID NO: 5 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5 and (b) a primer having the sequence of SEQ ID NO: 6 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 6; the pair of primers capable of hybridizing to the U57 gene region of HHV7 comprises (a) a primer having the sequence of SEQ ID NO: 9 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9 and (b) a primer having the sequence of SEQ ID NO: 10 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 10; the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 comprises (a) a primer having the sequence of SEQ ID NO: 13 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13 and (b) a primer having the sequence of SEQ ID NO: 14 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 14; the pair of primers capable of hybridizing to the NA1 gene region of EBV comprises (a) a primer having the sequence of SEQ ID NO: 17 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17 and (b) a primer having the sequence of SEQ ID NO: 18 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 18; and/or the pair of primers capable of hybridizing to the 5'-UTR region of HAV comprises (a) a primer having the sequence of SEQ ID NO: 21 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21 and (b) a primer having the sequence of SEQ ID NO: 22 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 22.

[0013] In some embodiments, determining the presence or amount of the one or more amplicons comprises contacting the amplicons with one or more oligonucleotide probes,

wherein the one or more oligonucleotide probes comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23. In some embodiments, each of the one or more oligonucleotide probes comprises the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23; or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3, 7, 11, 15, 19, or 23.

[0014] In some embodiments, the sample comprises one or more linear dsDNAs each comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24. In some embodiments, each of the one or more linear dsDNAs comprises the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24; or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 4, 8, 12, 16, 20, or 24.

[0015] The oligonucleotide probe can, for example, comprise a fluorescence emitter moiety, a fluorescence quencher moiety, or both. In some embodiments, each oligonucleotide probe is flanked by complementary sequences at the 5' end and 3' end. In some embodiments, one of the complementary sequences comprises a fluorescence emitter moiety and the other complementary sequence comprises a fluorescence quencher moiety.

[0016] The amount (e.g., copy numbers) of the linear dsDNA present in the sample can vary, for example in an amount of no more than 100 copies. In some embodiments, (c) determining the presence or amount of the amplicons comprises using a reference standard curve. In some embodiments, the reference standard curve is generated based on serial dilutions of the linear dsDNA. In some embodiments, the serial dilutions of the linear dsDNA comprise a logarithmic dilution series from about 10 copies of the linear dsDNA to about 1×10^8 copies of the linear dsDNA in reference samples.

[0017] The sample can, for example, comprise one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV viruses (e.g., nucleic acids from one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV viruses). In some embodiments, the sample is, or is derived from, a biological sample or an environmental sample. In some embodiments, the biological sample is obtained from a tissue sample, saliva, blood, plasma, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, swab of skin or a mucosal membrane surface, cultures thereof, or any combination thereof. In some embodiments, the biological sample comprises genetically modified cells. In some embodiments, the sample is a food sample, a beverage sample, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic surface, a soil sample, a fresh water sample, a waste water sample, a saline water sample, a gas sample, cultures thereof, or any combination thereof. In some embodiments, the sample comprises DNA or RNA.

[0018] The amplification can be carried out using a method selected from the group

consisting of polymerase chain reaction (PCR), ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), replicase-mediated amplification, Immuno-amplification, nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), rolling circle amplification, transcription-mediated amplification (TMA), or a combination thereof. In some embodiments, the PCR is quantitative real-time PCR (qPCR). In some embodiments, the qPCR is reverse-transcription qPCR (RT-qPCR).

[0019] Disclosed herein include compositions for detecting B19 virus in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO:2. The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3.

[0020] Disclosed herein include compositions for detecting HHV6 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6. The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 7.

[0021] Disclosed herein include compositions for detecting HHV7 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10. The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 11.

[0022] Disclosed herein include compositions for detecting HHV8 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14. The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 15.

[0023] Disclosed herein include compositions for detecting EBV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18. The

composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 19.

[0024] Disclosed herein include compositions for detecting HAV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22. The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 23.

[0025] Disclosed herein include compositions for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

[0026] In some embodiments, the pair of primers capable of hybridizing to the NS1 gene region of B19 comprises (a) a primer comprising the sequence of SEQ ID NO: 1 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1, and (b) a primer comprising the sequence of SEQ ID NO: 2 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 2; the pair of primers capable of hybridizing to the U31 gene region of HHV6 comprises (a) a primer comprising the sequence of SEQ ID NO: 5 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5, and (b) a primer comprising the sequence of SEQ ID NO: 6 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 6; the pair of primers capable of hybridizing to

the U57 gene region of HHV7 comprises (a) a primer comprising the sequence of SEQ ID NO: 9 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9, and (b) a primer comprising the sequence of SEQ ID NO: 10 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 10; the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 comprises (a) a primer comprising the sequence of SEQ ID NO: 13 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13, and (b) a primer comprising the sequence of SEQ ID NO: 14 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 14; the pair of primers capable of hybridizing to the NA1 gene region of EBV comprises (a) a primer comprising the sequence of SEQ ID NO: 17 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17, and (b) a primer comprising the sequence of SEQ ID NO: 18 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 18; and the pair of primers capable of hybridizing to the 5'-UTR region of HAV comprises (a) a primer comprising the sequence of SEQ ID NO: 21 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21, and (b) a primer comprising the sequence of SEQ ID NO: 22 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 22.

[0027] The composition can comprise one or more oligonucleotide probes each comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23. In some embodiments, each of the one or more oligonucleotide probes comprises the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3, 7, 11, 15, 19, or 23. In some embodiments, at least one of the one or more oligonucleotide probes comprises a fluorescence emitter moiety, a fluorescence quencher moiety, or both.

[0028] The composition can comprise one or more linear dsDNAs each comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24. In some embodiments, each of the one or more linear dsDNAs comprises the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 4, 8, 12, 16, 20, or 24.

[0029] Disclosed herein includes a composition comprising one or more compositions disclosed herein for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV; and a DNA polymerase. The composition, in some embodiments, comprises a reverse transcriptase. In some embodiments, the composition comprises a buffer and/or $MgCl_2$. In some embodiments, the composition comprises nucleic acids from B19, HHV6, HHV7, HHV8, EBV, and HAV, or any combination thereof. The nucleic acids can, for example, comprise a B19 DNA, an HHV6 DNA, an HHV7 DNA, an HHV8 DNA, an EBV DNA, an HAV RNA, cDNA

of an HAV RNA, or any combination thereof. In some embodiments, the composition is a reaction mixture (e.g., an amplification reaction mixture).

[0030] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the NS1 gene region of Human Parvovirus B19 (B19). In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 1-3, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 1-3.

[0031] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the U31 gene region of Human Herpes Virus 6 (HHV6). In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 5-7, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 5-7.

[0032] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the U57 gene region of Human Herpes Virus 7 (HHV7). In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 9-11, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 9-11.

[0033] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the BSLF1 gene region of Human Herpes Virus 8 (HHV8). In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 13-15, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 13-15.

[0034] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the NA1 gene region of Epstein Barr Virus (EBV). In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 17-19, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 17-19.

[0035] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the 5'-UTR of Hepatitis A Virus (HAV). In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 21-23, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 21-23.

[0036] Disclosed herein include compositions comprising two or more of the oligonucleotide probe or primer described herein.

[0037] Disclosed herein include linear double-stranded DNAs (dsDNAs) up to about 300 bp in length. In some embodiments the linear dsDNA comprises: the sequence of SEQ ID NO: 4 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4; the sequence of SEQ ID NO: 8 or a sequence having at least about 85% identity to the sequence of

SEQ ID NO: 8; the sequence of SEQ ID NO: 12 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12; the sequence of SEQ ID NO: 16 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16; the sequence of SEQ ID NO: 20 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20; or the sequence of SEQ ID NO: 24 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24.

[0038] Disclosed herein include compositions comprising one or more of the linear dsDNA described herein. Disclosed herein include compositions comprising two or more of the oligonucleotide probe or primer and one or more of the linear dsDNA described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] **FIG. 1** depicts a graph showing that an assay for detecting Human Herpes Virus 6 (HHV6) as disclosed herein can produce a linear amplification trend over an 8-fold dilution series, showing that the reaction is efficient for amplifying HHV6 target sequence.

[0040] **FIG. 2** depicts a graph showing that an assay for detecting Human Parvovirus B19 as disclosed herein can produce a linear amplification trend over an 8-fold dilution series, showing that the reaction is efficient for amplifying B19 target sequence.

DETAILED DESCRIPTION

[0041] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein and made part of the disclosure herein.

[0042] All patents, published patent applications, other publications, and sequences from GenBank, and other databases referred to herein are incorporated by reference in their entirety with respect to the related technology.

[0043] Methods, compositions, kits and systems for nucleic acid amplifications disclosed herein can be performed to determine the presence, absence, type, and/or level of one or more of Human Parvovirus B19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), and Hepatitis A Virus (HAV).

[0044] Disclosed herein include methods for detecting Human Parvovirus B19 (B19) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of B19 genome, wherein the target region comprises the NS1 gene region of B19, and wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; (b) generating amplicons of the target region of B19 from the sample, if the sample comprises B19; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of B19 in the sample.

[0045] Disclosed herein include methods for detecting Human Herpes Virus 6 (HHV6) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV6 genome, wherein the target region comprises the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; (b) generating amplicons of the target region of HHV6 from the sample, if the sample comprises HHV6; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV6 in the sample.

[0046] Disclosed herein include methods for detecting Human Herpes Virus 7 (HHV7) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV7 genome, wherein the target region comprises the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; (b) generating amplicons of the target region of HHV7 from the sample, if the sample comprises HHV7; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV7 in the sample.

[0047] Disclosed herein include methods for detecting Human Herpes Virus 8 (HHV8) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV8 genome, wherein the target region comprises the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; (b) generating amplicons of the target region of HHV8 from the sample, if the sample comprises HHV8; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV8 in the sample.

[0048] Disclosed herein include methods for detecting Epstein Barr Virus (EBV) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of EBV genome, wherein the target region

comprises the NA1 gene of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; (b) generating amplicons of the target region of EBV from the sample, if the sample comprises EBV; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of EBV in the sample.

[0049] Disclosed herein include methods for detecting Hepatitis A Virus (HAV) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HAV genome, wherein the target region is the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22; (b) generating amplicons of the target region of HAV from the sample, if the sample comprises HAV; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HAV in the sample.

[0050] Disclosed herein include methods for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. In some embodiments, the method comprises: a) contacting the sample with a plurality of pairs of primers, wherein the plurality of pairs of primers comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22; b) generating amplicons of the NS1 gene region of B19, amplicons of the U31 gene region of HHV6, amplicons of the U57 gene region of HHV7, amplicons of the BSLF1 gene region of HHV8, amplicons of the NA1 gene region of EBV, amplicons of the 5'-UTR region of HAV, or any combination thereof, if the sample comprises one or more of B19,

HHV6, HHV7, HHV8, EBV, and HAV; and c) determining the presence or amount of one or more amplicons as an indication of the presence of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in the sample.

[0051] Disclosed herein include compositions for detecting B19 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO:2.

[0052] Disclosed herein include compositions for detecting HHV6 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

[0053] Disclosed herein include compositions for detecting HHV7 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

[0054] Disclosed herein include compositions for detecting HHV8 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

[0055] Disclosed herein include compositions for detecting EBV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18.

[0056] Disclosed herein include compositions for detecting HAV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the 5'-UTR of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

[0057] Disclosed herein include compositions for detecting one or more (e.g., two or three) of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; a pair of primers capable of hybridizing to the U57 gene region of

HHV7, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

[0058] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the NS1 gene region of B19. In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 1-3, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 1-3.

[0059] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the U31 gene region of HHV6. In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 5-7, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 5-7.

[0060] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the U57 gene region of HHV7. In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 9-11, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 9-11.

[0061] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the BSLF1 gene region of HHV8. In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 13-15, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 13-15.

[0062] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the NA1 gene region of EBV. In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 17-19, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 17-19.

[0063] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the 5'-UTR of HAV. In some embodiments, the probe or primer comprises the sequence of any one of SEQ ID NOs: 21-23, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 21-23. Disclosed herein include compositions comprising two or more of the oligonucleotide probe or primer described herein.

[0064] Disclosed herein include linear double-stranded DNAs (dsDNAs) up to about 300 bp in length. In some embodiments the linear dsDNA comprises: the sequence of SEQ ID NO: 4 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4; the sequence of SEQ ID NO: 8 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 8; the sequence of SEQ ID NO: 12 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12; the sequence of SEQ ID NO: 16 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16; the sequence of SEQ ID NO: 20 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20; or the sequence of SEQ ID NO: 24 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24.

[0065] Some embodiments provide compositions comprising one or more of the linear dsDNA described herein. Some embodiments provide compositions comprising two or more of the oligonucleotide probe or primer and one or more of the linear dsDNA described herein.

Definitions

[0066] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. *See, e.g.* Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY 1989). For purposes of the present disclosure, the following terms are defined below.

[0067] As used herein, the term "nucleic acid" can refer to a polynucleotide sequence, or fragment thereof. A nucleic acid can comprise nucleotides. A nucleic acid can be exogenous or endogenous to a cell. A nucleic acid can exist in a cell-free environment. A nucleic acid can be a gene or fragment thereof. A nucleic acid can be DNA. A nucleic acid can be RNA. A nucleic acid can comprise one or more analogs (e.g., altered backbone, sugar, or nucleobase). Some non-limiting examples of analogs include: 5-bromouracil, peptide nucleic acid, xeno nucleic acid, morpholinos, locked nucleic acids, glycol nucleic acids, threose nucleic acids, dideoxynucleotides, cordycepin, 7-deaza-GTP, fluorophores (e.g., rhodamine or fluorescein linked to the sugar), thiol containing nucleotides, biotin linked nucleotides, fluorescent base analogs, CpG islands, methyl-7-guanosine, methylated nucleotides, inosine, thiouridine, pseudouridine, dihydrouridine, queuosine, and wyosine. "Nucleic acid", "polynucleotide, "target polynucleotide", "target nucleic acid", and "target sequence" can be used interchangeably. As used herein, a "nucleic acid" can refer to a polymeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases, or

base analogs, linked together by nucleic acid backbone linkages (e.g., phosphodiester bonds) to form a polynucleotide. Non-limiting examples of nucleic acid include RNA, DNA, and analogs thereof. The nucleic acid backbone can include a variety of linkages, for example, one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds, phosphorothioate or methylphosphonate linkages or mixtures of such linkages in a single oligonucleotide. Sugar moieties in the nucleic acid can be either ribose or deoxyribose, or similar compounds with known substitutions. Conventional nitrogenous bases (e.g., A, G, C, T, U), known base analogs (e.g., inosine), derivatives of purine or pyrimidine bases and "abasic" residues (i.e., no nitrogenous base for one or more backbone positions) are included in the term nucleic acid. That is, a nucleic acid can include only conventional sugars, bases and linkages found in RNA and DNA, or include both conventional components and substitutions (e.g., conventional bases and analogs linked via a methoxy backbone, or conventional bases and one or more base analogs linked via an RNA or DNA backbone).

[0068] A nucleic acid can comprise one or more modifications (e.g., a base modification, a backbone modification), to provide the nucleic acid with a new or enhanced feature (e.g., improved stability). A nucleic acid can comprise a nucleic acid affinity tag. A nucleoside can be a base-sugar combination. The base portion of the nucleoside can be a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides can be nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', the 3', or the 5' hydroxyl moiety of the sugar. In forming nucleic acids, the phosphate groups can covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound; however, linear compounds are generally suitable. In addition, linear compounds may have internal nucleotide base complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded compound. Within nucleic acids, the phosphate groups can commonly be referred to as forming the internucleoside backbone of the nucleic acid. The linkage or backbone can be a 3' to 5' phosphodiester linkage.

[0069] A nucleic acid can comprise a modified backbone and/or modified internucleoside linkages. Modified backbones can include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. Suitable modified nucleic acid backbones containing a phosphorus atom therein can include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkyl phosphotriesters, methyl and other alkyl phosphonate such as 3'-alkylene

phosphonates, 5'-alkylene phosphonates, chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkyl phosphoramidates, phosphorodiamidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', a 5' to 5' or a 2' to 2' linkage.

[0070] A nucleic acid can comprise polynucleotide backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These can include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0071] A nucleic acid can comprise a nucleic acid mimetic. The term "mimetic" can be intended to include polynucleotides wherein only the furanose ring or both the furanose ring and the internucleotide linkage are replaced with non-furanose groups, replacement of only the furanose ring can also be referred as being a sugar surrogate. The heterocyclic base moiety or a modified heterocyclic base moiety can be maintained for hybridization with an appropriate target nucleic acid. One such nucleic acid can be a peptide nucleic acid (PNA). In a PNA, the sugar-backbone of a polynucleotide can be replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleotides can be retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone The backbone in PNA compounds can comprise two or more linked aminoethylglycine units which gives PNA an amide containing backbone. The heterocyclic base moieties can be bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone.

[0072] A nucleic acid can comprise a morpholino backbone structure. For example, a nucleic acid can comprise a 6-membered morpholino ring in place of a ribose ring. In some of these embodiments, a phosphorodiamidate or other non-phosphodiester internucleoside linkage can replace a phosphodiester linkage. A nucleic acid can comprise linked morpholino units (e.g., morpholino nucleic acid) having heterocyclic bases attached to the morpholino ring. Linking groups can link the morpholino monomeric units in a morpholino nucleic acid. Non-ionic morpholino-based oligomeric compounds can have less undesired interactions with cellular proteins. Morpholino-based polynucleotides can be nonionic mimics of nucleic acids. A variety

of compounds within the morpholino class can be joined using different linking groups. A further class of polynucleotide mimetic can be referred to as cyclohexenyl nucleic acids (CeNA). The furanose ring normally present in a nucleic acid molecule can be replaced with a cyclohexenyl ring. CeNA DMT protected phosphoramidite monomers can be prepared and used for oligomeric compound synthesis using phosphoramidite chemistry. The incorporation of CeNA monomers into a nucleic acid chain can increase the stability of a DNA/RNA hybrid. CeNA oligoadenylates can form complexes with nucleic acid complements with similar stability to the native complexes. A further modification can include Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 4' carbon atom of the sugar ring thereby forming a 2'-C, 4'-C-oxymethylene linkage thereby forming a bicyclic sugar moiety. The linkage can be a methylene (-CH_2), group bridging the 2' oxygen atom and the 4' carbon atom wherein *n* is 1 or 2. LNA and LNA analogs can display very high duplex thermal stabilities with complementary nucleic acid (Tm=+3 to +10 °C), stability towards 3'-exonucleolytic degradation and good solubility properties.

[0073] A nucleic acid may also include nucleobase (often referred to simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases can include the purine bases, (e.g., adenine (A) and guanine (G)), and the pyrimidine bases, (e.g., thymine (T), cytosine (C) and uracil (U)). Modified nucleobases can include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (—C=C—CH3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-aminoadenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Modified nucleobases can include tricyclic pyrimidines such as phenoxazine cytidine(1H-pyrimido(5,4-b)(1,4)benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido(5,4-b)(1,4)benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g., 9-(2-aminoethoxy)-H-pyrimido(5,4-(b) (1,4)benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido(5,4-b)(1,4)benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g., 9-(2-aminoethoxy)-H-pyrimido(5,4-(b) (1,4)benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido(4,5-b)indol-2-one), pyridoindole cytidine (H-pyrido(3',2':4,5)pyrrolo[2,3-d]pyrimidin-2-one).

[0074] As used herein, the term "isolate nucleic acids" can refer to the purification of nucleic acids from one or more cellular components. One of skill in the art will appreciate that samples processed to "isolate nucleic acids" therefrom can include components and impurities other than nucleic acids. Samples that comprise isolated nucleic acids can be prepared from specimens using any acceptable method known in the art. For example, cells can be lysed using known lysis agents, and nucleic acids can be purified or partially purified from other cellular components. In nucleic acid testing (e.g., amplification and hybridization methods discussed in further detail below), the extracted nucleic acid solution can be added directly to a reagents (e.g., either in liquid, bound to a substrate, in lyophilized form, or the like, as discussed in further detail below), required to perform a test according to the embodiments disclosed herein.

[0075] As used herein, "template" can refer to all or part of a polynucleotide containing at least one target nucleotide sequence.

[0076] As used herein, a "primer" can refer to a polynucleotide that can serve to initiate a nucleic acid chain extension reaction. The length of a primer can vary, for example, from about 5 to about 100 nucleotides, from about 10 to about 50 nucleotides, from about 15 to about 40 nucleotides, or from about 20 to about 30 nucleotides. The length of a primer can be about 10 nucleotides, about 20 nucleotides, about 25 nucleotides, about 30 nucleotides, about 35 nucleotides, about 40 nucleotides, about 50 nucleotides, about 75 nucleotides, about 100 nucleotides, or a range between any two of these values. In some embodiments, the primer has a length of 10 to about 50 nucleotides, *i.e.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more nucleotides. In some embodiments, the primer has a length of 18 to 32 nucleotides.

[0077] As used herein, a "probe" can refer to an polynucleotide that can hybridize (e.g., specifically) to a target sequence in a nucleic acid, under conditions that allow hybridization, thereby allowing detection of the target sequence or amplified nucleic acid. A probe's "target" generally refers to a sequence within or a subset of an amplified nucleic acid sequence which hybridizes specifically to at least a portion of a probe oligomer by standard hydrogen bonding (i.e., base pairing). A probe may comprise target-specific sequences and other sequences that contribute to three-dimensional conformation of the probe. Sequences are "sufficiently complementary" if they allow stable hybridization in appropriate hybridization conditions of a probe oligomer to a target sequence that is not completely complementary to the probe's target-specific sequence. The length of a probe can vary, for example, from about 5 to about 100 nucleotides, from about 10 to about 50 nucleotides, from about 15 to about 40 nucleotides, or from about 20 to about 30 nucleotides. The length of a probe can be about 10 nucleotides, about 20 nucleotides, about 25 nucleotides, about 30 nucleotides, about 35

nucleotides, about 40 nucleotides, about 50 nucleotides, about 100 nucleotides, or a range between any two of these values. In some embodiments, the probe has a length of 10 to about 50 nucleotides. For example, the primers and or probes can be at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more nucleotides. In some embodiments, the probe can be non-sequence specific.

[0078] Preferably, the primers and/or probes can be between 8 and 45 nucleotides in length. For example, the primers and/or probes can be at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or more nucleotides in length. The primer and probe can be modified to contain additional nucleotides at the 5' or the 3' terminus, or both. One of skill in the art will appreciate that additional bases to the 3' terminus of amplification primers (not necessarily probes) are generally complementary to the template sequence. The primer and probe sequences can also be modified to remove nucleotides at the 5' or the 3' terminus. One of skill in the art will appreciate that in order to function for amplification, the primers or probes will be of a minimum length and annealing temperature as disclosed herein.

[0079] Primers and probes can bind to their targets at an annealing temperature, which is a temperature less than the melting temperature (T_m). As used herein, "T_m" and "melting temperature" are interchangeable terms which refer to the temperature at which 50% of a population of double-stranded polynucleotide molecules becomes dissociated into single strands. The formulae for calculating the T_m of polynucleotides are well known in the art. For example, the T_m may be calculated by the following equation: $T_m = 69.3+0.41 \times (G+C)\%-6-50/L$, wherein L is the length of the probe in nucleotides. The T_m of a hybrid polynucleotide may also be estimated using a formula adopted from hybridization assays in 1 M salt, and commonly used for calculating T_m for PCR primers: [(number of A+T) × 2°C + (number of G+C) x 4°C]. *See*, *e.g.*, C. R. Newton et al. PCR, 2nd ed., Springer-Verlag (New York: 1997), p.24 (incorporated by reference in its entirety, herein). Other more sophisticated computations exist in the art, which take structural as well as sequence characteristics into account for the calculation of T_m. The melting temperature of an oligonucleotide can depend on complementarity between the oligonucleotide primer or probe and the binding sequence, and on salt conditions. In some embodiments, an oligonucleotide primer or probe provided herein has a T_m of less than about 90°C in 50mM KCl, 10 mM Tris-HCl buffer, for example about 89°C, 88, 87, 86, 85, 84, 83, 82, 81, 80 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39°C, or less, including ranges between any two of the listed values.

[0080] In some embodiments, the primers disclosed herein, *e.g.*, amplification primers, can be provided as an amplification primer pair, *e.g.*, comprising a forward primer and a reverse primer (first amplification primer and second amplification primer). Preferably, the forward and reverse primers have T_m's that do not differ by more than 10°C, *e.g.*, that differ by less than 10°C, less than 9°C, less than 8°C, less than 7°C, less than 6°C, less than 5°C, less than 4°C, less than 3°C, less than 2°C, or less than 1°C.

[0081] The primer and probe sequences may be modified by having nucleotide substitutions (relative to the target sequence) within the oligonucleotide sequence, provided that the oligonucleotide contains enough complementarity to hybridize specifically to the target nucleic acid sequence. In this manner, at least 1, 2, 3, 4, or up to about 5 nucleotides can be substituted. As used herein, the term "complementary" can refer to sequence complementarity between regions of two polynucleotide strands or between two regions of the same polynucleotide strand. A first region of a polynucleotide is complementary to a second region of the same or a different polynucleotide if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide of the first region is capable of base pairing with a base of the second region. Therefore, it is not required for two complementary polynucleotides to base pair at every nucleotide position. "Fully complementary" can refer to a first polynucleotide that is 100% or "fully complementary" to a second polynucleotide and thus forms a base pair at every nucleotide position. "Partially complementary" also can refer to a first polynucleotide that is not 100% complementary (e.g., 90%, or 80% or 70% complementary) and contains mismatched nucleotides at one or more nucleotide positions. In some embodiments, an oligonucleotide includes a universal base.

[0082] As used herein, an "exogenous nucleotide sequence" can refer to a sequence introduced by primers or probes used for amplification, such that amplification products will contain exogenous nucleotide sequence and target nucleotide sequence in an arrangement not found in the original template from which the target nucleotide sequence was copied.

[0083] As used herein, "sequence identity" or "percent identical" as applied to nucleic acid molecules can refer to the percentage of nucleic acid residues in a candidate nucleic acid molecule sequence that are identical with a subject nucleic acid molecule sequence, after aligning the sequences to achieve the maximum percent identity, and not considering any nucleic acid residue substitutions as part of the sequence identity. Nucleic acid sequence identity can be determined using any method known in the art, for example CLUSTAL OMEGA, T-COFFEE, BLASTN.

[0084] As used herein, the term "sufficiently complementary" can refer to a contiguous nucleic acid base sequence that is capable of hybridizing to another base sequence by

hydrogen bonding between a series of complementary bases. Complementary base sequences can be complementary at each position in the oligomer sequence by using standard base pairing (e.g., G:C, A:T or A:U) or can contain one or more residues that are not complementary (including abasic positions), but in which the entire complementary base sequence is capable of specifically hybridizing with another base sequence in appropriate hybridization conditions. Contiguous bases can be at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100% complementary to a sequence to which an oligomer is intended to hybridize. Substantially complementary sequences can refer to sequences ranging in percent identity from 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 75, 70 or less, or any number in between, compared to the reference sequence. A skilled artisan can readily choose appropriate hybridization conditions which can be predicted based on base sequence composition, or be determined by using routine testing (*see e.g.*, Green and Sambrook, Molecular Cloning, A Laboratory Manual, 4th ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2012)).

[0085] As used herein, the term "multiplex PCR" refers to a type of PCR where more than one set of primers is included in a reaction allowing one single target, or two or more different targets, to be amplified in a single reaction vessel (e.g., tube). The multiplex PCR can be, for example, a real-time PCR.

Oligonucleotides

[0086] As described herein, nucleic acid amplifications can be performed to determine the presence, absence, type, and/or level of one or more of Human Parvovirus B19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), and Hepatitis A Virus (HAV) in a sample. In some embodiments, the presence, absence and/or level of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV viruses is determined by detecting one or more target gene regions of the target organism(s) using methods known in the art, such as nucleic acid (e.g., DNA or cDNA) amplifications. In some embodiments, an individual amplification reaction (e.g., PCR) is performed to detect the presence, absence and/or level of one of B19, HHV6, HHV7, HHV8, EBV, and HAV viruses in a sample. For example, the presence, absence and/or level of B19 virus is determined by an amplification reaction assay separate from the amplification reaction assay(s) used for other viruses. In some embodiments, a multiplex PCR is performed to detect the presence, absence or level of two or more (e.g., two or three) of B19, HHV6, HHV7, HHV8, EBV, and HAV viruses in a sample. For example, an amplification reaction assay can be used to detect simultaneously the presence, absence and/or level of HHV6 and B19 viruses. As another

example, an amplification reaction assay can be used to detect simultaneously the presence, absence and/or level of HHV7, HHV8, and EBV viruses.

[0087] There are provided, in some embodiments, real-time PCR (Polymerase Chain Reaction) primers and probes combinations as well as detection methods for identification of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. There are provided, in some embodiments, methods (e.g., qPCR assays) and compositions (e.g., primers and probes) targeting species-specific gene regions present in B19, HHV6, HHV7, HHV8, EBV, and HAV. In addition, in some embodiments of the methods, compositions and kits provided herein, linear dsDNAs are provided. The dsDNAs can comprise consensus standard sequences from each species-specific gene region. In some embodiments, the linear dsDNA can be used to generate a standard curve for quantification. In some embodiments, the linear dsDNA can be used as an internal control (e.g., spike-in control). Spike-in controls can overcome the assumption that DNA or RNA yields are equal in different experimental conditions and across all genomic regions. Spike-in controls can also be used to monitor the amplification reactions (e.g., amplification efficiency).

[0088] Disclosed herein include methods and compositions (e.g., reagents utilizing fluorogenic sequence-specific hybridization probes) which provide a rapid and economical solution to: (1) identification of viral species; (2) quantification of the viral genomes in the sample; (3) monitoring the quality of samples; and/or (4) quality control of the nucleic acid extraction and real-time PCR processes. The methods provided herein can comprise: subjecting the nucleic acid from a sample or culture suspected of containing B19, HHV6, HHV7, HHV8, EBV, and HAV to a PCR amplification utilizing sets of concentration optimized primer pairs and probes; treating the reaction mixture under the optimum thermal condition, and detecting amplified gene targets by monitoring fluorescence signals of the hydrolysis (TaqMan®) probes at each cycle and interpreting the data at the end of the program to report the final results.

[0089] Disclosed herein include probe-based (e.g., TaqMan probe-based) real-time PCR compositions (e.g., reagents) and methods (e.g., assays) for rapid identification of B19, HHV6, HHV7, HHV8, EBV, and HAV. As compared to currently available methods, the advantages of the disclosed methods include: (1) the designed dsDNA internal control can monitor the quality of the sample, indicate false-negative results that are mainly caused by the PCR inhibitors, instrument or reagent failure, and be used to generated a standard curve for quantification; and (2) the primers/probes provided herein can achieve high linearity, precision, accuracy, robustness, and specificity. Moreover, the disclosed methods are both fast and easy to perform.

[0090] Oligonucleotides (e.g., amplification primers and probes) that are capable of

specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to a target gene region, or complement thereof, in B19, HHV6, HHV7, HHV8, EBV, and HAV are provided (Table 1). Amplification of the target gene region of an organism in a sample can, in some embodiments, be indicative of the presence, absence, and/or level of the organism in the sample.

TABLE 1: SEQUENCES

NAME	SEQ ID NO:	SEQUENCE
B19-Forward Primer (FP)	1	ATTAATGCAGATGCCCTCCA
B19-Reverse Primer (RP)	2	GGGATGGGCGTACTAGAGC
B19 Probe	3	TTTAACCTCATCACCCCAGG
B19-NS1 linear fragment	4	TTTGATTTCCCTGGAATTAATGCAGATGCCCTCCACCCAGA CCTCCAAACCACCCCAATTGTCACAGACACCAGTATCAGCA GCAGTGGTGGTGAAAGCTCTGAAGAACTCAGTGAAAGCAGC TTTTTTAACCTCATCACCCCAGGCGCCTGGAACACTGAAAC CCCGCGCTCTAGTACGCCCATCCCCGGGACCAGTTCAGGAG AATCAT
HHV6- FP	5	CACAAATGACGATTCTAGCAAACAA
HHV6- RP	6	GTGGTTTTGCACAGGTAATTATTGT
HHV6 Probe	7	AGCTTGTCAAGAAAACAATCTGGA
HHV6-U31 linear fragment	8	CACAAATGACGATTCTAGCAAACAAAAGCAGCAAACGATTT TCATAAATTATTTATTGCCTTTCAAGGACTTTTCCGAAGTA TTCAACGAGGCGTTGACAGCTTGTCAAGAAAACAATCTGGA CATCCTCTTGATTTACAATAATTACCTGTGCAAAACCAC
HHV7- FP	9	TAGTTCCAGCACTGCAATCG
HHV7- RP	10	GACAACCTCGCAGATTGCTT
HHV7 Probe	11	GTTGGGTTAGGCATCACGTT
HHV7-U57 region dsDNA, 256 bp linear fragment	12	TAGTTCCAGCACTGCAATCGCAAATTTGAAAAGGGTAAAAA CTGATATGGGAAGTAAAGTGCAAGATCTATTTTCGGTCTTT CCAATGCACGCATACACCAACCCTACTGTAAATAGTTGGGT TAGGCATCACGTTGGCATTGAAAAACCCAATCCTTCCGAAA CCGATGCTTTAAACATCCTTTCTTTCGGTAAGATAAATAAG CAATCACAATCAATACTTTTACATGGCCAACAAGCAATCTG CGAGGTTGTC
HHV8- FP	13	ACCATGACCTCAGCCTATCG
HHV8- RP	14	CACCTCCTCGAATGTCCTGT
HHV8 Probe	15	TGCCAACACTGAAGGAACAG
HHV8-BSLF1 region dsDNA, 240 bp linear fragment	16	TATTTCCAGGCGACCAGGACTGGGATATGTTATCTGACAAA GACCTCACCTACCGAATTTTTTACCATGACCTCAGCCTATC GCTGCCAACACTGAAGGAACAGCTCCTTGTTTCAAGACACG AATACTTCAACCCTCGCTTGCCAGTGTATAGATGGGTATTA GACTTTGACCTGCCCGTCTGCCGCGACATTGACAGGACATT CGAGGAGGTGCACTCTCTCTGTTGTTCCCTGCGTG
EBV- FP	17	TACAACCTCAGGCGAGGAAT
EBV- RP	18	GCATCCTTCAAAACCTCAGC
EBV Probe	19	ACACCATTGAGTCGTCTCCC
EBV-NA1 region dsDNA, 232bp linear fragment	20	CCCTTTACAACCTCAGGCGAGGAATTGCCCTTGCTGTTCCA CAATGTCGTATTACACCATTGAGTCGTCTCCCCTTTGGAAT GGCCCCTGGACCCGGCCCACAACCTGGCCCACTAAGGGAGT CCATTGTCTGTTATTTCATGGTCTTTTTACAAACTCATATA

		TTTGCTGAGGTTTTGAAGGATGCGATTAAGGACCTTGTTAT GACAAAGCCCGCTCCTACCTGCAATAT
HAV- FP	21	TCAGGGCTGTCTCTAGGTTTA
HAV- RP	22	CCTCTCACAGGATCCCATTTAAG
HAV Probe	23	ACCTCTCTGTGCTTAGGGCAAACA
HAV-5'-UTR region dsDNA, 213 bp linear fragment	24	CGGCGGATATTGGTGAGTTGTTAAGACAAAAACCATTCAAC GCCGGAGGACTGGCTCTCATCCAGTGGATGCATTGAGTGAA TTGATTGTCAGGGCTGTCTCTAGGTTTAATCTCAGACCTCT CTGTGCTTAGGGCAAACACTATTTGGCCTTAAATGGGATCC TGTGAGAGGGGGTCCCTCCATTGACAGCTGGACTGTTCTTT GGGGCCTT

[0091] The target gene region of a virus under detection can vary. In some embodiments, the target gene region of B19 virus comprises, or is, the NS1 gene region. In some embodiments, the target gene region of HHV6 comprises, or is, the U31 gene region. In some embodiments, the target gene region of HHV7 comprises, or is, the U57 gene region. In some embodiments, the target gene region of HHV8 comprises, or is, the BSLF1 gene region. In some embodiments, the target gene region of EBV comprises, or is, the NA1 gene region. In some embodiments, the target gene region of HAV comprises, or is, the 5'-UTR region.

[0092] As disclosed herein, nucleic acid amplification (e.g., PCR) can be used for generating amplicons of the species-specific NS1 gene region of B19 for detecting B19 (e.g., the absence, presence and/or amount of B19) in a sample. In some embodiments, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to the NS1 gene region in B19 are provided. In some embodiments, primers and probes that can specifically bind to the NS1 region of B19 are used in detection of the presence, absence and/or level of B19 in a biological sample. Examples of oligonucleotides capable of specifically hybridizing to the NS1 gene region of B19 include, but are not limited to, SEQ ID NOs: 1-3 as provided in Table 1 and sequences that exhibits at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 1-3. There are also provided, in some embodiments, linear double-stranded DNAs (dsDNAs) which can be used as, e.g., as an internal control and/or quantification standard for the amplification. In some embodiments, the linear dsDNA comprises a consensus sequence for a target gene region, e.g., the NS1 gene region of different B19 variants. In some embodiments, the dsDNA comprises the sequence of SEQ ID NO: 4 or a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 4.

[0093] As disclosed herein, nucleic acid amplification (e.g., PCR) can be used for generating amplicons of the species-specific US31 gene region of HHV6 for detecting HHV6 (e.g., the absence, presence and/or amount of HHV6) in a sample. In some embodiments,

oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, including standard PCR conditions, and/or stringent hybridization conditions) to the U31 gene region in HHV6 are provided. In some embodiments, U31 gene region is used as the target gene region for the DNA amplification to detect the presence, absence and/or level of HHV6 in the sample. In some embodiments, primers and probes that can specifically bind to the U31 region of HHV6 are used in detection of the presence, absence and/or amount of HHV6 in a biological sample. Examples of oligonucleotides capable of specifically hybridizing to the U31 gene region of HHV6 include, but are not limited to, SEQ ID NOs: 5-7 as provided in Table 1 and sequences that exhibits at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 5-7. There is also provided, in some embodiments, linear dsDNAs which can be used as, e.g., as an internal control and/or quantification standard. In some embodiments, the linear dsDNA comprises a consensus sequence for a target gene region, e.g., the U31 gene region of HHV6 variants. In some embodiments, the dsDNA can comprise the sequence of SEQ ID NO: 8 or a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 8.

[0094] As disclosed herein, nucleic acid amplification (e.g., PCR) can be used for generating amplicons of the species-specific U57 gene region of HHV7 for detecting HHV7 (e.g., the absence, presence and/or amount of HHV7) in a sample. In some embodiments, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to U57 gene region in HHV7 are provided. In some embodiments, U57 gene region is used as the target gene region for the DNA amplification to detect the presence, absence and/or level of HHV7 in the sample. In some embodiments, primers and probes that can specifically bind to the U57 gene region of HHV7 are used in detection of the presence, absence and/or level of HHV7 in a biological sample. Examples of oligonucleotides capable of specifically hybridizing to the U57 gene region of HHV7 include, but are not limited to, SEQ ID NOs: 9-11 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 9-11. There is also provided, in some embodiments, linear dsDNAs which can be used as, e.g., as an internal control and/or quantification standard. In some embodiments, the linear dsDNA comprises a consensus sequence for a target gene region, e.g., the U57 gene region of HHV7 variants. In some embodiments, the dsDNA can comprise the sequence of SEQ ID NO: 12 or a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 12.

[0095] As disclosed herein, nucleic acid amplification (e.g., PCR) can be used for generating amplicons of the species-specific BSLF1 gene region of HHV8 for detecting HHV8

(e.g., the absence, presence and/or amount of HHV8) in a sample. In some embodiments, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to BSLF1 gene region in HHV8 are provided. In some embodiments, BSLF1 gene region is used as the target gene region for the DNA amplification to detect the presence, absence and/or level of HHV8 in the sample. In some embodiments, primers and probes that can specifically bind to the BSLF1region of HHV8 are used in detection of the presence, absence and/or level of HHV8 in a biological sample. Examples of oligonucleotides capable of specifically hybridizing to the BSLF1 gene region of HHV8 include, but are not limited, SEQ ID NOs: 13-15 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 13-15. There is also provided, in some embodiments, linear dsDNAs which can be used as, e.g., as an internal control and/or quantification standard. In some embodiments, the linear dsDNAs comprise a sequence comprising a consensus sequence for a target gene region, e.g., the BSLF1 gene region of HHV8. In some embodiments, the dsDNA can comprise the sequence of SEQ ID NO: 16 or a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 16.

[0096] As disclosed herein, nucleic acid amplification (e.g., PCR) can be used for generating amplicons of the species-specific NA1 gene region of EBV for detecting EBV (e.g., the absence, presence and/or amount of EBV) in a sample. In some embodiments, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to the NA1 gene region in EBV are provided. In some embodiments, NA1 gene region is used as the target gene region for the DNA amplification to detect the presence, absence and/or level of EBV in the sample. In some embodiments, primers and probes that can specifically bind to the NA1 region of EBV are used in detection of the presence, absence and/or level of EBV in a biological sample. Examples of oligonucleotides capable of specifically hybridizing to the NA1 gene region of EBV include, but are not limited to, SEQ ID NOs: 17-19 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 17-19. There is also provided, in some embodiments, linear dsDNAs which can be used as, e.g., as an internal control and/or quantification standard. In some embodiments, the linear dsDNAs comprise a consensus sequence for a target gene region, e.g., the NA1 gene region of EBV. In some embodiments, the dsDNA comprises the sequence of SEQ ID NO: 20 or a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 20.

[0097] As disclosed herein, nucleic acid amplification (e.g., PCR) can be used for generating amplicons of the species-specific 5'-UTR region of HAV for detecting HAV (e.g., the absence, presence and/or amount of HAV) in a sample. In some embodiments, oligonucleotides (e.g., amplification primers and probes) that are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to the 5'-UTR region in HAV are provided. In some embodiments, 5'-UTR region is used as the target for the nucleic acid amplification (e.g., cDNA) to detect the presence, absence and/or level of HAV in the sample. In some embodiments, primers and probes that can specifically bind to the 5'-UTR region of HAV are used in detection of the presence, absence and/or level of HAV in a biological sample. Examples of oligonucleotides capable of specifically hybridizing to the 5'-UTR region of HAV include, but are not limited, SEQ ID NOs: 21-23 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 21-23. There is also provided, in some embodiments, linear dsDNAs which can be used as, e.g., as an internal control and/or quantification standard. In some embodiments, the linear dsDNAs comprise a consensus sequence for a target gene region, e.g., the 5'-UTR region of HAV. In some embodiments, the dsDNA comprises the sequence of SEQ ID NO: 24 or a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 24.

[0098] Some embodiments provide primer/probe combinations. A primer/probe combination can comprise a forward primer, a reverse primer, and a probe (e.g., B19-FP, B19-RP, and B19 Probe in tandem). The compositions and methods provided herein can comprise one or more of the primer/probe combinations provided in Table 1. For example, a method or composition can comprise a primer/probe combination (e.g., B19-FP, B19-RP, and B19 Probe in tandem). Disclosed herein are methods and compositions comprising two or more primer/probe combinations (e.g., multiplexed reactions). For example, a method or composition can comprise multiple primer/probe combinations (e.g., B19-FP, B19-RP, and B19 Probe, HHV6-FP, HHV6-RP, and HHV6 Probe; HHV7-FP, HHV7-RP, and HHV7 Probe; HHV8-FP, HHV8-RP, and HHV8 Probe; EBV-FP, EBV-RP, and EBV Probe; HAV-FP, HAV-RP, and HAV Probe described in Table 1 in tandem). Disclosed herein are methods and compositions comprising: (1) one or more primer/probe combinations capable of specifically hybridizing to the sequence of the NS1 gene region, or a complement thereof, of B19 (e.g., B19-FP, B19-RP, and B19 Probe described in Table 1); (2) one or more primer/probe combinations capable of specifically hybridizing to the sequence of the U31 gene region, or a complement thereof, of HHV6 (e.g., HHV6-FP, HHV6-RP, HHV6 Probe described in Table 1); (3) one or more primer/probe combinations capable of specifically hybridizing to the sequence of the U57 gene region, or a

complement thereof, of the U57 gene region of HHV7 (e.g., HHV7-FP, HHV7-RP, HHV7 Probe described in Table 1); (4) one or more primer/probe combinations capable of specifically hybridizing to the sequence of the BSLF1 gene region, or a complement thereof, of HHV8 (e.g., HHV8-FP, HHV8-RP, HHV8 Probe described in Table 1); (5) one or more primer/probe combinations capable of specifically hybridizing to the sequence of the NA1 gene region, or a complement thereof, of EBV (e.g., EBV-FP, EBV-RP, EBV Probe described in Table 1); and/or (6) one or more primer/probe combinations capable of specifically hybridizing to the sequence of the 5'-UTR region, or a complement thereof, of HAV (e.g., HAV-FP, HAV-RP, HAV Probe described in Table 1).

Linear dsDNA standards

[0099] There are provided, in some embodiments, a plurality of linear dsDNAs that comprise a consensus sequence of, e.g., the NS1 gene region of B19, the U31 gene region in HHV6, the U57 gene region of HHV7, the BSLF1 gene region of HHV8, the NA1 gene region in EBV, or the 5'-UTR region in HAV. The term "consensus sequence," as used herein in the context of nucleic acid sequences, can refer to a calculated sequence representing the most frequent nucleotide residues found at each position in a plurality of similar sequences. Typically, a consensus sequence is determined by sequence alignment in which similar sequences are compared to each other and similar sequence motifs are calculated. In some embodiments, linear dsDNAs are used to generate, e.g., a standard curve for quantification. In some embodiments, the linear dsDNAs are used as spike-in controls for, e.g., monitoring the amplification efficiency.

[0100] Conserved consensus sequences identified were generated and are used as disclosed herein as standards for amplifications (e.g., qPCR assay). Primers and Probes sequences were designed for these sequences (Table 1). Synthetic DNA oligos were generated to use as a Standard (conserved sequence), and a Primer probe mix was generated with desired fluorophores.

[0101] Linear dsDNAs up to about 300 bp in length are disclosed herein, which can be used as, e.g., "spike-in controls" for evaluating, monitoring, observing, and/or tracking an amplification reaction disclosed herein, for example, to monitor amplification efficiency of a reaction. As used herein, the term "amplification efficiency" shall have its ordinary meaning, and can also refer to a determination of the capacity of the amplification reaction to synthesize nucleic acids. Presence of amplification inhibitors, missing or defective amplification reaction components, nuclease contamination, and defective equipment comprise non-limiting examples of causes of reduced amplification efficiency, and can be detected by the methods and

compositions disclosed herein. In some embodiments, the methods and compositions provided herein can be used for quantification purposes, e.g., linear dsDNAs can be used to generate a standard curve for quantification.

[0102] In some embodiments, the primer/probe provided herein are capable of specifically hybridizing (e.g., under standard nucleic acid amplification conditions, *e.g.*, standard PCR conditions, and/or stringent hybridization conditions) to the linear dsDNAs provided herein (Table 1). Examples of oligonucleotides capable of hybridizing to a sense or antisense sequence of SEQ ID NO: 4 include, but are not limited to, SEQ ID NOs: 1-3 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 1-3. Examples of oligonucleotides capable of hybridizing to a sense or antisense sequence of SEQ ID NO: 8 include, but are not limited to, SEQ ID NOs: 5-7 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 5-7. Examples of oligonucleotides capable of hybridizing to a sense or antisense sequence of SEQ ID NO: 12 include, but are not limited to, SEQ ID NOs: 9-11 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 9-11. Examples of oligonucleotides capable of hybridizing to a sense or antisense sequence of SEQ ID NO: 16 include, but are not limited to, SEQ ID NOs: 13-15 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 13-15. Examples of oligonucleotides capable of hybridizing to a sense or antisense sequence of SEQ ID NO: 20 include, but are not limited to, SEQ ID NOs: 17-19 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 17-19. Examples of oligonucleotides capable of hybridizing to a sense or antisense sequence of SEQ ID NO: 24 include, but are not limited to, SEQ ID NOs: 21-23 as provided in Table 1 and sequences that exhibit at least about 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 21-23.

[0103] Table 2 shows an exemplary dilution standard curve for detection of HHV6. Shown are Ct values over a logarithmic dilution series from 10 copies/reaction to 1×10^8 copies of dsDNA standard sequence (Also, *See*, FIG. 1).

TABLE 2: DILUTION CURVE AND EFFICIENCY OF HHV6 ASSAY

DNA Standard	Virus (copies/rxn)	Log concentration	Average Cq	Delta Cq
1	100,000,000	8.00	11.53	-
2	10,000,000	7.00	14.95	3.41
3	1,000,000	6.00	18.47	3.53
4	100,000	5.00	21.99	3.51
5	10,000	4.00	25.63	3.64
6	1,000	3.00	29.22	3.59

7	100	2.00	32.55	3.33
8	10	1.00	35.83	3.28
Efficiency	93% (calculated)		Should be between 90 and 110%	
Slope	-3.4994 (calculated)		Value must be the same as on the graph (*See*, FIG. 1)	
R-squared	0.9998 (calculated)		Should be between 0.99 and 1.00	
Intercept	39.517			
Lf slope	-3.4994			
Then efficiency=	93% (calculated)			

[0104] In some embodiments, the methods disclosed herein can have a Lower Limit of Quantification (LLOQ) (e.g., about 10 copies of an analyte of interest per sample) to a Upper Limit of Quantification (ULOQ) (e.g., about 1×10^6 copies of an analyte of interest per sample). In some embodiments, the methods disclosed herein have a LLOQ of about 10 viral genome copies per samples and a ULOQ of about 1×10^8 copies per sample (Table 3). The analyte can be the virus or the multiple viruses under detection, the internal control dsDNA, and/or a spike in nucleic acid. The LLOQ can vary in different embodiments, for example 10 copies, 20 copies, 30 copies, 40 copies, 50 copies, 100 copies, 150 copies, 200 copies, 300 copies, 400 copies, 500 copies, 750 copies, 1000 copies, 5000 copies, or 10000 copies.

TABLE 3: NON-LIMITING EXEMPLARY LLOQS FOR VIRAL DETECTION ASSAYS

Virus	ULOQ	LLOQ
EBV	1e6 copies	10 copies
HHV7	1e6 copies	10 copies
HHV8	1e6 copies	10 copies
HAV	1e6 copies	10 copies
HHV6	1e8 copies	10 copies
B19	1e8 copies	10 copies

[0105] Disclosed herein include linear double-stranded DNAs (dsDNAs) up to about 300 bp in length (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 128, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 bp in length or a number or a range between any two of these values). In some embodiments the linear dsDNA comprises: the sequence of SEQ ID NO: 4 or a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 4; the sequence of SEQ ID NO: 8 or a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 8; the sequence of SEQ ID NO: 12 or a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of

SEQ ID NO: 12; the sequence of SEQ ID NO: 16 or a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 16; the sequence of SEQ ID NO: 20 or a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 20; or the sequence of SEQ ID NO: 24 or a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 24. Disclosed herein include compositions comprising one or more of the linear dsDNA described herein.

[0106] The nucleic acids provided herein can be in various forms. For example, in some embodiments, the nucleic acids are dissolved (either alone or in combination with various other nucleic acids) in solution, for example buffer. In some embodiments, nucleic acids are provided, either alone or in combination with other isolated nucleic acids, as a salt. In some embodiments, nucleic acids are provided in a lyophilized form that can be reconstituted. For example, in some embodiments, the isolated nucleic acids disclosed herein can be provided in a lyophilized pellet alone, or in a lyophilized pellet with other isolated nucleic acids. In some embodiments, nucleic acids are provided affixed to a solid substance, such as a bead, a membrane, or the like. In some embodiments, nucleic acids are provided in a host cell, for example a cell line carrying a plasmid, or a cell line carrying a stably integrated sequence.

[0107] In some embodiments, the composition, reaction mixture, and kit comprise one or more pairs of amplification primers capable of specifically hybridizing to the sequence of the NS1 gene region of B19, the sequence of the U31 gene region of HHV6, the sequence of the U57 gene region of HHV7, the sequence of the BSLF1 gene region of HHV8, the sequence of the NA1 gene region of EBV, and the sequence of the 5'-UTR region of HAV. In some embodiments, the composition, reaction mixture, and kit comprise one or more probes capable of specifically hybridizing to the sequence of the NS1 gene region of B19, the sequence of the U31 gene region of HHV6, the sequence of the U57 gene region of HHV7, the sequence of the BSLF1 gene region of HHV8, the sequence of the NA1 gene region of EBV, and the sequence of the 5'-UTR region of HAV.

[0108] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the NS1 gene region of B19 virus. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 1-3, or a sequence having at least about 85% identity, at least about 90% identity, at least about 95% identity, at least about 98% identity, or at least about 99% identity to any one of SEQ

ID NOs: 1-3. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 1-3.

[0109] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the U31 gene region of HHV6. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 5-7, or a sequence having at least about 85% identity, at least about 90% identity, at least about 95% identity, at least about 98% identity, or at least about 99% identity to any one of SEQ ID NOs: 5-7. In some embodiments, the probe or primer comprises, or consists of, a sequence selected from SEQ ID NOs: 5-7.

[0110] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the U57 gene region of HHV7. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 9-11, or a sequence having at least about 85% identity, at least about 90% identity, at least about 95% identity, at least about 98% identity, or at least about 99% identity to any one of SEQ ID NOs: 9-11. In some embodiments, the probe or primer comprises, or consists of, a sequence selected from SEQ ID NOs: 9-11.

[0111] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the BSLF1 gene region of HHV8. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 13-15, or a sequence having at least about 85%, at least about 90% identity, at least about 95% identity, at least about 98% identity, or at least about 99% identity to any one of SEQ ID NOs: 13-15. In some embodiments, the probe or primer comprises, or consists of, a sequence selected from SEQ ID NOs: 13-15.

[0112] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the NA1 gene region of EBV. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 17-19, or a sequence having at least about 85% identity, at least about 90% identity, at least about 95% identity, at least about 98% identity, or at least about 99% identity to any one of SEQ ID NOs: 17-19. In some embodiments, the probe or primer comprises, or consists of, a sequence selected from SEQ ID NOs: 17-19.

[0113] Disclosed herein include oligonucleotide probes or primers up to about 100 nucleotides in length capable of hybridizing to the 5'-UTR region of HAV. In some embodiments, the probe or primer comprises, or consists of, the sequence of any one of SEQ ID NOs: 21-23, or a sequence having at least about 85% identity, at least about 90% identity, at least about 95% identity, at least about 98% identity, or at least about 99% identity to any one of

SEQ ID NOs: 21-23. In some embodiments, the probe or primer comprises, or consists of, a sequence selected from the group consisting of SEQ ID NOs: 21-23.

[0114] There are provided, in some embodiments, compositions comprising one or more, or two or more, of the oligonucleotide probes and/or primers disclosed herein. Disclosed herein include compositions comprising two or more of the oligonucleotide probe or primer and one or more of the linear dsDNA described herein.

[0115] Oligonucleotide probes can, in some embodiments, include a detectable moiety. For example, the oligonucleotide probes disclosed herein can comprise a radioactive label. Non-limiting examples of radioactive labels include ^{3}H, ^{14}C, ^{32}P, and ^{35}S. In some embodiments, oligonucleotide probes can include one or more non-radioactive detectable markers or moieties, including but not limited to ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radio-nucleotides. It will become evident to the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe. For example, oligonucleotide probes labeled with one or more dyes, such that upon hybridization to a template nucleic acid, a detectable change in fluorescence is generated. While non-specific dyes may be desirable for some applications, sequence-specific probes can provide more accurate measurements of amplification. One configuration of sequence-specific probe can include one end of the probe tethered to a fluorophore, and the other end of the probe tethered to a quencher. When the probe is unhybridized, it can maintain a stem-loop configuration, in which the fluorophore is quenched by the quencher, thus preventing the fluorophore from fluorescing. When the probe is hybridized to a template nucleic sequence, it is linearized, distancing the fluorophore from the quencher, and thus permitting the fluorophore to fluoresce. Another configuration of sequence-specific probe can include a first probe tethered to a first fluorophore of a FRET pair, and a second probe tethered to a second fluorophore of a FRET pair. The first probe and second probe can be configured to hybridize to sequences of an amplicon that are within sufficient proximity to permit energy transfer by FRET when the first probe and second probe are hybridized to the same amplicon.

[0116] The probe can be, for example, a TaqMan probe. TaqMan probes can comprise a fluorophore and a quencher. The quencher molecule can quench the fluorescence emitted by the fluorophore when excited by the cycler's light source via Förster resonance energy transfer (FRET). As long as the fluorophore and the quencher are in proximity, quenching can inhibit any detectable (e.g., fluorescence) signals. TaqMan probes provided herein can designed such that they anneal within a DNA region amplified by primers provided

herein. Without being bound by any particular theory, in some embodiments, as a PCR polymerase (e.g., Taq) extends the primer and synthesizes a nascent strand on a single-strand template, the 5' to 3' exonuclease activity of the PCR polymerase degrades the probe that has annealed to the template. Degradation of the probe can release the fluorophore from it and break the proximity to the quencher, thereby relieving the quenching effect and allowing fluorescence of the fluorophore. Hence, fluorescence detected in the quantitative PCR thermal cycler can, in some embodiments, be directly proportional to the fluorophore released and the amount of DNA template present in the PCR.

[0117] In some embodiments, the sequence specific probe comprises an oligonucleotide as disclosed herein conjugated to a fluorophore. In some embodiments, the probe is conjugated to two or more fluorophores. Non-limiting examples of fluorophores include: xanthene dyes, e.g., fluorescein and rhodamine dyes, such as fluorescein isothiocyanate (FITC), 2-[ethylamino)-3-(ethylimino)-2-7-dimethyl-3H-xanthen-9-yl]benzoic acid ethyl ester monohydrochloride (R6G)(emits a response radiation in the wavelength that ranges from about 500 to 560 nm), 1,1,3,3,3',3'-Hexamethylindodicarbocyanine iodide (HIDC) (emits a response radiation in the wavelength that ranged from about 600 to 660 nm), 6-carboxyfluorescein (commonly known by the abbreviations FAM and F), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE or J), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA or T), 6-carboxy-X-rhodamine (ROX or R), 5-carboxyrhodamine-6G (R6G5 or G5), 6-carboxyrhodamine-6G (R6G6 or G6), and rhodamine 110; cyanine dyes, e.g. Cy3, Cy5 and Cy7 dyes; coumarins, e.g., umbelliferone; benzimide dyes, e.g. Hoechst 33258; phenanthridine dyes, e.g. Texas Red; ethidium dyes; acridine dyes; carbazole dyes; phenoxazine dyes; porphyrin dyes; polymethine dyes, e.g. cyanine dyes such as Cy3 (emits a response radiation in the wavelength that ranges from about 540 to 580 nm), Cy5 (emits a response radiation in the wavelength that ranges from about 640 to 680 nm), etc; BODIPY dyes and quinoline dyes. Specific fluorophores of interest include: Pyrene, Coumarin, Diethylaminocoumarin, FAM, Fluorescein Chlorotriazinyl, Fluorescein, R110, Eosin, JOE, R6G, HIDC, Tetramethylrhodamine, TAMRA, Lissamine, ROX, Napthofluorescein, Texas Red, Napthofluorescein, Cy3, and Cy5, CAL fluor orange, and the like. Other examples of fluorescein dyes include 6-carboxyfluorescein (6-FAM), 2′,4′,1,4,-tetrachlorofluorescein (TET), 2′,4′,5′,7′,1,4-hexachlorofluorescein (HEX), 2′,7′-dimethoxy-4′,5′-dichloro-6-carboxyrhodamine (JOE), 2′-chloro-5′-fluoro-7′,8′-fused phenyl-1,4-dichloro-6-carboxyfluorescein (NED), and 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC). Probes can comprise SpC6, or functional equivalents and derivatives thereof. Probes can comprise a spacer moiety. A spacer moiety can comprise an alkyl group of at least 2 carbons to

about 12 carbons. A probe can comprise a spacer comprising an abasic unit. A probe can comprise a spacer selected from the group comprising of idSp, iSp9, iS18, iSpC3, iSpC6, iSpC12, or any combination thereof.

[0118] In some embodiments, the probe is conjugated to a quencher (e.g., in addition to a fluorophore). A quencher can absorb electromagnetic radiation and dissipate it as heat, thus remaining dark. Example quenchers include Dabcyl, NFQ's, such as BHQ-1 or BHQ-2 (Biosearch), IOWA BLACK FQ (IDT), and IOWA BLACK RQ (IDT). In some embodiments, the quencher is selected to pair with a fluorophore so as to absorb electromagnetic radiation emitted by the fluorophore. Fluorophore/quencher pairs useful in the compositions and methods disclosed herein are well-known in the art, and can be found, e.g., described in Marras, "Selection of Fluorophore and Quencher Pairs for Fluorescent Nucleic Acid Hybridization Probes" available at www.molecular-beacons.org/download/marras,mmb06%28335%293.pdf. Examples of quencher moieties include, but are not limited to: a dark quencher, a Black Hole Quencher® (BHQ®) (e.g., BHQ-0, BHQ-1, BHQ-2, BHQ-3), a Qxl quencher, an ATTO quencher (e.g., ATTO 540Q, ATTO 580Q, and ATTO 612Q), dimethylaminoazobenzenesulfonic acid (Dabsyl), Iowa Black RQ, Iowa Black FQ, IRDye QC-1, a QSY dye (e.g., QSY 7, QSY 9, QSY 21), AbsoluteQuencher, Eclipse, and metal clusters such as gold nanoparticles, and the like. Examples of an ATTO quencher include, but are not limited to: ATTO 540Q, ATTO 580Q, and ATTO 612Q. Examples of a Black Hole Quencher® (BHQ®) include, but are not limited to: BHQ-0 (493 nm), BHQ-1 (534 nm), BHQ-2 (579 nm) and BHQ-3 (672 nm).

[0119] The detectable label can be a fluorescent label selected from: an Alexa Fluor® dye (e.g., Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 430, Alexa Fluor® 488, Alexa Fluor® 500, Alexa Fluor® 514, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 610, Alexa Fluor® 633, Alexa Fluor® 635, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, Alexa Fluor® 700, Alexa Fluor® 750, Alexa Fluor® 790), an ATTO dye (e.g., ATTO 390, ATTO 425, ATTO 465, ATTO 488, ATTO 495, ATTO 514, ATTO 520, ATTO 532, ATTO Rho6G, ATTO 542, ATTO 550, ATTO 565, ATTO Rho3B, ATTO Rhol 1, ATTO Rhol2, ATTO Thiol 2, ATTO RholOl, ATTO 590, ATTO 594, ATTO Rhol3, ATTO 610, ATTO 620, ATTO Rhol4, ATTO 633, ATTO 647, ATTO 647N, ATTO 655, ATTO Oxal2, ATTO 665, ATTO 680, ATTO 700, ATTO 725, ATTO 740), a DyFight dye, a cyanine dye (e.g., Cy2, Cy3, Cy3.5, Cy3b, Cy5, Cy5.5, Cy7, Cy7.5), a FluoProbes dye, a Sulfo Cy dye, a Seta dye, an IRIS Dye, a SeTau dye, an SRfluor dye, a Square dye, fluorescein (FITC), tetramethylrhodamine (TRITC), Texas Red, Oregon Green, Pacific Blue, Pacific Green, Pacific Orange, a quantum dot, and a tethered fluorescent

protein.

[0120] In some embodiments, a fluorophore is attached to a first end of the probe, and a quencher is attached to a second end of the probe. In some embodiments, a probe can comprise two or more fluorophores. In some embodiments, a probe can comprise two or more quencher moieties. In some embodiments, a probe can comprise one or more quencher moieties and/or one or more fluorophores. A quencher moiety or a fluorophore can be attached to any portion of a probe (e.g., on the 5' end, on the 3' end, in the middle of the probe). Any probe nucleotide can comprise a fluorophore or a quencher moiety, such as, for example, BHQ1dT. Attachment can include covalent bonding, and can optionally include at least one linker molecule positioned between the probe and the fluorophore or quencher. In some embodiments, a fluorophore is attached to a 5' end of a probe, and a quencher is attached to a 3' end of a probe. In some embodiments, a fluorophore is attached to a 3' end of a probe, and a quencher is attached to a 5' end of a probe. Examples of probes that can be used in quantitative nucleic acid amplification include molecular beacons, SCORPION™ probes (Sigma), TAQMAN™ probes (Life Technologies) and the like. Other nucleic acid detection technologies that are useful in the embodiments disclosed herein include, but are not limited to nanoparticle probe technology (*See,* Elghanian, et al. (1997) *Science* 277:1078-1081.) and Amplifluor probe technology (*See,* U.S. Pat. Nos: 5,866,366; 6,090,592; 6,117,635; and 6,117,986).

[0121] Disclosed herein include compositions for detecting B19 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 1 or SEQ ID NO:2. In some embodiments, the pair of primers capable of hybridizing to the NS1 gene region of B19 comprises a primer comprising the sequence of SEQ ID NO: 1 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity or a number or a range between any two of these values to the sequence of SEQ ID NO: 1) and another primer comprising the sequence of SEQ ID NO:2 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity or a number or a range between any two of these values to the sequence of SEQ ID NO: 2). The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 3. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 3.

[0122] Disclosed herein include compositions for detecting HHV6 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, the pair of primers capable of hybridizing to the U31 gene region of HHV6 comprises a primer comprising the sequence of SEQ ID NO: 5 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity or a number or a range between any two of these values to the sequence of SEQ ID NO: 5) and another primer comprising the sequence of SEQ ID NO:6 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity or a number or a range between any two of these values to the sequence of SEQ ID NO: 6). The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 7. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 7.

[0123] Disclosed herein include compositions for detecting HHV7 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10. In some embodiments, the pair of primers capable of hybridizing to the U57 gene region of HHV7 comprises a primer comprising the sequence of SEQ ID NO: 9 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity or a number or a range between any two of these values to the sequence of SEQ ID NO: 9) and another primer comprising the sequence of SEQ ID NO:10 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity or a number or a range between any two of these values to the sequence of SEQ ID NO: 10). The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 11. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 11

[0124] Disclosed herein include compositions for detecting HHV8 in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14. In some embodiments, the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 comprises a primer comprising the sequence of SEQ ID NO: 13 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity, or a number or a range between any two of these values to the sequence of SEQ ID NO: 13) and another primer comprising the sequence of SEQ ID NO:14 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity, or a number or a range between any two of these values to the sequence of SEQ ID NO: 14). The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 15. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 15.

[0125] Disclosed herein include compositions for detecting EBV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18. In some embodiments, the pair of primers capable of hybridizing to the NA1 gene region of EBV comprises a primer comprising the sequence of SEQ ID NO: 17 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity, or a number or a range between any two of these values to the sequence of SEQ ID NO: 17) and another primer comprising the sequence of SEQ ID NO:18 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity, or a number or a range between any two of these values to the sequence of SEQ ID NO: 18). The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 19. In some embodiments, the oligonucleotide probe comprises the sequence of

SEQ ID NO: 19.

[0126] Disclosed herein include compositions for detecting HAV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22. In some embodiments, the pair of primers capable of hybridizing to the 5'-UTR region of HAV comprises a primer comprising the sequence of SEQ ID NO: 21 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity, or a number or a range between any two of these values to the sequence of SEQ ID NO: 21) and another primer comprising the sequence of SEQ ID NO:22 (or a sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100% identity, or a number or a range between any two of these values to the sequence of SEQ ID NO: 22). The composition can comprise an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 23. In some embodiments, the oligonucleotide probe comprises the sequence of SEQ ID NO: 23.

[0127] Provided herein are compositions comprising two or more primers and/or probes (e.g., for multiplexed reactions). Disclosed herein include compositions for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. In some embodiments, the composition comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; a pair of

primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

[0128] The pair of primers capable of hybridizing to the NS1 gene region of B19 can comprise (a) a primer comprising the sequence of SEQ ID NO: 1 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 1, and (b) a primer comprising the sequence of SEQ ID NO: 2 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 2; the pair of primers capable of hybridizing to the U31 gene region of HHV6 can comprise (a) a primer comprising the sequence of SEQ ID NO: 5 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 5, and (b) a primer comprising the sequence of SEQ ID NO: 6 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 6; the pair of primers capable of hybridizing to the U57 gene region of HHV7 can comprise (a) a primer comprising the sequence of SEQ ID NO: 9 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 9, and (b) a primer comprising the sequence of SEQ ID NO: 10 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 10; the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 can comprise (a) a primer comprising the sequence of SEQ ID NO: 13 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 13, and (b) a primer comprising the sequence of SEQ ID NO: 14 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 14; the pair of primers capable of hybridizing to the NA1 gene region of EBV can comprise (a) a primer comprising the sequence of SEQ ID NO: 17 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 17, and (b) a primer

comprising the sequence of SEQ ID NO: 18 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 18; and the pair of primers capable of hybridizing to the 5'-UTR region of HAV can comprise (a) a primer comprising the sequence of SEQ ID NO: 21 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 21, and (b) a primer comprising the sequence of SEQ ID NO: 22 or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 22.

[0129] The composition can comprise one or more oligonucleotide probes each comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23. Each of the one or more oligonucleotide probes can comprise the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23, or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 3, 7, 11, 15, 19, or 23. In some embodiments, each of the one or more oligonucleotide probes consists of a sequence selected from SEQ ID NO: 3, 7, 11, 15, 19, or 23. At least one of the one or more oligonucleotide probes can comprise a fluorescence emitter moiety, a fluorescence quencher moiety, or both.

[0130] The composition can comprise one or more linear dsDNAs each comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24. Each of the one or more linear dsDNAs can comprise the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24, or a sequence differing by one, two, three, four, five, six, or more nucleotide mismatches relative to SEQ ID NO: 4, 8, 12, 16, 20, or 24. In some embodiments, each of the one or more dsDNAs consists of a sequence selected from SEQ ID NO: 4, 8, 12, 16, 20, or 24.

Samples

[0131] The methods and compositions disclosed herein are suitable for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and/or HAV in a wide variety of samples. As used herein, a "sample" can refer to any type of material of biological origin. The sample can comprise, for example, fluids, tissues or cells. The sample can comprise a biological material taken directly from a subject, or cultured cells or tissues, or any fraction or products produced from or derived from biological materials. A sample can be purified, partially purified, unpurified, enriched, or amplified. In some embodiments, the sample comprises genetically modified cells. In some embodiments, the sample is or comprises a therapeutical or

pharmaceutical composition.

[0132] The sample can be a biological sample, for example a clinical sample. In some embodiments, the sample is taken from a biological source, such as vagina, urethra, penis, anus, throat, cervix, fermentation broths, cell cultures, and the like. The biological sample can be used (i) directly as obtained from the subject or source, or (ii) following a pre-treatment to modify the character of the sample. Thus, the test sample can be pre-treated prior to use, for example, by disrupting cells or viral particles, preparing liquids from solid materials, diluting viscous fluids, filtering liquids, concentrating liquids, inactivating interfering components, adding reagents, purifying nucleic acids, and the like. Accordingly, a "biological sample" as used herein includes nucleic acids (DNA, RNA or total nucleic acids) extracted from a clinical or biological specimen. Sample preparation can also include using a solution that contains buffers, salts, detergents, and/or the like which are used to prepare the sample for analysis. In some embodiments, the sample is processed before molecular testing. In some embodiments, the sample is analyzed directly, and is not pre-processed prior to testing.

[0133] In some embodiments, a sample to be tested is processed prior to performing the methods disclosed herein. For example, in some embodiments, the sample can be isolated, concentrated, or subjected to various other processing steps prior to performing the methods disclosed herein. For example, in some embodiments, the sample can be processed to isolate nucleic acids from the sample prior to contacting the sample with the oligonucleotides, as disclosed herein. In some embodiments, the methods disclosed herein are performed on the sample without culturing the sample *in vitro*. In some embodiments, the methods disclosed herein are performed on the sample without isolating nucleic acids from the sample prior to contacting the sample with oligonucleotides as disclosed herein.

[0134] A sample can comprise one or more nucleic acids (e.g., a plurality of nucleic acids). The term "plurality" as used herein can refer two or more. Thus, in some embodiments, a sample includes two or more (e.g., 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more, 500 or more, 1,000 or more, or 5,000 or more) nucleic acids (e.g., gDNA, RNA). A disclosed method can be used as a very sensitive way to detect a target nucleic acid (e.g., the NS1 gene region of B19) present in a sample (e.g., in a complex mixture of nucleic acids such as gDNAs). In some embodiments, the sample includes 5 or more nucleic acids (e.g., 10 or more, 20 or more, 50 or more, 100 or more, 500 or more, 1,000 or more, or 5,000 or more nucleic acids) that differ from one another in sequence. In some embodiments, the sample includes 10 or more, 20 or more, 50 or more, 100 or more, 500 or more, 10^3 or more, 5×10^3 or more, 10^4 or more, 5×10^4 or more, 10^5 or more, 5×10^5 or more, 10^6 or more 5×10^6 or more, or 10^7 or more, nucleic acids.

[0135] In some embodiments, the sample comprises from 10 to 20, from 20 to 50, from 50 to 100, from 100 to 500, from 500 to 10^3, from 10^3 to 5 x 10^3, from 5 x 10^3 to 10^4, from 10^4 to 5 x 10^4, from 5 x 10^4 to 10^5, from 10^5 to 5 x 10^5, from 5 x 10^5 to 10^6, from 10^6 to 5 x 10^6, or from 5 x 10^6 to 10^7, or more than 10^7, copies of target nucleic acids. In some embodiments, the sample comprises from 5 to 10^7 nucleic acids (e.g., that differ from one another in sequence)(e.g., from 5 to 10^6, from 5 to 10^5, from 5 to 50,000, from 5 to 30,000, from 10 to 10^6, from 10 to 10^5, from 10 to 50,000, from 10 to 30,000, from 20 to 10^6, from 20 to 10^5, from 20 to 50,000, or from 20 to 30,000 nucleic acids, or a number or a range between any two of these values). In some embodiments, the sample includes 20 or more nucleic acids that differ from one another in sequence.

[0136] The sample can be derived from any source, e.g., the sample can be a synthetic combination of purified nucleic acids; the sample can be a cell lysate, an DNA-enriched cell lysate, or nucleic acids isolated and/or purified from a cell lysate. The sample can be from a patient (e.g., for the purpose of diagnosis). The sample can be from permeabilized cells. The sample can be from crosslinked cells. The sample can be in tissue sections. The sample can be from tissues prepared by crosslinking followed by delipidation and adjustment to make a uniform refractive index.

[0137] A sample can include a target nucleic acid (e.g., the NS1 gene region of B19) and a plurality of non-target nucleic acids. In some embodiments, the target nucleic acid is present in the sample at one copy per 10 non-target nucleic acids, one copy per 20 non-target nucleic acids, one copy per 25 non-target nucleic acids, one copy per 50 non-target nucleic acids, one copy per 100 non-target nucleic acids, one copy per 500 non-target nucleic acids, one copy per 10^3 non-target nucleic acids, one copy per 5 x 10^3 non-target nucleic acids, one copy per 10^4 non-target nucleic acids, one copy per 5 x 10^4 non-target nucleic acids, one copy per 10^5 non-target nucleic acids, one copy per 5 x 10^5 non-target nucleic acids, one copy per 10^6 non-target nucleic acids, less than one copy per 10^6 non-target nucleic acids, or a number or a range between any two of these values. In some embodiments, the target nucleic acid is present in the sample at from one copy per 10 non-target nucleic acids to 1 copy per 20 non-target nucleic acids, from 1 copy per 20 non-target nucleic acids to 1 copy per 50 non target nucleic acids, from 1 copy per 50 non-target nucleic acids to 1 copy per 100 non-target nucleic acids, from 1 copy per 100 non-target nucleic acids to 1 copy per 500 non-target nucleic acids, from 1 copy per 500 non target nucleic acids to 1 copy per 10^3 non-target nucleic acids, from 1 copy per 10^3 non-target nucleic acids to 1 copy per 5 x 10^3 non-target nucleic acids, from 1 copy per 5 x 10^3 non-target nucleic acids to 1 copy per 10^4 non target nucleic acids, from 1 copy per 10^4 non-target nucleic acids to 1 copy per 10^5 non-target nucleic acids, from 1 copy per 10^5 non-

target nucleic acids to 1 copy per 10^6 non-target nucleic acids, or from 1 copy per 10^6 non target nucleic acids to 1 copy per 10^7 non-target nucleic acids, or a number or a range between any two of these values.

[0138] Suitable samples include but are not limited to saliva, blood, serum, plasma, urine, aspirate, cerebral spinal fluid (CSF) and biopsy samples. Thus, the term "sample" with respect to a patient encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents; washed; or enrichment for certain cell populations, such as cancer cells. Cells can be genetically modified (e.g., by a CRISPR-Cas9 gene editing system). The sample can be a sample that have been enriched for particular types of molecules, e.g., nucleic acids.

[0139] A sample for use in the methods disclosed herein can include any conventional biological sample obtained from an organism or a part thereof, such as animal. In particular embodiments, the biological sample is obtained from an animal subject, such as a human subject. A sample can also be a sample obtained from any organ or tissue (including a biopsy or autopsy specimen, such as a tumor biopsy) or can include a cell (whether a primary cell or cultured cell) or medium conditioned by any cell, tissue or organ. Exemplary samples include, without limitation, cells, cell lysates, blood smears, cytocentrifuge preparations, cytology smears, bodily fluids (e.g., blood, plasma, serum, saliva, sputum, urine, bronchoalveolar lavage, semen, or any combination thereof), tissue biopsies (e.g., tumor biopsies), fine-needle aspirates, and/or tissue sections (e.g., cryostat tissue sections and/or paraffin-embedded tissue sections). In other examples, the sample includes circulating tumor cells (which can be identified by cell surface markers). In particular examples, samples are used directly (e.g., fresh or frozen), or can be manipulated prior to use, for example, by fixation (e.g., using formalin) and/or embedding in wax (such as formalin-fixed paraffin-embedded (FFPE) tissue samples).

[0140] In some embodiments, a sample is, or comprises, an environmental sample, such as water, soil, or a surface such as industrial or medical surface.

[0141] Owing to the increased sensitivity of the embodiments disclosed herein, in certain example embodiments, the assays and methods may be run on crude samples or samples where the target molecules to be detected are not further fractionated or purified from the sample.

[0142] Cell lysis procedures and reagents are known in the art and may generally be performed by chemical (e.g., detergent, hypotonic solutions, enzymatic procedures, and the like,

or combination thereof), physical (e.g., French press, sonication, and the like), or electrolytic lysis methods. Any suitable lysis procedure can be utilized. For example, chemical methods generally employ lysing agents to disrupt cells and extract nucleic acids from the cells, followed by treatment with chaotropic salts. In some embodiments, cell lysis comprises use of detergents (e.g., ionic, nonionic, anionic, zwitterionic). In some embodiments, cell lysis comprises use of ionic detergents (e.g., SDS, sodium lauryl sulfate (SLS), deoxycholate, cholate, sarkosyl). Physical methods such as freeze/thaw followed by grinding, the use of cell presses and the like also may be useful. High salt lysis procedures also may be used. For example, an alkaline lysis procedure may be utilized. The latter procedure traditionally incorporates the use of phenol-chloroform solutions, and an alternative phenol-chloroform-free procedure involving three solutions may be utilized.

[0143] In typical sample extractions, cells are lysed by mechanical shearing with glass beads as described in US Patent No. 7,494,771, incorporated by reference in its entirety herein, to lyse the target organisms. As disclosed in WO2003/008636, such a generic method of cell lysis is efficient for a wide variety of target organisms and specimen matrices. There are also other less universal lysis methods that are designed specifically to target a certain species or group of organisms, or which exploit specific enzymatic or chemical activities. For example, ACP enzyme is commonly used to lyse of Gram-positive organisms.

Nucleic acid testing

[0144] The methods described herein can include, for example, nucleic acid testing. For example, the test can include testing for target nucleic acid sequences in a sample. Various forms of nucleic acid testing can be used in the embodiments disclosed herein, including but not limited to, testing that involves nucleic acid amplification. A target nucleic acid (e.g., viral DNA, RNA) can be single-stranded or double-stranded. The source of the target nucleic acid can be any source (e.g., any sample). In some embodiments, the target nucleic acid is a viral nucleic acid (e.g., DNA or RNA). As such, the compositions and methods provided herein can be employed for detecting the presence of a viral nucleic acid amongst a population of nucleic acids (e.g., in a sample).

[0145] Provided herein are compositions and methods for detecting a target nucleic acid (e.g., the NS1 gene region of B19, the U31 gene region of HHV6, the U57 gene region of HHV7, the BSLF1 gene region of HHV8, the NA1 gene region of EBV, or the 5'-UTR region of HAV) in a sample that can detect said target nucleic acid with a high degree of sensitivity. In some embodiments, the compositions and methods provided herein can be used to detect a target nucleic acid present in a sample comprising a plurality of nucleic acids (including the target

nucleic acid and a plurality of non-target nucleic acids), wherein the target nucleic acid is present at one or more copies per 10^7 non-target nucleic acids (e.g., one or more copies per 10^6 non-target nucleic acids, one or more copies per 10^5 non-target nucleic acids, one or more copies per 10^4 non-target nucleic acids, one or more copies per 10^3 non-target nucleic acids, one or more copies per 10^2 non-target nucleic acids, one or more copies per 50 non-target nucleic acids, one or more copies per 20 non-target nucleic acids, one or more copies per 10 non-target nucleic acids, one or more copies per 5 non-target nucleic acids, or any range between two of these values). In some embodiments, the disclosed methods can be used to detect a target nucleic acid present in a sample comprising a plurality of nucleic acids (including the target nucleic acid and a plurality of non-target nucleic acids), wherein the target nucleic acid is present at one or more copies per 10^{18} non-target nucleic acids, per 10^{15} non-target nucleic acids, per 10^{12} non-target nucleic acids, per 10^9 non-target nucleic acids, per 10^6 non-target nucleic acids, per 10^5 non-target nucleic acids, per 10^4 non-target nucleic acids, per 10^3 non-target nucleic acids, per 10^2 non-target nucleic acids, per 50 non-target nucleic acids, per 20 non-target nucleic acids, per 10 non-target nucleic acids, or per 5 non-target nucleic acids, or a number or a range between any two of these values.

[0146] The concentration at which a target nucleic acid can be detected comprises a range from about 10 copies of target nucleic acid sequence per sample to about 1×10^8 copies of target nucleic acid sequence per sample (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 copies per sample, or a number or a range between any two of these values).

[0147] As used herein, nucleic acid amplification can refer to any known procedure for obtaining multiple copies of a target nucleic acid sequence or its complement or fragments thereof, using sequence-specific methods. Examples of known amplification methods include, but are not limited to, polymerase chain reaction (PCR), ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA) (e.g., multiple displacement amplification (MDA)), replicase-mediated amplification, immuno-amplification, nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), rolling circle amplification, and transcription-mediated amplification (TMA). In some embodiments, e.g., where a target nucleic acid is RNA, a DNA copy (cDNA) of the target RNA is synthesized by reverse transcription prior to the amplification step. In some embodiments, two or more of the aforementioned nucleic acid amplification methods can be performed, for example sequentially.

[0148] For example, LCR amplification uses at least four separate oligonucleotides to amplify a target and its complementary strand by using multiple cycles of hybridization, ligation, and denaturation. SDA amplifies by using a primer that contains a recognition site for

a restriction endonuclease which nicks one strand of a hemimodified DNA duplex that includes the target sequence, followed by amplification in a series of primer extension and strand displacement steps.

[0149] PCR is a method well-known in the art for amplification of nucleic acids. PCR involves amplification of a target sequence using two or more extendable sequence-specific oligonucleotide primers that flank the target sequence. The nucleic acid containing the target sequence of interest is subjected to a program of multiple rounds of thermal cycling (denaturation, annealing and extension) in the presence of the primers, a thermostable DNA polymerase (e.g., Taq polymerase) and various dNTPs, resulting in amplification of the target sequence. PCR uses multiple rounds of primer extension reactions in which complementary strands of a defined region of a DNA molecule are simultaneously synthesized by a thermostable DNA polymerase. At the end of each cycle, each newly synthesized DNA molecule acts as a template for the next cycle. During repeated rounds of these reactions, the number of newly synthesized DNA strands increases exponentially such that after 20 to 30 reaction cycles, the initial template DNA will have been replicated several thousand-fold or million-fold. PCR can generate double-stranded amplification products suitable for post-amplification processing. If desired, amplification products can be detected by visualization with agarose gel electrophoresis, by an enzyme immunoassay format using probe-based colorimetric detection, by fluorescence emission technology, or by other detection means known to one of skill in the art.

[0150] A wide variety of PCR methods are known in the art. Examples of PCR method include, but not limited to, Real-Time PCR, End-Point PCR, Amplified fragment length polymorphism PCR (AFLP-PCR), Alu-PCR, Asymmetric PCR, Colony PCR, DD-PCR, Degenerate PCR, Hot-start PCR, In situ PCR, Inverse PCR Long-PCR, Multiplex PCR, Nested PCR, PCR-ELISA, PCR-RFLP, PCR-single strand conformation polymorphism (PCR-SSCP), quantitative competitive PCR (QC-PCR), rapid amplification of cDNA ends-PCR (RACE-PCR), Random Amplification of Polymorphic DNA-PCR (RAPD-PCR), Real-Time PCR, Repetitive extragenic palindromic-PCR (Rep-PCR), reverse transcriptase PCR (RT-PCR), TAIL-PCR, Touchdown PCR and Vectorette PCR.

[0151] Real-time PCR, such as quantitative real time PCR (qPCR), can be used to simultaneously quantify and amplify a specific part of a given nucleic acid molecule. It can be used to determine whether a specific sequence is present in the sample; and if it is present, the number of copies of the sequence that are present. The term "real-time" can refer to periodic monitoring during PCR. Certain systems such as the ABI 7700 and 7900HT Sequence Detection Systems (Applied Biosystems, Foster City, Calif.) conduct monitoring during each

thermal cycle at a pre-determined or user-defined point. Real-time analysis of PCR with fluorescence resonance energy transfer (FRET) probes measures fluorescent dye signal changes from cycle-to-cycle, preferably minus any internal control signals. The real-time procedure follows the general pattern of PCR, but the nucleic acid is quantified after each round of amplification. Two examples of methods of quantification are the use of fluorescent dyes (e.g., SYBRGreen) that intercalate into double-stranded DNA, and modified DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA. Intercalating agents have a relatively low fluorescence when unbound, and a relatively high fluorescence upon binding to double-stranded nucleic acids. As such, intercalating agents can be used to monitor the accumulation of double strained nucleic acids during a nucleic acid amplification reaction. Examples of such non-specific dyes useful in the embodiments disclosed herein include intercalating agents such as SYBR Green I (Molecular Probes), propidium iodide, ethidium bromide, and the like.

[0152] Samples can be infected and/or contaminated with multiple organisms. The disclosed primers and probes are tolerant to mixed infections of a sample. Because of the specific target sequences, primers and probes, the methods and compositions disclosed herein can be used to detect the presence/absence or level of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample with high sensitivity, specificity and accuracy.

[0153] The primers disclosed herein can be paired with additional PCR systems using a uniform chemistry and thermal PCR profile to provide a panel of assays for the detection of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV to improve overall assay sensitivity and robustness.

[0154] In some embodiments, multiplex PCR is performed to amplify and detect, *e.g.*, by direct or indirect means, the presence or absence of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV to allow identification and determination of, e.g., viral contamination of a sample in one test. In some embodiments of the multiplex PCR, the presence or absence of B19 can be determined by amplifying and detecting the presence or absence of the NS1 gene; the presence or absence of HHV6 can be determined by amplifying and detecting the presence or absence of the U31 gene; the presence or absence of HHV7 can be determined by amplifying and detecting the presence or absence of the U57 gene; the presence or absence of HHV8 can be determined by amplifying and detecting the presence or absence of the BSLF1 gene; the presence or absence of EBV can be determined by amplifying and detecting the presence or absence of the NA1 gene; and the presence or absence of HAV can be determined by amplifying and detecting the presence or absence of the 5'-UTR region.

[0155] Accordingly, some embodiments for the detection and/or identification of

B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample include the steps of providing a test sample; and contacting the sample with oligonucleotide primers that can specifically hybridize and amplify (1) the NS1 gene region of B19; (2) the U31 gene region of HHV6; (3) the U57 gene region of HHV7; (4) the BSLF1 gene region of HHV8; (5) the NA1 gene region of EBV; and/or (6) the 5'-UTR region of HAV, and oligonucleotide probes that can specifically hybridize to (1) the NS1 gene region of B19; (2) the U31 gene region of HHV6; (3) the U57 gene region of HHV7; (4) the BSLF1 gene region of HHV8; (5) the NA1 gene region of EBV; and/or (6) the 5'-UTR region of HAV under standard nucleic acid amplification conditions and/or stringent hybridization conditions. As described herein, the sample can be contacted with all of the primers and probes at once, or can be contacted with some of the primers and probes first and subsequently contacted by the remainder of the primers and probes. In some embodiments, the sample is contacted with one primer/probe set.

[0156] The oligonucleotide probe can be, for example, between about 10 and about 45 nucleotides in length, and comprises a detectable moiety (e.g., a signal moiety, a detectable label). In some embodiments, the contacting is performed under conditions allowing for the specific hybridization of the primers to the corresponding targeted gene region if the target organism is present in the sample. The presence and/or amount of probe that is specifically bound to the corresponding targeted gene region (if present in the sample being tested) can be determined, wherein bound probe is indicative of the presence of the corresponding target organism in the sample. In some embodiments, the amount of bound probe is used to determine the amount of the corresponding target organism in the sample.

[0157] The determining step can be achieved using any methods known to those skilled in the art, including but not limited to, *in situ* hybridization, following the contacting step. The detection of hybrid duplexes (*i.e.*, of a probe specifically bound to the targeted gene region) can be carried out by a number of methods. Typically, hybridization duplexes are separated from unhybridized nucleic acids and the labels bound to the duplexes are then detected. Such labels refer to radioactive, fluorescent, biological or enzymatic tags or labels of standard use in the art. A label can be conjugated to either the oligonucleotide probes or the nucleic acids derived from the biological sample. Those of skill in the art will appreciate that wash steps may be employed to wash away excess sample/target nucleic acids or oligonucleotide probes (as well as unbound conjugate, where applicable). Further, standard heterogeneous assay formats are suitable for detecting the hybrids using the labels present on the oligonucleotide primers and probes. Determining the presence or amount of one or more amplicons can comprise contacting said amplicons with a plurality of oligonucleotide probes. At least one of the plurality of oligonucleotide probes comprises a fluorescence emitter moiety and

a fluorescence quencher moiety. In some embodiments, determining the presence or amount of one or more amplicons comprises measuring a detectable signal, such as, for example, a detectable signal from a probe.

[0158] In some embodiments, determining the presence or amount of one or more amplicons comprises measuring a detectable signal, such as, for example, a detectable signal from a probe (e.g., after cleavage of the probe by the 5′–3′ exonuclease activity of a PCR polymerase (e.g., Taq)). The measuring can in some embodiments be quantitative, e.g., in the sense that the amount of signal detected can be used to determine the amount of target nucleic acid (e.g., the NA1 gene region of EBV) present in the sample. The measuring can in some embodiments be qualitative, e.g., in the sense that the presence or absence of detectable signal can indicate the presence or absence of targeted nucleic acid (e.g., virus). In some embodiments, a detectable signal will not be present (e.g., above a given threshold level) unless the targeted nucleic acid (e.g., virus) is present above a particular threshold concentration. In some embodiments, a disclosed method can be used to determine the amount of a target nucleic acid (e.g., the NA1 gene region of EBV) in a sample (e.g., a sample comprising the target nucleic acid and a plurality of non-target nucleic acids). Determining the amount of a target nucleic acid in a sample can comprise comparing the amount of detectable signal generated from a test sample to the amount of detectable signal generated from a reference sample. Determining the amount of a target nucleic acid in a sample can comprise: measuring the detectable signal to generate a test measurement; measuring a detectable signal produced by a reference sample to generate a reference measurement; and comparing the test measurement to the reference measurement to determine an amount of target nucleic acid present in the sample. The reference standard curve can be generated based on serial dilutions of the linear dsDNA provided herein. The serial dilutions of the linear dsDNA can comprise a logarithmic dilution series from about 10 copies of the linear dsDNA to about 1×10^8 copies of the linear dsDNA in reference samples (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 copies per sample, or a number or a range between any two of these values). Determining the amount of a target nucleic acid in a sample can be used to derive the presence and/or amount of an organism comprising said target nucleic acid in a sample.

[0159] The detectable signal can be produced by the fluorescence-emitting dye pair of a probe. For example, in some embodiments, a disclosed method includes contacting amplicons with a probe comprising a FRET pair or a quencher/fluor pair, or both. In some embodiments, a disclosed method includes contacting amplicons with a probe comprising a FRET pair. In some embodiments, a disclosed method includes contacting amplicons with a probe comprising a fluor/quencher pair.

[0160] Fluorescence-emitting dye pairs comprise a FRET pair or a quencher/fluor pair. In both embodiments of a FRET pair and a quencher/fluor pair, the emission spectrum of one of the dyes overlaps a region of the absorption spectrum of the other dye in the pair. As used herein, the term "fluorescence-emitting dye pair" is a generic term used to encompass both a "fluorescence resonance energy transfer (FRET) pair" and a "quencher/fluor pair," both of which terms are discussed in more detail below. The term "fluorescence-emitting dye pair" is used interchangeably with the phrase "a FRET pair and/or a quencher/fluor pair."

[0161] In some embodiments (e.g., when the probe includes a FRET pair) the probe produces an amount of detectable signal prior to being cleaved, and the amount of detectable signal that is measured is reduced when the probe is cleaved. In some embodiments, the probe produces a first detectable signal prior to being cleaved (e.g., from a FRET pair) and a second detectable signal when the probe is cleaved (e.g., from a quencher/fluor pair). As such, in some embodiments, the probe comprises a FRET pair and a quencher/fluor pair.

[0162] In some embodiments, the probe comprises a FRET pair. FRET is a process by which radiationless transfer of energy occurs from an excited state fluorophore to a second chromophore in close proximity. The range over which the energy transfer can take place is limited to approximately 10 nanometers (100 angstroms), and the efficiency of transfer is extremely sensitive to the separation distance between fluorophores. Thus, as used herein, the term "FRET" ("fluorescence resonance energy transfer"; also known as "Forster resonance energy transfer") can refer to a physical phenomenon involving a donor fluorophore and a matching acceptor fluorophore selected so that the emission spectrum of the donor overlaps the excitation spectrum of the acceptor, and further selected so that when donor and acceptor are in close proximity (usually 10 nm or less) to one another, excitation of the donor will cause excitation of and emission from the acceptor, as some of the energy passes from donor to acceptor via a quantum coupling effect. Thus, a FRET signal serves as a proximity gauge of the donor and acceptor; only when they are in close proximity to one another is a signal generated. The FRET donor moiety (e.g., donor fluorophore) and FRET acceptor moiety (e.g., acceptor fluorophore) are collectively referred to herein as a "FRET pair".

[0163] The donor-acceptor pair (a FRET donor moiety and a FRET acceptor moiety) is referred to herein as a "FRET pair" or a "signal FRET pair." Thus, in some embodiments, a probe includes two signal partners (a signal pair), when one signal partner is a FRET donor moiety and the other signal partner is a FRET acceptor moiety. A probe that includes such a FRET pair (a FRET donor moiety and a FRET acceptor moiety) will thus exhibit a detectable signal (a FRET signal) when the signal partners are in close proximity (e.g., while on the same RNA molecule), but the signal will be reduced (or absent) when the partners are separated (e.g.,

after cleavage of the probe by the 5′–3′ exonuclease activity of a PCR polymerase (e.g., Taq)). FRET donor and acceptor moieties (FRET pairs) will be known to one of ordinary skill in the art and any convenient FRET pair (e.g., any convenient donor and acceptor moiety pair) can be used.

[0164] In some embodiments, one signal partner of a signal quenching pair produces a detectable signal and the other signal partner is a quencher moiety that quenches the detectable signal of the first signal partner (e.g., the quencher moiety quenches the signal of the signal moiety such that the signal from the signal moiety is reduced (quenched) when the signal partners are in proximity to one another, e.g., when the signal partners of the signal pair are in close proximity).

[0165] For example, in some embodiments, an amount of detectable signal increases when the probe is cleaved. For example, in some embodiments, the signal exhibited by one signal partner (a signal moiety, a fluorescence emitter moiety) is quenched by the other signal partner (a quencher signal moiety, a fluorescence quencher moiety), e.g., when both are present on the same ssDNA molecule prior to cleavage by the 5′–3′ exonuclease activity of a PCR polymerase (e.g., Taq). Such a signal pair is referred to herein as a "quencher/fluor pair", "quenching pair", or "signal quenching pair." For example, in some embodiments, one signal partner (e.g., the first signal partner) is a signal moiety that produces a detectable signal that is quenched by the second signal partner (e.g., a quencher moiety). The signal partners of such a quencher/fluor pair will thus produce a detectable signal when the partners are separated (e.g., after cleavage of the probe by the 5′–3′ exonuclease activity of a PCR polymerase (e.g., Taq)), but the signal will be quenched when the partners are in close proximity (e.g., prior to cleavage of the probe by the 5′–3′ exonuclease activity of a PCR polymerase (e.g., Taq)).

[0166] A quencher moiety can quench a signal from the signal moiety (e.g., prior to cleavage of the probe by the 5′–3′ exonuclease activity of a PCR polymerase (e.g., Taq)) to various degrees. In some embodiments, a quencher moiety quenches the signal from the signal moiety where the signal detected in the presence of the quencher moiety (when the signal partners are in proximity to one another) is 95% or less of the signal detected in the absence of the quencher moiety (when the signal partners are separated). For example, in some embodiments, the signal detected in the presence of the quencher moiety can be 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, 40% or less, 30% or less, 20% or less, 15% or less, 10% or less, or 5% or less of the signal detected in the absence of the quencher moiety. In some embodiments, no signal (e.g., above background) is detected in the presence of the quencher moiety.

[0167] In some embodiments, the signal detected in the absence of the quencher

moiety (when the signal partners are separated) is at least 1.2 fold greater (e.g., at least 1.3fold, at least 1.5 fold, at least 1.7 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 3.5 fold, at least 4 fold, at least 5 fold, at least 7 fold, at least 10 fold, at least 20 fold, or at least 50 fold greater, or a number or a range between any two of these values) than the signal detected in the presence of the quencher moiety (when the signal partners are in proximity to one another).

[0168] In some embodiments, the signal moiety is a fluorescent label. In some such embodiments, the quencher moiety quenches the signal (e.g., the light signal) from the fluorescent label (e.g., by absorbing energy in the emission spectra of the label). Thus, when the quencher moiety is not in proximity with the signal moiety, the emission (the signal) from the fluorescent label can be detectable because the signal is not absorbed by the quencher moiety. Any convenient donor acceptor pair (signal moiety /quencher moiety pair) can be used and many suitable pairs are known in the art.

[0169] In some embodiments, the quencher moiety absorbs energy from the signal moiety (also referred to herein as a "detectable label" or a "detectable moiety") and then emits a signal (e.g., light at a different wavelength). Thus, in some embodiments, the quencher moiety is itself a signal moiety (e.g., a signal moiety can be 6- carboxyfluorescein while the quencher moiety can be 6-carboxy-tetramethylrhodamine), and in some such embodiments, the pair can also be a FRET pair. In some embodiments, a quencher moiety is a dark quencher. A dark quencher can absorb excitation energy and dissipate the energy in a different way (e.g., as heat). Thus, a dark quencher has minimal to no fluorescence of its own (does not emit fluorescence).

[0170] In some embodiments, cleavage of a probe can be detected by measuring a colorimetric read-out. For example, the liberation of a fluorophore (e.g., liberation from a FRET pair, liberation from a quencher/fluor pair, and the like) can result in a wavelength shift (and thus color shift) of a detectable signal. Thus, in some embodiments, cleavage of a probe can be detected by a color-shift. Such a shift can be expressed as a loss of an amount of signal of one color (wavelength), a gain in the amount of another color, a change in the ration of one color to another, and the like.

[0171] Disclosed herein include methods and compositions for real-time PCR capable of detecting one or more of 6 gene targets, which enables detection of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample.

[0172] Disclosed herein include methods for detecting B19 in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of B19 genome, wherein the target region comprises the NS1 gene region of B19, and wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; (b) generating amplicons of the target region of B19 from the sample, if the sample comprises B19; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of B19 in the sample.

[0173] A primer in the pair of primers can comprise the sequence of SEQ ID NO: 1 or SEQ ID NO: 2, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1 or SEQ ID NO: 2. Determining the presence or amount of the amplicons of the target region can comprise contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 3. The oligonucleotide probe can comprise the sequence of SEQ ID NO: 3, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3. The sample can comprise a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 4 to the sample. The linear dsDNA can comprise the sequence of SEQ ID NO: 4, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 4. In some embodiments, the linear dsDNA is present in the sample in an amount of about 10 copies, about 20 copies, about 30 copies, about 40 copies, about 50 copies, about 60 copies, about 70 copies, about 80 copies, about 90 copies, or about 100 copies. The sample can comprise at least 10 copies of B19 genome. The sample can comprise no more than 1×10^8 copies of B19 genome (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 copies per sample, or a number or a range between any two of these values).

[0174] Disclosed herein include methods for detecting HHV6 in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV6 genome, wherein the target region comprises the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; (b) generating amplicons of the target region of HHV6 from the sample, if the sample comprises HHV6; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV6 in the sample.

[0175] A primer in the pair of primers can comprise the sequence of SEQ ID NO: 5 or SEQ ID NO: 6, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5 or SEQ ID NO: 6. Determining the presence or amount of the amplicons of the target region can comprise contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 7. The oligonucleotide probe can comprise the sequence of SEQ ID NO: 7, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 7. The sample can comprise a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 8. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 8 to the sample. The linear dsDNA can comprise the sequence of SEQ ID NO: 8, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 8. In some embodiments, the linear dsDNA is present in the sample in an amount of about 10 copies, about 20 copies, about 30 copies, about 40 copies, about 50 copies, about 60 copies, about 70 copies, about 80 copies, about 90 copies, or about 100 copies. The sample can comprise at least 10 copies of HHV6 genome. The sample can comprise no more than 1×10^8 copies of HHV6 genome (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 copies per sample, or a number or a range between any two of these values).

[0176] Disclosed herein include methods for detecting HHV7 in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV7 genome, wherein the target region comprises the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; (b) generating amplicons of the target region of HHV7 from the sample, if the sample comprises HHV7; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV7 in the sample.

[0177] A primer in the pair of primers can comprise the sequence of SEQ ID NO: 9 or SEQ ID NO: 10, or a sequence differing by one or two nucleotide mismatches relative to SEQ

ID NO: 9 or SEQ ID NO: 10. Determining the presence or amount of the amplicons of the target region can comprise contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 11. The oligonucleotide probe can comprise the sequence of SEQ ID NO: 11, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 11. The sample can comprise a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 12 to the sample. The linear dsDNA can comprise the sequence of SEQ ID NO: 12, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 12. In some embodiments, the linear dsDNA is present in the sample in an amount of about 10 copies, about 20 copies, about 30 copies, about 40 copies, about 50 copies, about 60 copies, about 70 copies, about 80 copies, about 90 copies, or about 100 copies. The sample can comprise at least 10 copies of HHV7 genome. The sample can comprise no more than 1×10^6 copies of HHV7 genome (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 copies per sample, or a number or a range between any two of these values).

[0178] Disclosed herein include methods for detecting HHV8 in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV8 genome, wherein the target region comprises the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; (b) generating amplicons of the target region of HHV8 from the sample, if the sample comprises HHV8; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV8 in the sample.

[0179] A primer in the pair of primers can comprise the sequence of SEQ ID NO: 13 or SEQ ID NO: 14, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13 or SEQ ID NO: 14. Determining the presence or amount of the amplicons of the target region can comprise contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these

values) to the sequence of SEQ ID NO: 15. The oligonucleotide probe can comprise the sequence of SEQ ID NO: 15, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 15. In some embodiments, the sample can comprise a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 16. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 16 to the sample. The linear dsDNA can comprise the sequence of SEQ ID NO: 16, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 16. In some embodiments, the linear dsDNA is present in the sample in an amount of about 10 copies, about 20 copies, about 30 copies, about 40 copies, about 50 copies, about 60 copies, about 70 copies, about 80 copies, about 90 copies, or about 100 copies. The sample can comprise at least 10 copies of HHV8 genome. The sample can comprise no more than 1×10^6 copies of HHV8 genome (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 copies per sample, or a number or a range between any two of these values).

[0180] Disclosed herein include methods for detecting EBV in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of EBV genome, wherein the target region comprises the NA1 gene of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; (b) generating amplicons of the target region of EBV from the sample, if the sample comprises EBV; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of EBV in the sample.

[0181] A primer in the pair of primers can comprise the sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18. Determining the presence or amount of the amplicons of the target region can comprise contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 19. The oligonucleotide probe can comprise the sequence of SEQ ID NO: 19, or a sequence differing by one or two nucleotide mismatches

relative to SEQ ID NO: 19. The sample can comprise a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 20. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 20 to the sample. The linear dsDNA can comprise the sequence of SEQ ID NO: 20, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 20. In some embodiments, the linear dsDNA is present in the sample in an amount of about 10 copies, about 20 copies, about 30 copies, about 40 copies, about 50 copies, about 60 copies, about 70 copies, about 80 copies, about 90 copies, or about 100 copies. The sample can comprise at least 10 copies of EBV genome. The sample can comprise no more than 1×10^6 copies of EBV genome (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 copies per sample, or a number or a range between any two of these values).

[0182] Disclosed herein include methods for detecting Hepatitis A Virus (HAV) in a sample. In some embodiments, the method comprises: (a) contacting a sample with a pair of primers capable of hybridizing to a target region of HAV genome, wherein the target region is the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence that exhibits at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22; (b) generating amplicons of the target region of HAV from the sample, if the sample comprises HAV; and (c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HAV in the sample. In some embodiments, contacting the sample with the pair of primers comprises subjecting the sample to a reverse transcription condition to reverse transcribe the target region of HAV. It can be advantageous in some embodiments that the reverse transcription condition occurs in the same reaction vessel as the amplification reaction. The reverse transcription condition can comprise a reverse transcriptase and/or one or more of RNAse inhibitor and bovine serum albumin,

[0183] A primer in the pair of primers can comprise the sequence of SEQ ID NO: 21 or SEQ ID NO: 22, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21 or SEQ ID NO: 22. Determining the presence or amount of the amplicons of the target region can comprise contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 23. The oligonucleotide probe can comprise the sequence of SEQ ID NO: 23, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 23. The sample can comprise a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24. The method can comprise adding a linear dsDNA comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 24 to the sample. The linear dsDNA can comprise the sequence of SEQ ID NO: 24, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 24. In some embodiments, the linear dsDNA is present in the sample in an amount of about 10 copies, about 20 copies, about 30 copies, about 40 copies, about 50 copies, about 60 copies, about 70 copies, about 80 copies, about 90 copies, or about 100 copies. The sample can comprise at least 10 copies of HAV genome. The sample can comprise no more than 1×10^6 copies of HAV genome (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6 copies per sample, or a number or a range between any two of these values).

[0184] There are provided, in some embodiments, methods and compositions for multiplex real-time PCR capable of simultaneously detecting 6 gene targets, which can accomplish detection of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. Disclosed herein include methods for detecting one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in a sample. In some embodiments, the method comprises: a) contacting the sample with a plurality of pairs of primers, wherein the plurality of pairs of primers comprises: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2; a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6; a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10; a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14; a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22; b) generating amplicons of the NS1 gene region of B19, amplicons of the U31 gene region of HHV6, amplicons of the U57 gene region of HHV7, amplicons of the BSLF1 gene region of HHV8, amplicons of the NA1 gene region of EBV, amplicons of the 5'-UTR region of HAV, or any combination thereof, if the sample comprises one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV; and c) determining the presence or amount of one or more amplicons as an indication of the presence of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in the sample.

[0185] The pair of primers capable of hybridizing to the NS1 gene region of B19 can comprise (a) a primer having the sequence of SEQ ID NO: 1 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1 and (b) a primer having the sequence of SEQ ID NO: 2 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 2; the pair of primers capable of hybridizing to the U31 gene region of HHV6 can comprise (a) a primer having the sequence of SEQ ID NO: 5 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5 and (b) a primer having the sequence of SEQ ID NO: 6 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 6; the pair of primers capable of hybridizing to the U57 gene region of HHV7 can comprise (a) a primer having the sequence of SEQ ID NO: 9 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9 and (b) a primer having the sequence of SEQ ID NO: 10 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 10; the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 can comprise (a) a primer having the sequence of SEQ ID NO: 13 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13 and (b) a primer having the sequence of SEQ ID NO: 14 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 14; the pair of primers capable of hybridizing to the NA1 gene region of EBV can comprise (a) a primer having the sequence of SEQ ID NO: 17 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17 and (b) a primer having the sequence of SEQ ID NO: 18 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 18; and/or

the pair of primers capable of hybridizing to the 5'-UTR region of HAV can comprise (a) a primer having the sequence of SEQ ID NO: 21 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21 and (b) a primer having the sequence of SEQ ID NO: 22 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 22.

[0186] Determining the presence or amount of the one or more amplicons can comprise contacting the amplicons with one or more oligonucleotide probes, wherein the one or more oligonucleotide probes can comprise a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23. Each of the one or more oligonucleotide probes can comprise the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23; or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3, 7, 11, 15, 19, or 23. In some embodiments, each of the one or more oligonucleotide probes consists of a sequence selected from the group consisting of SEQ ID NO: 3, 7, 11, 15, 19, or 23.

[0187] The sample can comprise one or more linear dsDNAs each comprising a sequence having at least about 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or a number or a range between any two of these values) to the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24. Each of the one or more linear dsDNAs can comprise the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24; or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 4, 8, 12, 16, 20, or 24. In some embodiments, each of the one or more linear dsDNAs consists of a sequence selected from the group consisting of SEQ ID NO: 4, 8, 12, 16, 20, or 24.

[0188] The oligonucleotide probe can comprise a fluorescence emitter moiety, a fluorescence quencher moiety, or both. Each oligonucleotide probe can be flanked by complementary sequences at the 5' end and 3' end. In some embodiments, one of the complementary sequences can comprise a fluorescence emitter moiety and the other complementary sequence can comprise a fluorescence quencher moiety.

[0189] The linear dsDNA can be present in the sample in an amount of no more than 100 copies (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 copies, or a number or a range between any two of these values). In some embodiments, (c) determining the presence or amount of the amplicons comprises using a reference standard curve. The reference standard curve can be generated based on serial dilutions of the linear

dsDNA. The serial dilutions of the linear dsDNA can comprise a logarithmic dilution series from about 10 copies of the linear dsDNA to about 1×10^8 copies of the linear dsDNA in reference samples (e.g., about 10, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 copies per sample, or a number or a range between any two of these values).

[0190] The sample can comprise one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV. The sample can be a biological sample or an environmental sample. The biological sample can be obtained from a tissue sample, saliva, blood, plasma, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, swab of skin or a mucosal membrane surface, cultures thereof, or any combination thereof. The biological sample can comprise genetically modified cells. The sample can be a food sample, a beverage sample, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic surface, a soil sample, a fresh water sample, a waste water sample, a saline water sample, a gas sample, cultures thereof, or any combination thereof. The sample can comprise DNA or RNA.

[0191] The amplification can be carried out using, for example, PCR, ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), replicase-mediated amplification, immuno-amplification, nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), rolling circle amplification, transcription-mediated amplification (TMA), or a combination thereof. The PCR can be quantitative real-time PCR (qPCR). The qPCR can be reverse-transcription qPCR (RT-qPCR).

EXAMPLES

[0192] Some aspects of the embodiments discussed above are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the present disclosure.

Materials and Methods

[0193] The following experimental materials and methods were used for Examples 1-4 described below.

[0194] Qiagen DNAeasy Blood and Tissue Kit was used to isolate DNA from cell samples. 300 µl of cells from a 50×10^6 cells/ml aliquot were used to prepare each sample. During sample preparation, an internal control DNA template (Genesig) was added according to manufacturer's instructions. Final DNA sample was diluted in TE buffer for a final concentration of 100 ng/µl.

[0195] For extraction of RNA, the Qiagen RNAeasy kit was used. 300 µl of cells from a 50×10^6 cells/ml aliquot were used to prepare each sample. Extraction control RNA was

added during processing as provided in the kit. Final RNA was extracted in RNAse-free water for a final concentration of 100 ng/µl. Final concentrations of nucleic acids were measured using a Dropsense instrument.

Example 1

Human Parvovirus B19 and Human Herpes Virus 6

[0196] This example describes use of the compositions and methods described herein for detecting Human Parvovirus B19 in a sample.

qPCR Setup

[0197] B19 (targeting the NS1 gene region) and HHV6 (targeting the U31 gene region) FAM-labeled Primer Probe mix used for the assay was synthesized by IDT and the sequences are shown in Table 1 above. 100 µL or 500 µL of IDTE buffer was dispensed into the IDT Mini or Std qPCR Taqman Primer and Probe mix for B19, HHV6 FAM-Primer/Probe mix to make 20X Primer/probe Stock concentration.

Preparation of Standard

[0198] Linear dsDNA fragments synthesized using GeneArt Gene Synthesis (Thermoscientific) were used as standards and the sequences are shown in Table 1 above.

[0199] The amount of nuclease free water to be added to reach a final concentration of 20 ng/µl was calculated. The lyophilized Standard was resuspended in nuclease free water, and dilutions were calculated to achieve 1e8 copies per reaction in the first tube.

Preparation of Standard Dilution Curve

[0200] Nuclease free water was pipetted into 11 tubes and labeled as described below (Table 4-Table 5). The working concentration of the lyophilized tube reconstituted in water was 20 ng/µL. The volume of template from the calculated concentration was pipetted into tube 1 and mixed. 10 µL was pipetted from tube 2 to tube 3 and mixed. Serial dilution steps were repeated up until tube 8 to complete the dilution series. 5 µl of Tube 1 through Tube 8 was used for qPCR runs.

TABLE 4: STANDARD DILUTION CURVE FOR B19-NS1

Standard Curve	Stock Standard (µl)	Water (µl)	Total volume (µl)	B19 Copy number	B19 Copy number per 5 (µl) reaction
Manufacturer tube (450 ng)	Lyophilized	22.5	22	9.0e +10	4.5e +11
Tube -2	10	90	50	9.0e+09	4.5e+10
Tube -1	22.5	77.5	100	2.0e+09	1.01e+10
Tube 0	10	90	100	2.0e+08	1.01e+09
Tube 1 (Standard-1)	10	90	100	2.0e+07	1.01e+08
Tube 2 (Standard-2)	10	90	100	2.0e+06	1.01e+07
Tube 3 (Standard-3)	10	90	100	2.0e+05	1.01e+06
Tube 4 (Standard-4)	10	90	100	2.0e+04	1.01e+05

Tube 5 (Standard-5)	10	90	100	2.0e+03	1.01e+04
Tube 6 (Standard-6)	10	90	100	2.0e+02	1.01e+03
Tube 7 (Standard-7)	10	90	100	2.0e+01	1.01e+02
Tube 8 (Standard-8)	10	90	100	2.0e+00	1.01e+01

TABLE 5: STANDARD DILUTION CURVE FOR HHV-U31

Standard Curve	Stock Standard (µl)	Water (µl)	Total volume (µl)	HHV6 Copy number	HHV6 Copy number per 5 µl reaction
Manufacturer tube (450ng)	Lyophilized	22.5	22	1.1e+11	5.70e+11
Tube -2	10	90	50	1.1e+10	5.70e+10
Tube -1	18	82	100	2.1e+09	1.03e+10
Tube 0	10	90	100	2.1e+08	1.03e+09
Tube 1 (Standard-1)	10	90	100	2.1e+07	1.03e+08
Tube 2 (Standard-2)	10	90	100	2.1e+06	1.03e+07
Tube 3 (Standard-3)	10	90	100	2.1e+05	1.03e+06
Tube 4 (Standard-4)	10	90	100	2.1e+04	1.03e+05
Tube 5 (Standard-5)	10	90	100	2.1e+03	1.03e+04
Tube 6 (Standard-6)	10	90	100	2.1e+02	1.03e+03
Tube 7 (Standard-7)	10	90	100	2.1e+01	1.03e+02
Tube 8 (Standard-8)	10	90	100	2.1e+00	1.03e+01

Control(s) used in the assay

[0201] 5 µl of Tube 1 was used as a positive control. 5µL of water was used as a negative control. 5 µl of control DNA other than used in the assay was used as a specificity control. 5 µL of mastermix was used as a non-template control. DNA extraction control added during lysis of cells was quantified with VIC internal extraction control.

Preparation of Spike samples

[0202] Two spike samples were tested in each run, including positive control, negative control, non-template control, specificity control, and a DNA extraction control. All the samples were tested in triplicates.

[0203] Spike samples were prepared by spiking DNA samples with Standards at Level 1 (LV1-100 copies), and Level 2 (LV2-10 copies), as shown in Table 6 below. Spike samples dilutions were stored at -80°C.

TABLE 6: SPIKE SAMPLES PREPARATION

Level	HHV6 or B19 Standard to be spiked in	Copy number in Standard	Volume of Standard (µl)	DNA Volume (µl)	Total Volume (µl)	Dilution Factor	Copies in spike sample
Level 1 (LV1)	Standard-6	1000	20	180	200	10	100
Level 2 (LV2)	Standard-7	100	20	180	200	10	10
Unspiked (UNS)	N/A	N/A	N/A	15	15	1	N/A

[0204] 5 µL each of spike and unspiked DNA were used for qPCR reactions.

Sample Reaction and qPCR Run Setup

[0205] For each DNA sample a reaction mix was prepared according to Table 7 below.

TABLE 7: WORKING MASTER MIX PREPARATION

Component	**Volume**
IDT PrimeTime Gene Expression Master mix	10 µL
HHV6 or B19 primer/probe mix (FAM),	1 µL
Genesig DNA extraction control primer/probe mix (VIC)	1 µL
RNAse/DNase free water	3 µL
Final Volume	15 µL

[0206] 20 µl of low reference dye was added to 5 ml of IDT PrimeTime Gene Expression Master mix. Using the volumes above, a working master mix was prepared based upon number of samples. Appropriate volumes of RNAse/DNase free water, HHV6 or B19 primer/probe mix (FAM), DNA extraction control primer/probe mix (VIC) and IDT Primetime Gene Expression Master Mix for each sample were added to a centrifuge tube and the tube was mixed by vortexing. 5 µL of DNA template for each of the standards/controls/and samples were loaded next. 15 µl of Working Master Mix was pipetted into each well. The final volume in each well was 20 µL. B19/HHV6 assay run conditions are shown below in Table 8.

TABLE 8: QPCR RUN SETUP

Number of Cycles	**Step**	**HHV6**		**B19**	
		Temp	**Time**	**Temp**	**Time**
	Enzyme Activation	95° C	3 min	95° C	3 min
50 cycles	Denaturation	95° C	15 sec	95° C	15 sec
	Data Collection	55° C	1 min	60° C	1 min

Assay Acceptance Criteria

[0207] Below are the acceptance criteria used for the assay.

[0208] **Standards**

[0209] At least 2 out of 3 replicates had to be detected in order to be considered the specific sample or standard. %CV of CT values of all replicates was less than 15%, ranges were established using Mean ± 3SD. 8 of 8 standards must have passed the above criterion.

[0210] **Spike Samples**

[0211] For spike samples, Level 1> Level 2. The target genes must have been detected in at least 2 of 3 replicates across all two levels of spike samples. The acceptable ranges for the spike samples were calculated as Mean of all replicates ± 3SD, from at least 3 independent runs. %CV of CT values of all replicates was less than 15%, ranges were established using Mean ± 3SD.

[0212] **Controls**

[0213] The negative control (water) was expected to show undetectable signal in at least 3/3 replicates per target. The unspiked sample was expected to show undetectable signal in at least 2/3 replicates per target. The non-template control sample was expected to show undetectable signal in at least 3/3 replicates per target. The Specificity sample was expected to show undetectable signal in 3/3 replicates per target. DNA extraction control was expected to show detectable signal of VIC in 2/3 replicates per target. For other Parameters (Specific to the test), the R^2 of the standard curve was required to be $\geq$ 0.995. All reported Ct values were derived from the linear phase of the exponential amplification when viewed using the log view of the amplification (ΔRn vs. Cycle). Results were reported as Ct values or log transformed copies/well (quantity values).

Example 2

Human Herpes Virus 7, Human Herpes Virus 8, and Epstein Barr Virus

[0214] This example describes use of the compositions and methods described herein for detecting Human Herpes Virus 6, Human Herpes Virus 8, and Epstein Barr Virus in a sample.

qPCR Setup

[0215] 165 µL of nuclease free water was pipetted into the HHV7 or HHV8 or EBV FAM-Primer/Probe mix (brown tube) and Internal extraction control VIC- Primer/Probe mix (brown tube). The positive control template (red tube) was resuspended in 500 pL of the template preparation buffer supplied (yellow tube).

Preparation of Standard Dilution Curve

[0216] See Table 9 for the copy numbers in each dilution standard. 90 µL of template preparation buffer was pipetted into 5 tubes and labeled 2-6. 10 µL of positive control template was pipetted into tube 2. 10 µL was pipetted from tube 2 to tube 3. Serial dilution steps were repeated up until tube 6 to complete the dilution series. 5 µl of Tube 1 through Tube 6 was used in qPCR reactions.

TABLE 9: STANDARD DILUTION CURVE

Standard Curve	**Copy number**	**Copy number per 5 µl reaction**
Tube 1 (Positive control)	2e5 copies/µL	1e6 copies/µL
Tube 2 (Standard-2)	2e4 copies/µL	1e5 copies/µL
Tube 3 (Standard-3)	2e3 copies/µL	1e4 copies/µL
Tube 4 (Standard-4)	2e2 copies/µL	1e3 copies/µL
Tube 5 (Standard-5)	20 copies/µL	1e2 copies/µL
Tube 6 (Standard-6)	2 copies/µL	10 copies/µL

Control(s) used in the assay

[0217] 5 µl of Tube 1 was used as a positive control. 5µL of water was used as a

negative control. 5 µl of control DNA other than used in the assay was used as a specificity control. 5µL of mastermix was used as a non-template control. DNA extraction control added during lysis of cells was quantified with VIC internal extraction control.

Preparation of Spike Samples

[0218] Two spike samples were tested in each run, including positive control, negative control, non-template control, specificity control, and a DNA extraction control. All the samples were tested in triplicates. Spike samples were prepared by spiking DNA samples with Standards at Level 1 (LV1-100 copies), and Level 2 (LV2-10 copies), as shown in Table 10. Spike samples dilutions were prepared freshly on the day of the run.

TABLE 10: SPIKE SAMPLES PREPARATION

Level	HHV7 or HHV8 or EBV Standard to be spiked in	Copies in Standard	Volume Standard	DNA Volume	Total Volume	Dilution Factor	Copies in QC
Level 1 (LV1)	Standard-4	1000	2	18	20	10	100
Level 2 (LV2)	Standard-5	100	3	27	30	10	10
Unspiked (UNS)	N/A	N/A	N/A	15	15	1	N/A

[0219] 30 µL aliquots of Level 1 and Level 2 were prepared. Example: 3µL of Standard-4 was added to 27µL of the DNA sample (100ng) at a 10-fold dilution to achieve 100 copies of HHV7 in level 1. An unspiked sample was also used in the experiment. 5µL each of spike and unspiked DNA was used.

Sample Reaction Setup

[0220] For each DNA sample a reaction mix was prepared according to Table 11 below.

TABLE 11: WORKING MASTER MIX PREPARATION

Component	Volume
Precision Plus LR qPCR Mastermix	10 µL
HHV7 or HHV8 or EBV primer/probe mix (FAM)	1 µL
DNA extraction control primer/probe mix (VIC)	1 µL
RNAse/DNase free water	3 µL
Final Volume	15 µL

[0221] Using the volumes above, a working master mix was prepared based upon number of samples. Appropriate volumes of RNAse/DNase free water, HHV7 or HHV8 or EBV primer/probe mix (FAM), DNA extraction control primer/probe mix (VIC) and PrecisionPlus LR qPCR Master Mix for each sample were mixed and centrifuged to prepare the Working Master Mix.

[0222] 5µL of DNA template for each of the standards/controls/and samples were

next loaded. 15µl of Working Master Mix was pipetted into each well. The final volume in each well was 20µL. qPCR reactions were run under conditions shown below in Table 12.

TABLE 12: ASSAY SETUP

Number of Cycles	Step	Time	Temp
	Enzyme Activation	2 min	95° C
50 cycles	Denaturation	10 sec	95° C
	Data Collection	60 sec	60° C

Assay Acceptance Criteria

[0223] Below are the acceptance criteria used for the assay.

[0224] Standards

[0225] At least 2 out of 3 replicates must have been detected in order to consider the specific sample or standard. %CV of CT values of all replicates was less than 15%, ranges were established using Mean ± 3SD. 6 of 6 standards must have passed the above criterion.

[0226] Spike Samples

[0227] The levels of spike samples were as follows: Level 1> Level 2. The target genes must have been detected in at least 2 of 3 replicates across all two levels of spike samples. The acceptable ranges for the spike samples were calculated as Mean of all replicates ± 3SD, from at least 3 independent runs. %CV of Ct values of all replicates was less than 15%, ranges were established using Mean ± 3SD. %RE of the spike samples must be <±20% for Level 1, except for the Level 2 which must be <±30 %.

[0228] Controls

[0229] The negative control (water) must have shown undetectable signal in 3/3 replicates for the virus being tested. The non-template control sample must have shown undetectable signal in at least 3/3 replicates for the virus being tested. The Specificity sample must have shown undetectable signal in 3/3 replicates for the virus being tested. The unspiked sample must have shown undetectable signal in at least 2/3 replicates for the virus being tested. DNA extraction control must have shown detectable signal of VIC in 2/3 replicates for the virus being tested.

[0230] Other Parameters (Specific to the test)

[0231] The R^2 of the standard curve must have been ≥ 0.995. All reported Ct values were expected to be derived from the linear phase of the exponential amplification when viewed using the log view of the amplification (ΔRn vs. Cycle). Results were reported as Ct values or log transformed copies/well (quantity values).

Example 3

Detection of Hepatitis A Virus

[0232] This example describes use of the compositions and methods described herein for detecting Hepatitis A Virus in a sample.

qPCR Setup

[0233] 165 µL of RNAase free water was pipetted into the HAV FAM- Primer/Probe mix (brown tube) or Internal extraction control VIC-Primer/Probe mix (brown tube). The positive control template (red tube) was resuspended in 500 µL of the template preparation buffer supplied (yellow tube).

Preparation of Standard Dilution Curve

[0234] Table 13 shows the copy numbers in each dilution standard. 90 µL of template preparation buffer was pipetted into 5 tubes labeled 2-6. 10 µL of positive control template was pipetted into tube 2. 10 µL was pipetted from tube 2 to tube 3. Serial dilution steps were repeated up until tube 6 to complete the dilution series. 5µl of Tube 1 through Tube 6 were used in qPCR assays.

TABLE 13: STANDARD DILUTION CURVE

Standard Curve	Copy number	Copy number per 5 µl reaction
Tube 1 (Positive control)	2e5 copies/µL	1e6 copies/µL
Tube 2 (Standard-2)	2e4 copies/µL	1e5 copies/µL
Tube 3 (Standard-3)	2e3 copies/µL	1e4 copies/µL
Tube 4 (Standard-4)	2e2 copies/µL	1e3 copies/µL
Tube 5 (Standard-5)	20 copies/µL	1e2 copies/µL
Tube 6 (Standard-6)	2 copies/µL	10 copies/µL

Control(s) used in the assay

[0235] 5 µl of Tube 1 was used as a positive control. 5µL of water was used as a negative control. 5µL of HEV RNA was used as a specificity control. 5µL of mastermix was used as a no-template control. RNA extraction control added to the sample was quantified with VIC internal extraction control.

Preparation of Spike samples

[0236] Two spike samples were tested in each run, including positive control, negative control, non- template control, specificity control, and RNA extraction control. All the samples were tested in triplicates. Spike samples were prepared by spiking HAV Standards at Level 1 (LV1-100 copies), and Level 2 (LV2-10 copies) into Extracted RNA samples, as shown in Table 14 below. Spike samples dilutions were freshly prepared on the day of the run.

TABLE 14: SPIKE SAMPLES PREPARATION

QC Level	HAV Standard to be spiked in	Copies in Standard	Volume Standard	Volume RNA	Total Volume	Dilution Factor	Copies in QC
Level 1 (LV1)	Standard-4	1000	2	18	20	10	100
Level 2	Standard-5	100	3	27	30	10	10

(LV2)							
Unspiked (UNS)	N/A	N/A	N/A	15	15	1	N/A

[0237] 30 µL aliquots of Level 1 and Level 2 were prepared. For example, Example: 3 µL of Standard-4 was added to 27µL of the RNA sample (100ng/µl) at a 10-fold dilution to achieve 100 copies of HAV in Level 1. The above step was repeated by following the above Table to generate Level 1 and Level 2. An unspiked sample was also used in the experiment and 5 ul of each spike and unspiked RNA was used for RT-qPCR reactions.

Sample Reaction and Run Setup

[0238] For each RNA a reaction mix was prepared according to Table 15.

TABLE 15: WORKING MASTER MIX PREPARATION

Component	Volume
PrecisionPlus OneStep 2X RT -qPCR Master Mix	10µL
HAV primer/probe mix (FAM)	1µL
RNA extraction control primer/probe mix (VIC)	1µL
RNase/DNase free water	3µL
Final Volume	15µL

[0239] Using the volumes above, a working master mix was prepared based upon number of samples. Appropriate volumes of RNase/DNase free water, HAV primer/probe mix (FAM), RNA extraction control primer/probe mix (VIC) and PrecisionPlus OneStep 2X RT-qPCR Master Mix for each sample were added to a centrifuge tube and vortexed to generate a Working Master Mix. 5µL of RNA template for each of the standards/controls/and samples were loaded next. 15µl of Working Master Mix was pipetted into each well. The final volume in each well was 20µL.

[0240] Reactions were run under conditions of: 10 minutes RT extension at 55°C, 95°C for 2 minutes, following by cycling of 95°C for 10 seconds and 55°C extension for 1 min, for 50 cycles.

Assay Acceptance Criteria

[0241] Below are the acceptance criteria used for the assay.

[0242] **Standards**

[0243] At least 2 out of 3 replicates must have been detected in order to consider the specific sample or standard. %CV of Ct values of all replicates was less than 15%, ranges may be established using Mean ± 3SD. 6 of 6 standards must have passed the above criterion.

[0244] **Spike Samples**

[0245] During qualification, the results of the spike samples were as follows: Level 1> Level 2. The target genes must have been detected in at least 2 of 3 replicates across all two

levels of spike samples. The acceptable ranges for the spike samples were calculated as Mean of all replicates ± 3SD, from at least 3 independent runs. %CV of Ct values of all replicates was less than 15%, ranges were established using Mean ± 3SD. %RE of the spike samples must have been <±20% for Level 1, except for the Level 2 which must be <±30 %.

[0246] Controls

[0247] The negative control (water) sample must have shown undetectable signal in 3/3 replicates for HAV virus. The non-template control sample must have shown undetectable signal in at least 3/3 replicates for HAV virus. The Specificity sample must have shown undetectable signal in 3/3 replicates for HAV virus. The unspiked sample must have shown undetectable signal in at least 2/3 replicates for HAV virus. RNA extraction control must have shown detectable signal of VIC in 2/3 replicates for HAV virus.

[0248] Other Parameters (Specific to the test)

[0249] The R^2 of the standard curve must have been $\geq$ 0.995. All reported Ct values must have been derived from the linear phase of the exponential amplification when viewed using the log view of the amplification (ΔRn vs. Cycle). Results were reported as Ct values or log transformed copies/well (quantity values).

Example 4

Assay Qualification

[0250] This example demonstrates that the compositions and methods described herein for detection of viral genomes generate results that are linear, accurate, precise, robust, specific, and exhibit an upper limit of quantification of about 10,000,000 copies/well and a lower limit of quantification of about 10 copies/well.

[0251] The HAV Viral copy number (in the total extracted RNA) or HHV6, HHV7, HHV8, EBV, B19 (Viral copy number in the extracted DNA) was quantified using qPCR. The Taqman based method uses sequence specific primer probe sets for accurate quantification. Viral copy number in samples was calculated based on a standard curve consisting of known concentrations of Synthetic RNA or DNA, and results are reported as copies per well.

[0252] The Taqman qPCR assay was used to quantify the of HHV7, HHV8, HHV6, B19, EBV, HAV viral copy number in the samples. The TaqMan based qPCR method utilizes a nucleic acid probe complementary to the target sequence of the DNA and a set of Forward and Reverse primers. The TaqMan probe is labeled with two fluorescent moieties (A FAM reporter dye and a TAMRA quencher dye). The Primers and probes are designed to bind to the DNA template during the annealing step. During polymerization, the Taq DNA polymerase with its 5' exonuclease activity degrades the probe upon strand synthesis and releases the reporter dye (FAM) from the quencher (TAMRA), thereby emitting fluorescence signal which is captured by

the qPCR instrument. The fluorescence increases with each cycle and is detectable against the threshold background. A threshold value is produced and defined as the cycle number where detectable fluorescence intersects the cycle threshold. Therefore, the fluorescence detected during qPCR is proportional to the fluorophore released and the amount of DNA template present in the PCR reaction.

[0253] Total DNA/RNA is extracted from the samples using the QIAGEN DNeasy Blood & Tissue Kits/ RNeasy® Plus Kit. DNA/RNA concentration is determined by measuring absorbance at 260 nm.

[0254] The Viral copy number in the total extracted RNA/DNA was quantified using qPCR. This method uses sequence specific primer probe sets for accurate quantification. Viral copy number in the samples were calculated based on a standard curve consisting of known concentrations of Plasmid DNA/RNA or synthetic linear DNA fragment, and results were reported as copies per well.

Spike study preparation for Qualification

[0255] Two levels of spike study samples (Level 1-100 copies, Level 2-10 copies) were prepared by spiking known concentration of HEV positive control into template preparation buffer or into RNA extracted from samples (0.5 micrograms of RNA per reaction was used in the assay). An unspiked sample was also be used in the assay. Samples from 3 different lots were used in the assay. These samples were prepared on the day of the run and used during the qualification process. For HHV7 and HHV8, if copies were in the unspiked samples, the quantity values were subtracted from the observed quantity values in the spiked samples (100 copies, 10 copies) and used for analysis.

[0256] Two levels of spike study samples (Level 1-100 copies, Level 2-10 copies) were prepared by spiking known concentration of viral (HHV7, HHV8, EBV, HAV) positive control into template preparation buffer or viral (HHV6, B19) synthetic DNA into water. RNA or DNA was extracted from samples (0.5 micrograms of RNA or DNA per reaction was used in the assay). An unspiked sample was also used in the assay to quantify the basal levels of the virus. Samples from three different preparations were used in the experiments described herein.

Statistical Methods and Acceptance Criteria

% CV = (Standard Deviation/Mean) * 100

% Recovery = (Observed / Expected) *100

[0257] **Acceptance/Rejection Criteria**

[0258] The Acceptance/Rejection Criteria for each parameter assessed during this qualification process are listed under each section in the discussion of results below.

[0259] The Acceptance/Rejection Criteria for each qualification run were as follows.

The R^2 of the standard curve must have been ≥ 0.995. The no-template control (DNA extraction control samples extracted during each run) must have shown positive signal for VIC extraction control in at least 2/3 replicates. The water no-template control must have shown undetectable signal in 3/3 replicates. The target genes were expected to be detected in at least 2/3 replicates across all two levels of spike samples. All reported Ct values were expected to be derived from linear phase of the exponential amplification when viewed using the log view of the amplification plot (ΔRn vs. Cycle).

Discussion of Results

[0260] The following parameters were determined in the qualification of the assay: Linearity, Accuracy, Limits of Quantification (LLOQ/ULOQ), Precision (Intra assay, Inter assay precision), Robustness, and Specificity.

Linearity

[0261] The Standard curve was generated using the positive control (HHV7, HHV8, EBV, HAV provided in the Genesig kit) and for HHV6, B19 use the linear synthetic DNA. 10-fold serial dilution was performed to generate 6 standard points starting from 1E+6 copies/rxn down to 10 copies/rxn to construct a standard curve. Linearity was determined by evaluating the standard curve from the first six qualification runs performed by multiple operators across multiple days.

[0262] The Standard curves from the six qualification runs were evaluated to determine linearity. Results from the linear regression analysis from these runs are shown in Table 16-Table 21. The standard curve demonstrated acceptable linearity with %CV of < 15% and an R^2 of ≥0.995. Further, the slope and y-intercept were evaluated to determine an acceptable range for the assay (Mean ± 3*SD) and the PCR efficiency was within 90%-110%.

TABLE 16: HAV LINEARITY

Run	Slope	y-intercept	R^2	PCR %Efficiency
1	-3.54	37.73	0.9999	92
2	-3.59	40.14	0.9991	90
3	-3.57	39.67	0.9978	90
4	-3.57	39.71	0.9989	90
5	-3.40	36.43	0.9983	97
6	-3.57	39.27	0.9998	91
Average	-3.54	38.82	NA	NA
SD	0.07	1.44	NA	NA
Avg -3*SD	-3.75	34.50	NA	NA
Avg +3*SD	-3.33	43.15	NA	NA

TABLE 17: EBV LINEARITY

Run	Slope	y-intercept	R^2	PCR %Efficiency

1	-3.56	38.88	0.9998	91
2	-3.53	36.75	0.9999	92
3	-3.48	38.66	1.0000	94
4	-3.56	38.81	0.9980	91
5	-3.44	37.91	0.9994	95
6	-3.52	38.21	0.9997	92
Average	-3.52	38.20	NA	NA
SD	0.05	0.80	NA	NA
Avg -3*SD	-3.66	35.79	NA	NA
Avg +3*SD	-3.37	40.62	NA	NA

TABLE 18: HHV6 LINEARITY

Run	Slope	y-intercept	R^2	PCR %Efficiency
1	-3.40	37.23	0.9991	97
2	-3.38	39.02	0.9963	98
3	-3.37	37.89	0.9994	98
4	-3.52	38.58	0.9980	92
5	-3.35	37.65	0.9995	99
6	-3.50	39.52	0.9998	93
Average	-3.42	38.31	NA	NA
SD	0.07	0.87	NA	NA
Avg -3*SD	-3.63	35.69	NA	NA
Avg +3*SD	-3.21	40.94	NA	NA

TABLE 19: HHV7 LINEARITY

Run	Slope	y-intercept	R^2	PCR %Efficiency
1	-3.53	33.85	0.9985	92
2	-3.52	36.79	0.9998	92
3	-3.52	38.40	0.9998	92
4	-3.40	36.16	0.9992	97
5	-3.54	37.95	0.9990	92
6	-3.45	36.14	0.9997	95
Average	-3.49	36.55	NA	NA
SD	0.06	1.61	NA	NA
Avg -3*SD	-3.66	31.71	NA	NA
Avg +3*SD	-3.32	41.39	NA	NA

TABLE 20: HHV8 LINEARITY

Run	Slope	y-intercept	R^2	PCR %Efficiency
1	-3.39	37.79	0.9997	97
2	-3.31	37.44	0.9982	101
3	-3.41	37.76	0.9999	97
4	-3.47	37.85	0.9984	94
5	-3.40	36.60	0.9988	97
6	-3.41	37.61	0.9998	97
Average	-3.40	37.51	NA	NA
SD	0.05	0.47	NA	NA

Avg -3*SD	-3.55	36.10	NA	NA
Avg +3*SD	-3.24	38.91	NA	NA

TABLE 21: B19 LINEARITY

Run	Slope	y-intercept	R^2	PCR %Efficiency
1	-3.21	37.33	0.9942	105
2	-3.28	37.91	0.9962	102
3	-3.50	37.60	0.9964	93
4	-3.33	36.91	0.9999	100
5	-3.37	37.14	0.9985	98
6	-3.36	36.40	0.9997	98
Average	-3.34	37.22	NA	NA
SD	0.10	0.53	NA	NA
Avg -3*SD	-3.64	35.63	NA	NA
Avg +3*SD	-3.05	38.81	NA	NA

Accuracy

[0263] To assess the accuracy, spike in recovery was performed where the extracted DNA/RNA or template buffer was spiked in with known concentrations (100 copies, 10 copies) of Virus standards. At least two different concentrations within the range of the standard curve were tested, over three independent runs.

[0264] To assess the accuracy, RNA/DNA was extracted from samples and their concentrations were measured, and RNA/DNA was diluted to 100 ng/µl. The diluted RNA/DNA (0.5 µg) was spiked with two different concentrations of standard, 100 copies/well and 10 copies/well, or with template dilution buffer with a known concentration (1e6, 1e5, 1e4, 1e3, 1e2, and 10 copies/well) of the target (HHV6, HHV7, HHV8, EBV, HAV, B19 positive control). Three replicates were measured per concentration over three independent runs.

[0265] To meet acceptance criteria for Accuracy, the mean observed value of the Log- transformed copies/well value was expected to be within 20% for all levels except at 10 copies, where it was expected to be within 30% and %Recovery of the Log- transformed copies/well value to be between 80-120%, except for the low level (10 copies), where it must have been within 70-130%.

[0266] Results are shown in Table 22 – Table 39C. Acceptance criteria were met for all concentrations tested for standards spiked into buffer and standards spiked into RNA/DNA (100 and 10 copies/well) tested, all levels demonstrated acceptable recovery and precision within the acceptance criteria. Therefore, the assay was considered to demonstrate acceptable accuracy for concentrations ranging from 1e6 to 10 copies/well.

TABLE 22: HAV ACCURACY – LEVEL 1 (100 COPIES)

HAV Runs	Sample 1			Sample 2			Sample 3		
	Copies	Avg	Log (10)	Copies	Avg	Log (10)	Copies/well	Avg	Log (10)

	/well	Copies /well	Copies /well	/well	Copies/well	Copies/well		Copies/well	Copies/well
Run 1	145	113	2.1	89	90	2.0	85	82	1.9
	94			79			78		
	101			101			83		
Run 2	74	76	1.9	44	56	1.7	48	65	1.8
	78			56			57		
	76			69			91		
Run 3	68	79	1.9	85	88	1.9	74	72	1.9
	72			81			52		
	98			98			89		
Avg		90	2.0		78	1.9		73	1.9
Expected		100	2.0		100	2.0		100	2.0
SD			0.10			0.12			0.05
%CV			5			6			3
%Recovery			98			95			93

TABLE 23: HAV ACCURACY – LEVEL 2 (10 COPIES)

HAV Runs	**Sample 1**			**Sample 2**			**Sample 3**		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	14	11	1.1	5	8	0.9	13	10	1.0
	10			8			8		
	10			12			8		
Run 2	12	9	0.9	2	5	0.7	6	6	0.8
	10			7			6		
	4			4			2		
Run 3	3	8	0.9	6	7	0.8	4	6	0.7
	13			3			6		
	9			11			7		
Avg		9	1.0		6	0.8		7	0.8
Expected		10	1.0		10	1.0		10	1.0
SD			0.08			0.15			0.17
%CV			8			18			21
%Recovery			97			81			82

TABLE 24A: ACCURACY –SPIKED IN BUFFER

HAV Runs	**Standard 1**			**Standard 2**			**Standard 3**		
	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	1135440	978286	6.0	97447	103951	5.0	9973	10209	4.0
	876745			95588			9959		
	922673			118818			10695		
Run 2	935176	1055373	6.0	108471	94484	5.0	9438	9318	4.0
	972321			90958			9495		
	1258622			84024			9021		
Run 3	1092680	1050643	6.0	99139	90421	5.0	10694	10021	4.0
	892020			87503			10223		
	1167228			84620			9145		
Avg		1028101	6.0		96285	5.0		9849	4.0
Expected		1000000	6.0		100000	5.0		10000	4.0
SD			0.02			0.03			0.02
%CV			0			1			1
%Recovery			100			100			100

TABLE 24B: ACCURACY –SPIKED IN BUFFER

HAV Runs	Standard 4			Standard 5			Standard 6		
	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	929	985	3.0	67	98	2.0	11	13	1.1
	991			133			13		
	1034			95			14		
Run 2	752	893	3.0	86	82	1.9	9	9	1.0
	875			92			12		
	1051			68			6		
Run 3	895	894	3.0	74	83	1.9	8	8	0.9
	974			85			7		
	814			91			9		
Avg		924	3.0		88	1.9		10	1.0
Expected		1000	3.0		100	2.0		10	1.0
SD			0.02			0.04			0.10
%CV			1			2			10
%Recovery			99			97			100

TABLE 25: EBV ACCURACY – LEVEL 1 (100 COPIES)

EBV Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	113	124	2.1	136	128	2.1	112	115	2.1
	116			128			119		
	143			120			113		
Run 2	94	93	2.0	89	78	1.9	88	84	1.9
	105			74			89		
	81			70			77		
Run 3	106	99	2.0	100	104	2.0	107	105	2.0
	110			112			91		
	81			99			115		
Avg		106	2.0		103	2.0		101	2.0
Expected		100	2.0		100	2.0		100	2.0
SD			0.07			0.11			0.07
%CV			3			5			3
%Recovery			101			101			100

TABLE 26: EBV ACCURACY – LEVEL 2 (10 COPIES)

EBV Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	5	6	0.8	10	10	1.0	15	11	1.0
	8			12			6		
	5			9			12		
Run 2	6	7	0.9	6	7	0.8	6	9	1.0
	8			7			18		
	8			7			5		
Run 3	9	10	1.0	10	8	0.9	11	14	1.1
	15			6			18		
	5			8			12		
Avg		8	0.9		8	0.9		11	1.1
Expected		10	1.0		10	1.0		10	1.0
SD			0.10			0.09			0.08
%CV			11			10			8
%Recovery			88			91			106

TABLE 27A: EBV ACCURACY – SPIKED IN DILUENT

EBV Runs	Standard 1			Standard 2			Standard 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	902715	882770	5.9	92533	94553	5.0	9843	10278	4.0
	902123			101979			11282		
	843470			89146			9710		
Run 2	1044422	1042860	6.0	122427	108656	5.0	10440	14600	4.2
	1064719			101809			22158		
	1019440			101732			11201		
Run 3	968532	971696	6.0	97328	100150	5.0	9760	9783	4.0
	970997			102312			9876		
	975561			100809			9714		
Avg		965775	6.0		101119	5.0		11554	4.1
Expected		1000000	6.0		100000	5.0		10000	4.0
SD			0.04			0.03			0.09
%CV			1			1			2
%Recovery			100			100			102

TABLE 27B: EBV ACCURACY – SPIKED IN DILUENT

EBV Runs	Standard 4			Standard 5			Standard 6		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	1071	1070	3.0	130	118	2.1	15	9	1.0
	1171			111			4		
	969			112			8		
Run 2	969	984	3.0	117	92	2.0	8	8	0.9
	1071			81			6		
	912			80			9		
Run 3	931	1015	3.0	92	101	2.0	13	9	0.9
	1045			109			8		
	1068			102			5		
Avg		1023	3.0		104	2.0		9	0.9
Expected		1000	3.0		100	2.0		10	1.0
SD			0.02			0.05			0.04
%CV			1			3			4
%Recovery			100			101			94

TABLE 28: HHV6 ACCURACY – LEVEL 1 (100 COPIES)

HHV6 Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	143	145	2.2	116	113	2.1	180	154	2.2
	141			115			134		
	151			108			148		
Run 2	103	133	2.1	191	147	2.2	98	140	2.1
	142			97			189		
	155			153			132		
Run 3	146	176	2.2	166	160	2.2	134	137	2.1
	214			146			157		
	169			168			121		
Avg		152	2.2		140	2.1		144	2.2
Expected		100	2.0		100	2.0		100	2.0
SD			0.06			0.08			0.03
%CV			3			4			1
%Recovery			109			107			108

TABLE 29: HHV6 ACCURACY – LEVEL 2 (10 COPIES)

HHV6 Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	17	23	1.4	16	17	1.2	9	12	1.1
	28			15			13		
	23			21			14		
Run 2	22	16	1.2	19	20	1.3	9	19	1.3
	10			14			20		
	17			26			27		
Run 3	8	13	1.1	35*	22	1.3	15	23	1.4
	18			20			25		
	1.2			24			30		
Avg		17	1.2		20	1.3		18	1.3
Expected		10	1.0		10	1.0		10	1.0
SD			0.12			0.05			0.15
%CV			10			4			12
%Recovery			124			129			125

TABLE 30A: HHV6 ACCURACY – SPIKED IN DILUENT

HHV6 Runs	Standard 1			Standard 2			Standard 3		
	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	85415388	94818039	8.0	10437781	9486040	7.0	1285688	1174220	6.1
	86801612			10181290			1090326		
	112237117			7839049			1146645		
Run 2	91489800	78076573	7.9	10570967	9117177	7.0	1084708	1129623	6.1
	78145199			8258582			1048504		
	64594720			8521982			1255656		
Run 3	109025692	118730984	8.1	10354150	10747195	7.0	1015806	1048626	6.0
	145115056			10845769			1074079		
	102052204			11041666			1055993		
Avg		97208532	8.0		9783471	7.0		1117489	6.0
Expected		100000000	8.0		10000000	7.0		1000000	6.0
SD			0.09			0.04			0.02
%CV			1			1			0
%Recovery			100			100			101

TABLE 30B: HHV6 ACCURACY – SPIKED IN DILUENT

HHV6 Runs	Standard 4			Standard 5			Standard 6		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	128644	127778	5.1	12321	12363	4.1	1206	1150	3.1
	127272			11812			1155		
	127418			12956			1089		
Run 2	130761	124206	5.1	10767	14750	4.2	2121	1473	3.2
	117171			13776			1132		
	124685			19706			1167		
Run 3	108615	108408	5.0	9309	9744	4.0	937	1030	3.0
	109027			9540			946		
	107582			10383			1206		
Avg		120131	5.1		12286	4.1		1218	3.1
Expected		100000	5.0		10000	4.0		1000	3.0
SD			0.04			0.09			0.08
%CV			1			2			3
%Recovery			102			102			103

TABLE 30C: HHV6 ACCURACY – SPIKED IN DILUENT

HHV6 Runs	Standard 7			Standard 8		
	Copies/well	Avg Copies/well	Log (10) Copies/well	Copies/well	Avg Copies/well	Log (10) Copies/well
Run 1	114	123	2.1	15	13	1.1
	117			17		
	138			7		
Run 2	116	109	2.0	40	23	1.4
	85			11		
	125			18		
Run 3	86	110	2.0	17	12	1.1
	123			6		
	121			14		
Avg		114	2.1		16	1.2
Expected		100	2.0		10	1.0
SD			0.03			0.15
%CV			1			12
%Recovery			103			121

TABLE 31: HHV7 ACCURACY – LEVEL 1 (100 COPIES)

HHV7 Runs	Sample 1				Sample 2				Sample 3			
	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	87	76	59	1.8	93	88	81	1.9	60	60	72	1.9
	62	51			76	71			85	85		
	60	49			90	85			72	72		
Run 2	92	81	82	1.9	180	176	139	2.1	83	83	96	2.0
	83	72			126	122			102	102		
	105	94			124	120			103	103		
Run 3	106	91	102	2.0	94	90	88	1.9	71	71	84	1.9
	114	99				71			103	103		
	131	116				104			79	79		
Avg			81	1.9			103	2.0			84	1.9
Expected			100	2.0			100	2.0			100	2.0
SD				0.12				0.13				0.06
%CV				6				6				3
%Recovery				95				101				96

TABLE 32: HHV7 ACCURACY – LEVEL 2 (10 COPIES)

HHV7 Runs	Sample 1				Sample 2				Sample 3			
	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	15	4	7	0.8	19	14	11	1.1	6	NA	5	0.7
	18	7			17	12			6	NA		
	19	8			13	8			4	NA		
Run 2	15	4	9	0.9	11	7	11	1.1	1	NA	10	1.0
	23	12			21	17			19	NA		
	22	11			14	10			10	NA		
Run 3	23	8	7	0.8	12	8	10	1.0	7	NA	8	0.9
	21	6			13	9			6	NA		
	21	6			15	11			12	NA		
Avg			7	0.9			11	1.0			8	0.9
Expected			10	1.0			10	1.0			10	1.0

SD				0.07				0.04				0.14
%CV				8				4				16
%Recovery				87				103				90

TABLE 33A: HHV7 ACCURACY – SPIKED IN DILUENT

HHV7 Runs	Standard 1			Standard 2			Standard 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	1022417	1048923	6.0	104134	108063	5.0	9528	9845	4.0
	956975			109011			9888		
	1167376			111044			10118		
Run 2	894167	908600	6.0	93959	96056	5.0	11118	11428	4.1
	932752			95023			11715		
	898882			99185			11451		
Run 3	996566	1042245	6.0	98912	102689	5.0	9403	9785	4.0
	1074845			104146			10210		
	1055324			105010			9743		
Avg		999923	6.0		102269	5.0		10353	4.0
Expected		1000000	6.0		100000	5.0		10000	4.0
SD			0.04			0.03			0.04
%CV			1			1			1
%Recovery			100			100			100

TABLE 33B: HHV7 ACCURACY – SPIKED IN DILUENT

HHV7 Runs	Standard 4			Standard 5			Standard 6		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	999	976	3.0	89	94	2.0	11	12	1.1
	1013			95			10		
	915			97			16		
Run 2	1094	1159	3.1	116	114	2.1	8	9	0.9
	1249			117			5		
	1133			108			13		
Run 3	919	939	3.0	88	95	2.0	10	7	0.9
	966			104			7		
	932			94			5		
Avg		1024	3.0		101	2.0		9	1.0
Expected		1000	3.0		100	2.0		10	1.0
SD			0.05			0.05			0.12
%CV			2			2			12
%Recovery			100			100			98

TABLE 34: HHV8 ACCURACY – LEVEL 1 (100 COPIES)

HHV8 Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	105	122	2.1	97	89	1.9	87	102	2.0
	105			98			110		
	155			72			109		
Run 2	97	111	2.0	104	104	2.0	107	116	2.1
	122			96			127		
	113			111			115		
Run 3	95	84	1.9	139	126	2.1	118	120	2.1
	62			114			101		
	95			125			140		
Avg		105	2.0		106	2.0		113	2.1
Expected		100	2.0		100	2.0		100	2.0

SD			0.08			0.08			0.04
%CV			4			4			2
%Recovery			101			101			103

TABLE 35: HHV8 ACCURACY – LEVEL 2 (10 COPIES)

HHV8 Runs	**Sample 1**			**Sample 2**			**Sample 3**		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	5	6	0.8	3	6	0.8	8	9	0.9
	6			8			7		
	6			6			11		
Run 2	7	10	1.0	29	17	1.2	10	16	1.2
	8			7			25		
	15			13			13		
Run 3	8	7	0.9	10	9	1.0	11	11	1.1
	5			9			12		
	9			8			12		
Avg		8	0.9		10	1.0		12	1.1
Expected		10	1.0		10	1.0		10	1.0
SD			0.12			0.23			0.14
%CV			14			23			13
%Recovery			88			102			108

TABLE 36A: HHV8 ACCURACY – SPIKED IN DILUENT

HHV8 Runs	**Standard 1**			**Standard 2**			**Standard 3**		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	975770	1013802	6.0	106465	104283	5.0	10092	10018	4.0
	991188			99193			10193		
	1074447			107192			9770		
Run 2	999489	951462	6.0	98297	93255	5.0	10030	9869	4.0
	926631			89072			9886		
	928267			92396			9690		
Run 3	1139589	1114378	6.0	108931	108319	5.0	10946	10658	4.0
	1122308			105501			10456		
	1081238			110524			10572		
Avg		1026547	6.0		101952	5.0		10182	4.0
Expected		1000000	6.0		100000	5.0		10000	4.0
SD			0.03			0.03			0.02
%CV			1			1			0
%Recovery			100			100			100

TABLE 36B: HHV8 ACCURACY – SPIKED IN DILUENT

HHV8 Runs	**Standard 4**			**Standard 5**			**Standard 6**		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	984	1005	3.0	61	83	1.9	6	8	0.9
	1019			112			7		
	1013			77			11		
Run 2	1015	1005	3.0	95	96	2.0	18	12	1.1
	986			96			11		
	1012			96			9		
Run 3	1034	1073	3.0	114	109	2.0	7	7	0.9
	1145			122			7		
	1041			92			7		
Avg		1028	3.0		96	2.0		9	1.0
Expected		1000	3.0		100	2.0		10	1.0

SD			0.02			0.06			0.12
%CV			1			3			13
%Recovery			100			99			97

TABLE 37: B19 ACCURACY – LEVEL 1 (100 COPIES)

B19 Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	102	82	1.9	120	99	2.0	89	86	1.9
	75			72			74		
	70			105			94		
Run 2	134	149	2.2	138	138	2.1	122	138	2.1
	119			134			133		
	193			143			158		
Run 3	223	189	2.3	242	218	2.3	209	206	2.3
	193			224			191		
	150			189			218		
Avg		140	2.1		152	2.2		143	2.2
Expected		100	2.0		100	2.0		100	2.0
SD			0.19			0.17			0.19
%CV			9			8			9
%Recovery			107			109			108

TABLE 38: B19 ACCURACY – LEVEL 2 (10 COPIES)

B19 Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	15	22	1.3	0.3	6	0.8	6	12	1.1
	25			5			12		
	25			12			17		
Run 2	18	14	1.1	13	8	0.9	10	10	1.0
	12			0.04			7		
	12			11			12		
Run 3	34	18	1.3	12	13	1.1	14	16	1.2
	6			12			18		
	14			16			12105062*		
Avg		18	1.3		9	1.0		12	1.1
Expected		10	1.0		10	1.0		10	1.0
SD			0.10			0.18			0.11
%CV			8			19			10
%Recovery			125			96			109

TABLE 39A: B19 ACCURACY – SPIKED IN DILUENT

B19 Runs	Standard 1			Standard 2			Standard 3		
	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	210724668	160218327	8.2	13456161.0	12445407	7.1	1184998	916525	6.0
	129386447			12421311			751021		
	140543866			11458749			813556		
Run 2	113878603	107063652	8.0	10659957	11304643	7.1	1124046	1137126	6.1
	106821237			11533385			1171900		
	100491117			11720588			1115431		
Run 3	107084090	106820240	8.0	9904297	11405052	7.1	869053	882816	5.9
	107597408			10132339			893465		
	105779223			14178519			885930		
Avg		124700740	8.1		11718367	7.1		978822	6.0

Expected		100000000	8.0		10000000	7.0		1000000	6.0
SD			0.10			0.02			0.06
%CV			1			0			1
%Recovery			101			101			100

TABLE 39B: B19 ACCURACY – SPIKED IN DILUENT

B19 Runs	Standard 4			Standard 5			Standard 6		
	Copies / well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	76333	74817	4.9	7990.0	6959	3.8	916	571	2.8
	74967			6585			424		
	73150			6301			373		
Run 2	120602	111568	5.0	12115	12113	4.1	1183	1022	3.0
	107027			11011			1091		
	107074			13212			791		
Run 3	99462	98760	5.0	9847	10760	4.0	957	990	3.0
	97032			10825			1062		
	99786			11607			952		
Avg		95048	5.0		9944	4.0		861	2.9
Expected		100000	5.0		10000	4.0		1000	3.0
SD			0.09			0.13			0.14
%CV			2			3			5
%Recovery			100			100			98

TABLE 39C: B19 ACCURACY – SPIKED IN DILUENT

Runs	Standard 7			Standard 8		
	Copies/well	Avg Copies/well	Log (10) Copies/well	Copies/well	Avg Copies/well	Log (10) Copies/well
Run 1	96	101	2.0	9.0	15	1.2
	95			18		
	111			19		
Run 2	96	94	2.0	13	11	1.0
	91			10		
	96			9		
Run 3	117	112	2.0	12	9	1.0
	98			11		
	120			5		
Avg		102	2.0		12	1.1
Expected		100	2.0		10	1.0
SD			0.04			0.11
%CV			2			10
%Recovery			100			107

Limits of Quantification (ULOQ/ LLOQ)

[0267] The results of the Linearity were evaluated to determine Lower Limit of Quantification (LLOQ). The Upper Limit of Quantification (ULOQ) was defined as the highest concentration (i.e., the Log-transformed copy/well value) that returned a result within 15% of the expected concentration and that also showed a precision with a CV of <15%. The LLOQ was defined as the lowest concentration that returned a result within 20% of the expected concentration and a %CV of <20% LLOQ. Range was determined from the standard curves of the linearity study.

[0268] The results of the Accuracy study (Table 22-Table 39C) were evaluated to

determine Upper Limit of Quantification (ULOQ) and Lower Limit of Quantification (LLOQ). The ULOQ was defined as 10,00,000 copies/well for the targets (HHV7, HHV8, EBV, HAV) and 10,00,00,000 copies/well for the targets (HHV6 and B19) or the highest concentration (i.e., the Log-transformed copy/well value) that returned a result within 20% of the expected concentration and that also showed precision with a %CV of ≤ 20%. The LLOQ was defined as 10 copies/well, the lowest concentration (i.e., the Log- transformed copy/well value) that returned a result within 30% of the expected concentration and that also showed precision with a %CV of ≤30% (see also Table 3).

Precision

[0269] Intra assay Precision

[0270] Repeatability of the method was ascertained by determining the coefficient of variation of the measured Ct value and concentration (i.e. Log-transformed copies/well value) obtained between six replicates of extracted DNA/RNA spiked in with known concentrations (100 copies, 10 copies) of Virus standards in a single assay.

[0271] To assess repeatability, RNA/DNA was extracted from samples, their concentrations were measured, and RNA/DNA was diluted to 100 ng/µl. The diluted RNA/DNA (0.5 µg) was spiked with 2 different concentrations of the target (HHV6, HHV7, HHV8, EBV, HAV, B19 positive control) within the range of the standard, including 100 copies/well and 10 copies/well. Six replicates per each spiked in concentrations were measured in a single assay. To meet acceptance criteria for repeatability, the measured concentration (Log- transformed copies/well value) was expected to demonstrate precision with ≤20% for the 100 copies/well spiked sample except for the 10 copies/well spiked sample, where it was expected to be within ≤30% CV. Acceptance criteria for intra assay repeatability were met for the HHV6, HHV7, HHV8, EBV, HAV, B19 targets and therefore the assay was considered to demonstrate acceptable repeatability.

[0272] Inter assay Precision

[0273] Intermediate precision was evaluated by determining the coefficient of variation from reported values across multiple operators across multiple days in at least three independent assays.

[0274] As the assays were found to be reproducible within the same run, intermediate (or inter assay) reproducibility was examined next. Diluted RNA/DNA (0.5 µg) spiked with 2 different concentrations of the target (HHV6, HHV7, HHV8, EBV, HAV, B19 positive control) within the range of the standard curve were tested, including 100 copies/well and 10 copies/well. Three replicates per each spiked in concentration were measured in three independent assays, performed by two different operators over multiple days. To meet

acceptance criteria for Intermediate precision, the measured concentration (Log-transformed copies/well value) was expected to demonstrate precision with ≤20% for the 100 copies/well spiked sample except for the 10 copies/well spiked sample, where it was expected to be within ≤30% CV. Results for Intermediate precision are shown in Table 40-Table 51 below. Acceptance criteria for Intermediate precision were met for the target (HHV6, HHV7, HHV8, EBV, HAV, B19), and therefore the assay was considered to demonstrate acceptable Intermediate precision over different runs, and operators.

TABLE 40: HAV INTERASSAY PRECISION – LEVEL 1 (100 COPIES)

HAV Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	55	64	1.8	44	50	1.7	59	83	1.9
	57			59			66		
	81			46			123		
Run 2	59	85	1.9	78	80	1.9	116	105	2.0
	96			72			98		
	102			92			100		
Run 3	141	109	2.0	96	102	2.0	97	97	2.0
	108			113			104		
	78			97			90		
Avg		86	1.9		77	1.9		95	2.0
SD			0.12			0.16			0.05
%CV			6			8			3

TABLE 41: HAV INTERASSAY PRECISION – LEVEL 2 (10 COPIES)

HAV Runs	Sample 1			Sample 2			Sample 3		
	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	10	10	1.0	8	9	0.9	4	9	1.0
	13			3			12		
	7			15			12		
Run 2	10	9	0.9	7	10	1.0	8	8	0.9
	10			18			10		
	6			5			6		
Run 3	10	10	1.0	12	9	1.0	16	13	1.1
	9			10			21		
	12			5			3		
Avg		10	1.0		9	1.0		10	1.0
SD			0.03			0.04			0.12
%CV			4			4			12

TABLE 42: EBV INTERASSAY PRECISION – LEVEL 1 (100 COPIES)

EBV Runs	Sample 1			Sample 2			Sample 3		
	Copies/ well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	126	117	2.1	92	104	2.0	88	89	1.9
	125			124			107		
	99			97			72		
Run 2	155	143	2.2	136	122	2.1	104	111	2.0
	164			106			126		
	111			125			102		
Run 3	183	150	2.2	118	127	2.1	145	114	2.1

	138			115			87		
	128			147			110		
Avg		137	2.1		118	2.1		105	2.0
SD			0.06			0.04			0.06
%CV			3			2			3

TABLE 43: EBV INTERASSAY PRECISION – LEVEL 2 (10 COPIES)

	Sample 1			**Sample 2**			**Sample 3**		
EBV Runs	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	13	15	1.2	8	9	0.9	4	11	1.0
	22			10			8		
	11			8			21		
Run 2	20	11	1.0	15	13	1.1	15	16	1.2
	5			12			29		
	6			11			4		
Run 3	21	13	1.1	12	7	0.8	4	8	0.9
	8			6			12		
	10			3					
Avg		13	1.1	11	9	1.0		12	1.1
SD			0.08			0.13			0.15
%CV			7			13			14

TABLE 44: HHV6 INTERASSAY PRECISION – LEVEL 1 (100 COPIES)

	Sample 1			**Sample 2**			**Sample 3**		
HHV6 Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	164	135	2.1	163	147	2.2	146	140	2.1
	125			148			133		
	115			131			140		
Run 2	98	92	2.0	107	121	2.1	126	119	2.1
	107			128			111		
	72			130			119		
Run 3	107	103	2.0	161	114	2.1	106	118	2.1
	8*			72			124		
	99			108			125		
Avg		110	2.0		127	2.1		126	2.1
SD			0.08			0.06			0.04
%CV			4			3			2

TABLE 45: HHV6 INTERASSAY PRECISION – LEVEL 2 (10 COPIES)

	Sample 1			**Sample 2**			**Sample 3**		
HHV6 Runs	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	19	21	1.3	20	14	1.2	27	18	1.3
	24			4			7		
	19			19			20		
Run 2	10	9	1.0	21	21	1.3	6	10	1.0
	14			7			13		
	3			35			11		
Run 3	17	16	1.2	11	9	1.0	11	10	1.0
	25			11			12		
	7			6			6		
Avg		15	1.2		15	1.2		13	1.1
SD			0.18			0.17			0.15
%CV			15			15			14

TABLE 46: HHV7 INTERASSAY PRECISION – LEVEL 1 (100 COPIES)

	Sample 1				Sample 2				Sample 3			
HHV7 Runs	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies/ well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	132	111	139	2.1	124	115	118	2.1	132	NA	129	2.1
	204	183			121	112			124	NA		
	143	122			138	129			130	NA		
Run 2	183	171	120	2.1	148	140	113	2.1	135	NA	113	2.1
	130	118			86	78			89	NA		
	82	70			130	122			114	NA		
Run 3	87	76	128	2.1	142	134	122	2.1	84	NA	101	2.0
	167	157			136	129			112	NA		
	162	151			111	104			107	NA		
Avg			129	2.1			118	2.1			114	2.1
SD				0.03				0.02				0.05
%CV				2				1				3

TABLE 47: HHV7 INTERASSAY PRECISION – LEVEL 2 (10 COPIES)

	Sample 1				Sample 2				Sample 3			
HHV 7 Runs	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	Copies /well	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	34	13	11	1.0	17	8	12	1.1	8	NA	10	1.0
	33	12			27	18			16	NA		
	27	6			20	11			7	NA		
Run 2	20	8	13	1.1	12	4	8	0.9	15	NA	9	0.9
	33	21			21	13			6	NA		
	23	11			15	7			5	NA		
Run 3	16	6	18	1.2	16	9	15	1.2	12	NA	12	1.1
	34	24			34	26			16	NA		
	34	24			17	9			8	NA		
Avg			14	1.1			12	1.1			10	1.0
SD				0.11				0.13				0.07
%CV				10				13				7

TABLE 48: HHV8 INTERASSAY PRECISION – LEVEL 1 (100 COPIES)

	Sample 1			Sample 2			Sample 3		
HHV8 Runs	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	56	66	1.8	62	63	1.8	66	62	1.8
	64			66			68		
	78			61			52		
Run 2	110	112	2.1	134	113	2.1	103	99	2.0
	103			95			106		
	124			112			87		
Run 3	90	87	1.9	86	83	1.9	103	99	2.0
	108			73			106		
	64			90			87		
Avg		89	1.9	90	86	1.9		87	1.9
SD			0.12			0.13			0.12
%CV			6			7			6

TABLE 49: HHV8 INTERASSAY PRECISION – LEVEL 2 (10 COPIES)

	Sample 1			Sample 2			Sample 3		
HHV8 Runs	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies /well	Log (10) Copies /well
Run 1	4	4	0.6	13	7	0.9	4	6	0.8
	6			2			5		
	2			8			8		
Run 2	15	12	1.1	14	12	1.1	3	11	1.0
	37*			14			18		
	9			10			11		
Run 3	10	10	1.0	7	6	0.8	1*	5	0.7
	9			3			5		
	11			9			4		
Avg		9	0.9		9	0.9		7	0.8
SD			0.26			0.15			0.19
%CV			27			16			23

TABLE 50: B19 INTERASSAY PRECISION – LEVEL 1 (100 COPIES)

	Sample 1			Sample 2			Sample 3		
B19 Runs	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	143	130	2.1	138	140	2.1	88	114	2.1
	134			135			124		
	112			148			131		
Run 2	148	159	2.2	178	175	2.2	169	299	2.5
	141			159			243		
	188			189			484		
Run 3	235	210	2.3	229	212	2.3	160	189	2.3
	176			190			219		
	219			217			189		
Avg		166	2.2		176	2.2		201	2.3
SD			0.11			0.09			0.21
%CV			5			4			9

TABLE 51: B19 INTERASSAY PRECISION – LEVEL 2 (10 COPIES)

	Sample 1			Sample 2			Sample 3		
B19 Runs	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well	Copies /well	Avg Copies/well	Log (10) Copies/well
Run 1	22	23	1.4	38	23	1.4	11	21	1.3
	22			10			44		
	26			21			8		
Run 2	10	12	1.1	5	29	1.5	5	10	1.0
	18			31			8		
	8			52			16		
Run 3	10	12	1.1	11	11	1.0	7	17	1.2
	16			12			12		
	11			10			32		
Avg		16	1.2		21	1.3		16	1.2
SD			0.16			0.22			0.17
%CV			14			17			15

Robustness

[0275] The Robustness of the assay was determined by running the Standard curve samples (1E6 copies down to 10 copies) at a different annealing temperature. 2 runs were performed and % Recovery of the spiked samples was calculated.

[0276] To determine Robustness of the assay, Standard curve samples and spike in samples (100 copies, 10 copies/well) were run at a different annealing temperature (Table 52). Three replicates per each spiked in concentrations or standards were measured in two independent assays, performed by two different operators. To meet acceptance criteria for Robustness of the assay, the mean observed Ct values must be ≤20%. The spike in samples showed unacceptable % CV and % Recovery and therefore, the assay is recommended to be performed at optimal annealing temperature. Results are shown in Table 53-Table 63.

TABLE 52: EXEMPLARY OPTIMAL AND TESTED ANNEALING TEMPERATURES

Virus tested	**Optimal Annealing temperature**	**Annealing temperature tested during robustness**
HHV6	55°C	60°C
HHV7	60°C	55°C
HHV8	60°C	55°C
EBV	60°C	55°C
HAV	55°C	60°C
B19	60°C	55°C

TABLE 53: HAV ROBUSTNESS – LEVEL 1 (100 COPIES)

HAV Runs	**Sample 1**					**Sample 2**					**Sample 2**				
	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	262	200	2.3	32.5	33.0	262	200	2.3	33.8	33.5	184	172	2.2	33.1	33.2
	139			33.5		139			33.4		183			33.1	
	200			32.9		200			33.2		149			33.4	
Run 2	130	126	2.1	32.7	32.7	167	170	2.2	32.3	32.3	100	91	2.0	33.1	33.2
	133			32.6		194			32.1		85			33.3	
	115			32.9		148			32.5		89			33.3	
Avg		163	2.2		32.9		185	2.3		32.9		132	2.1		33.2
SD			0.14		0.16			0.05		0.82			0.19		0.02
%CV			6		1			2		3			9		0
Expected		100	2.0				100	2.0				100	2.0		
% Recovery			110					113					105		

TABLE 54: HAV ROBUSTNESS – LEVEL 2 (10 COPIES)

HAV Runs	**Sample 1**					**Sample 2**					**Sample 3**				
	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	6	11	1.1	38.4	37.6	12	15	1.2	37.4	37.0	10	8	0.9	37.6	38.1
	18			36.7		17			36.9		6			38.5	
	10			37.7		18			36.8					Und	
Run 2	6	6	0.8	37.3	37.6	7	8	0.9	37	36.9	8	13	1.1	36.8	36.5
	10			36.5		6			37.2		6			37.4	
	2			38.9		11			36.5		24			35.3	
Avg		9	0.9		37.6		12	1.0		37.0		10	1.0		37.3
SD			0.20		0.02			0.20		0.09			0.13		1.10
%CV			22		0			19		0			13		3

Expected		10	1.0				10	1.0				10	1.0		
% Recovery			92					104					101		

TABLE 55: EBV ROBUSTNESS – LEVEL 1 (100 COPIES)

	Sample 1					Sample 2					Sample 3				
EBV Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	49	68	1.8	35	34.5	34	42	1.6	35.6	35.3	40	46	1.7	35.3	35.1
	71			34.4		34			35.6		48			35	
	83			34.1		56			34.8		49			35	
Run 2	69	66	1.8	34.9	35.0	59	57	1.8	35.2	35.3	61	68	1.8	35.2	34.0
	76			34.8		56			35.3		76			34.8	
	53			35.4		56			35.3		405*			31.9	
Avg		67	1.8		34.8		49	1.7		35.3		57	1.8		34.5
SD			0.01		0.38			0.10		0.05			0.12		0.80
%CV			0		1			6		0			7		2
Expected		100	2.0				100	2.0				100	2.0		
% Recovery			91					85					88		
*Outlier															

TABLE 56: EBV ROBUSTNESS – LEVEL 2 (10 COPIES)

	Sample 1					Sample 2					Sample 3				
EBV Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	0	1	-0.2	43.9	42.5	0	0	-0.3	45	43.2	0	1	0.0	44.1	42.1
	1			42.1		1			41.8		2			40.1	
	1			41.6		0			42.7		1			42	
Run 2	4	5	0.7	39.6	39.3	2	6	0.8	40.9	39.6	3	4	0.6	40.1	39.8
	5			39.4		6			39		4			39.7	
	7			38.9		6			39		4			39.6	
Avg		3	0.5		40.9		3	0.2		41.4		3	0.3		40.9
SD			0.68		2.29			0.81		2.50			0.42		1.60
%CV			142		6			360		6			134		4
Expected		10	1.0				10	1.0				10	1.0		
% Recovery			8					53					40		

TABLE 57: HHV6 ROBUSTNESS – LEVEL 1 (100 COPIES)

	Sample 1					Sample 2					Sample 3				
HHV6 Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	29	54	1.7	37.0	36.2	63	56	1.7	35.7	35.9	73	60	1.8	35.4	35.8
	Und			Und		Und			Und		65			35.7	
	78			35.3		48			36.1		41			36.4	
Run 2	15	24	1.4	34.9	34.3	12	27	1.4	35.3	34.2	8	21	1.3	35.9	34.7
	36			33.6		34			33.7		36			33.6	
	21			34.5		36			33.6		19			34.6	
Avg		39	1.6		35.2		42	1.6		35.1		40	1.5		35.3

SD			0.25		1.29			0.22		1.21			0.32		0.81
%CV			16		4			14		3			21		2
Expected		100	2.0				100	2.0				100	2.0		
% Recovery			78					80					77		

TABLE 58: HHV6 ROBUSTNESS – LEVEL 2 (10 COPIES)

	Sample 1					Sample 2					Sample 3				
HHV6 Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	9	15	1.2	38.9	38.3	und	74	1.9	Und	35.4	und	29	1.5	Und	37.0
	20			37.6		und			Und		und			Und	
	und			Und		74			35.4		29			37.0	
Run 2	7	12	1.1	36.2	35.5	20	13	1.1	38.0	37.4	3	4	0.6	37.6	37.2
	und			Und		und			Und		4			36.8	
	17			34.7		7			36.7		und			Und	
Avg		13	1.1		36.9		44	1.5		36.4		16	1.0		37.1
SD			0.06		1.98			0.52		1.38			0.64		0.15
%CV			6		5			35		4			64		0
Expected		10	1.0				10	1.0				10	1.0		
% Recovery			112					150					101		

TABLE 59: HHV7 ROBUSTNESS – LEVEL 1 (100 COPIES)

	Sample 1					Sample 2					Sample 3				
HHV7 Runs	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	91	83	1.9	29.7	29.8	82	76	1.9	29.9	30.0	48	57	1.8	30.8	30.5
	76			30.0		72			30.1		67			30.3	
	81			29.9		76			30.0		56			30.5	
Run 2	87	99	2.0	29.3	29.2	87	89	1.9	29.4	29.4	75	79	1.9	29.8	29.7
	79			29.5		79			29.6		94			29.4	
	130			28.8		99			29.3		67			29.9	
Avg		91	2.0		29.5		82	1.9		29.7		68	1.8		30.1
SD			0.05		0.46			0.05		0.38			0.10		0.59
%CV			3		2			2		1			5		2
Expected		100	2.0				100	2.0				100	2.0		
% Recovery			98					96					91		

TABLE 60: HHV7 ROBUSTNESS – LEVEL 2 (10 COPIES)

	Sample 1					Sample 2					Sample 3				
HHV7 Runs	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Adjusted Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	2	4	0.6	33.7	33.4	-2	2	0.3	35.0	33.9	4	5	0.7	34.6	34.7
	3			33.5		2			33.7		9			33.3	
	6			33.1		6			33.1		1			36.3	
Run 2	2	13	1.1	32.2	31.5	-1	0	#NUM!	34.1	33.8	1	2	0.3	36.9	35.5
	8			31.7		1			33.5		4			34.2	
	28			30.7		0			33.8		2			35.5	

Avg		8	0.8		32.5		1	#NUM!		33.9		3	0.5		35.1
SD			0.38		1.33			#NUM!		0.09			0.24		0.57
%CV			46		4			#NUM!		0			48		2
Expected		10	1.0				10	1.0				10	1.0		
% Recovery			83					#NUM!					51		

TABLE 61: HHV8 ROBUSTNESS – LEVEL 1 (100 COPIES)

	Sample 1					Sample 2					Sample 3				
HHV8 Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	54	61	1.8	29.6	29.5	112	99	2.0	28.5	28.7	60	57	1.8	29.5	29.6
	60			29.5		78			29.1		60			29.5	
	67			29.3		109			28.6		50			29.8	
Run 2	Und					54	49	1.7	30.2	30.3	94	83	1.9	29.4	29.6
	Und					47			30.4		68			29.9	
	Und					47			30.4		88			29.5	
Avg			NA		NA		74	1.8		29.5		70	1.8		29.6
SD								0.22		1.12			0.12		0.00
%CV								12		4			6		0
Expected		100	2.0				100	2.0				100	2.0		
% Recovery								92					92		

TABLE 62: HHV8 ROBUSTNESS – LEVEL 2 (10 COPIES)

	Sample 1					Sample 2					Sample 3				
HHV8 Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT
Run 1	2	4	0.6	34.7	33.8	2	3	0.4	34.8	35.6	4	3	0.5	33.8	34.0
	8			32.6		0			38.3		2			34.8	
	3			33.9		4			33.8		5			33.4	
Run 2	3	3	0.5	34.4	34.2	1	1	0.1	35.5	35.3	4	3	0.4	33.7	34.5
	2			34.6		2			35.1		3			34.3	
	5			33.5		1			35.3		1			35.4	
Avg		4	0.6		34.0		2	0.3		35.5		3	0.5		34.2
SD			0.08		0.29			0.22		0.24			0.08		0.34
%CV			15		1			78		1			17		1
Expected		10	1.0				10	1.0				10	1.0		
% Recovery			58					28					48		

TABLE 63: B19 ROBUSTNESS – LEVEL 1 (100 COPIES

	Sample 1					Sample 2					Sample 3				
B19 Runs	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies /well	Log (10) Copies /well	CT	Avg CT	Copies /well	Avg Copies/well	Log (10) Copies/well	CT	Avg CT
Run 1	90	85	1.9	29.7	29.9	127	72	1.9	29.2	30.2	59	63	1.8	30.3	30.3
	123			29.2		41			30.8		36			31.0	
	41			30.8		49			30.6		94			29.6	
Run 2	Und			Und		Und			Und		Und			Und	
	Und			Und		Und			Und		Und			Und	

	Und			Und		Und			Und		Und			Und	
Avg			NA	NA				NA	NA				NA	NA	
SD															
%CV															
Expected		100	2.0	NA			100	2.0	NA			100	2.0	NA	
% Recovery															

Specificity

[0277] To assess the specificity, specific primer probe sets other than the ones used in the assay were tested for off target amplification. Viral DNA/RNA other than the target was used to determine the specificity of the probes and primers used in the assay (Table 64). For example, for HHV7 assay, HHV8 standard 1 samples (copy number of 1,000,000) was used to determine specificity of the primers and probe sets.

[0278] To assess the specificity of the assay, Assay specific primer probe sets were tested for off-target amplification. RNA/DNA template (with the highest standard concentration-1e6 or 1e8 copies/well) was used to determine the specificity of the probes and primers used in the assay. Each sample was tested in three replicates in at least 5 runs by multiple operators. To meet acceptance criteria, the target (HHV6, HHV7, HHV8, EBV, HAV, B19) specific primer probe sets were expected to show undetectable signal for the off-target template.

TABLE 64: PRIMER/PROBE AND TEMPLATE PAIRS FOR SPECIFICITY TESTING

Assay specific Primers and probes	Template used
HHV6	HHV7
HHV7	HHV8
HHV8	HHV7
EBV	HHV7
HAV	HEV
B19	HHV7

[0279] Assays were not able to detect sequence for specificity test templates, indicting the assays are indeed specific for their targets. Therefore, acceptable criteria were met for the target, demonstrating that the assay demonstrates acceptable specificity for detecting the desired target (HHV6, HHV7, HHV8, EBV, HAV, B19).

[0280] The Acceptance/Rejection Criteria for each parameter assessed during this qualification process are listed under each section in the discussion of results.

[0281] The Acceptance/Rejection Criteria for each qualification run were defined as: (1) The R2 of the standard curve must be ≥ 0.995, (2) The NTC (DNA/RNA extraction control samples extracted during each run) must show positive signal for VIC in at least 2/3 replicates, (3), The water NTC must show undetectable signal in 3/3 replicates. (4) All reported Ct Values must be derived from linear phase of the exponential amplification when viewed using the log view of the amplification plot (ΔRn vs. Cycle), (5) The average Ct value for each DNA Standard

should between 3.1 - 3.6 cycles later than the next DNA Standard.

TABLE 65: CONCLUSIONS

Parameter	Result	Comments
Linearity	PASS	$R^2 \geq 0.995$
Range (ULOQ-LLOQ)	PASS	The assay was able to detect Viral copy number of samples ranging from 1e6 to 10 copies/well for HHV7, HHV8, EBV, HAV and 1e8 to 10 copies/well for HHV6 and B19.
Accuracy	PASS	Acceptable recovery (80-120%) from Spike Level 1. Acceptable recovery (70-130%) from Spike Level 2. Acceptable recoveries were obtained for all the levels of standards spiked in assay specific buffers.
Precision-Intra assay Precision-Inter assay	PASS	Acceptable precision % CV ≤ 20% for Spike Level 1 (100 copies), % CV ≤ 30% for Spike Level 2 (10 copies).
Specificity	PASS	The assay is specific for detecting the specific virus that was tested.
Robustness	NA	Based on the data, changing the primer annealing temperature is not efficient unacceptable recoveries and varying % PCR efficiency were observed.

Example 5

B19 Viral Assay

[0282] This example describes the superior performance of the compositions and methods disclosed herein for detection of a virus, e.g., Parvovirus B19, in a sample.

[0283] Shown in Table 66 are data from a representative experiment analyzing a dilution curve using standards and other reagents from the Genesig Parvovirus B19 kit. Experiments were performed according to manufacturer's instructions. As shown in Table 66, the observed concentration of standard DNA in the reactions can be almost 1000-fold higher than expected (compare, for example, column 2 of Table 66 to column 7).

TABLE 66: B19 GENESIG STANDARD

Expected B19 DNA conc (Copies/ml)	Expected B19 Copy number (Copies/ml)	Log (10) expected DNA conc (Copies/ml)	Sample	Observed B19 DNA Conc (Copies/ml)	Outliers	Avg Observed B19 DNA Conc (Copies/ml)	Log (10) Observed DNA conc (Copies/ml)	%RECOVERY
1E+08	100,000,000	8.0	Std1	82,122,006,528		82,122,006,528	10.9	136
				82,122,006,528				
				82,122,006,528				
1E+07	10,000,000	7.0	Std2	3,131,889,920		3,131,889,920	9.5	136
				3,131,889,920				
				3,131,889,920				
1E+06	1,000,000	6.0	Std3	109,628,608		109,628,608	8.0	134
				109,628,608				
				109,628,608				
1E+05	100,000	5.0	Std4	3,686,505		3,686,505	6.6	131
				3,686,505				
				3,686,505				
1E+04	10,000	4.0	Std5	123,788		123,788	5.1	127
				123,788				
				123,788				

1E+03	1,000	3.0	Std6	5,797		5,797	3.8	125
				5,797				
				5797				
1E+02	100	2.0	Std7			325	2.5	126
				325				
1E+01	10	1.0	Std8					

[0284] In contrast to the above, the presently disclosed compositions and methods produce data that is more accurate than previous methods. Table 67A-Table 67B and FIG. 2 show the results from a standard curve resulting from the B19 standard assays disclosed herein. As depicted below, the data show linear amplification resulting in ~100% efficiency.

TABLE 67A: B19 CRISPR ASSAY STANDARD CURVE

DNA Standard	[Virus] (copies/rxn)	Log conc	Average Cq	Delta Cq
1	100,000,000	8.00	12.77	
2	10,000,000	7.00	16.17	3.41
3	1,000,000	6.00	19.15	2.98
4	100,000	5.00	22.44	3.29
	10,000	4.00	25.99	3.55
	1,000	3.00	29.58	3.59
5	100	2.00	32.53	2.95
6	10	1.00	35.88	3.34

TABLE 67B: B19 ASSAY STANDARD CURVE SUMMARY

Statistic	Value	Note
Efficiency:	100%	Should be between 90 and 110%
Slope:	-3.3145	Value must be the same as on the graph (*See*, FIG. 2)
R-squared:	0.9996	Should be between 0.99 and 1.00

[0285] In these experiments, samples and qPCR reactions were set up as described above in Example 1.

[0286] Briefly, standard samples were prepared at concentrations shown in Table 67A. For PCR reactions, 20 µl of low reference dye was added to 5 ml of IDT PrimeTime Gene Expression Master mix. Using the volumes in Table 7, a working master mix was prepared based upon number of samples. Appropriate volumes of RNAse/DNase free water, B19 primer/probe mix (FAM), DNA extraction control primer/probe mix (VIC) and IDT Primetime Gene Expression Master Mix for each sample were added to a centrifuge tube and the tube was mixed by vortexing. 5 µL of DNA template for each of the standards/controls/and samples were loaded next. 15 µl of Working Master Mix was pipetted into each well. The final volume in each well was 20 µL. B19 assay run conditions are shown in Table 8.

[0287] Table 68 below shows that the assay is extremely accurate, even down to 10 cp/sample.

TABLE 68: B19 ASSAY RECOVERY

Level of Std Spiked	Expected B19 DNA conc (Copies/ml)	Log (10) expected DNA conc (Copies/ml)	Sample	Observed B19 DNA Conc (Copies/ml)	Outliers	Avg Observed B19 DNA Conc (Copies/ml)	Log (10) Observed DNA conc (Copies/ml)	%Recovery Log (10)
Std1	104,000,000	8.0	Std1	175983840		175983840	8.2	103
Std2	10,400,000	7.0	Std2	14824256		14824256	7.2	102
Std3	1,040,000	6.0	Std3	1648782		1648782	6.2	103
Std4	104,000	5.0	Std4	173157		173157	5.2	104
Std5	10,400	4.0	Std5	13914		13914	4.1	103
Std6	1,040	3.0	Std6	1186		1186	3.1	102
Std7	104	2.0	Std7	120		120	2.1	103
Std8	10.4	1.0	Std8	16		16	1.2	119

[0288] In at least some of the previously described embodiments, one or more elements used in an embodiment can interchangeably be used in another embodiment unless such a replacement is not technically feasible. It will be appreciated by those skilled in the art that various other omissions, additions and modifications may be made to the methods and structures described above without departing from the scope of the claimed subject matter. All such modifications and changes are intended to fall within the scope of the subject matter, as defined by the appended claims.

[0289] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Any reference to "or" herein is intended to encompass "and/or" unless otherwise stated.

[0290] It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (*e.g.*, bodies of the appended claims) are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation,

even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (*e.g.*, "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (*e.g.*, the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, " a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, " a system having at least one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms.

[0291] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0292] As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0293] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

WHAT IS CLAIMED IS:

1. A method of detecting Human Parvovirus B19 (B19) in a sample, comprising:

(a) contacting a sample with a pair of primers capable of hybridizing to a target region of B19 genome, wherein the target region comprises the NS1 gene region of B19, and wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2;

(b) generating amplicons of the target region of B19 from the sample, if the sample comprises B19; and

(c) determining the presence or amount of the amplicons of the target region as an indication of the presence of B19 in the sample.

2. The method of claim 1, wherein each primer in the pair of primers comprises the sequence of SEQ ID NO: 1 or SEQ ID NO: 2, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1 or SEQ ID NO: 2.

3. The method of any one of claims 1-2, wherein determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3.

4. The method of claim 3, wherein the oligonucleotide probe comprises the sequence of SEQ ID NO: 3, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3.

5. The method of any one of claims 1-4, wherein the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4.

6. The method of any one of claims 1-4, comprising adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4 to the sample.

7. The method of any one of claims 5-6, wherein the linear dsDNA comprises the sequence of SEQ ID NO: 4, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 4.

8. The method of any one of claims 1-7, wherein the sample comprises no more than 1×10^8 copies of B19 genome.

9. A method of detecting Human Herpes Virus 6 (HHV6) in a sample, comprising:

(a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV6 genome, wherein the target region comprises the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least

about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6;

(b) generating amplicons of the target region of HHV6 from the sample, if the sample comprises HHV6; and

(c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV6 in the sample.

10. The method of claim 9, wherein each primer in the pair of primers comprises the sequence of SEQ ID NO: 5 or SEQ ID NO: 6, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5 or SEQ ID NO: 6.

11. The method of any one of claims 9-10, wherein determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 7.

12. The method of claim 11, wherein the oligonucleotide probe comprises the sequence of SEQ ID NO: 7, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 7.

13. The method of any one of claims 9-12, wherein the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 8.

14. The method of any one of claims 9-12, comprising adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 8 to the sample.

15. The method of any one of claims 13-14, wherein the linear dsDNA comprises the sequence of SEQ ID NO: 8, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 8.

16. The method of any one of claims 9-15, wherein the sample comprises no more than 1×10^8 copies of HHV6 genome.

17. A method of detecting Human Herpes Virus 7 (HHV7) in a sample, comprising:

(a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV7 genome, wherein the target region comprises the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10;

(b) generating amplicons of the target region of HHV7 from the sample, if the sample comprises HHV7; and

(c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV7 in the sample.

18. The method of claim 17, wherein each primer in the pair of primers comprises the sequence of SEQ ID NO: 9 or SEQ ID NO: 10, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9 or SEQ ID NO: 10.

19. The method of any one of claims 17-18, wherein determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 11.

20. The method of claim 19, wherein the oligonucleotide probe comprises the sequence of SEQ ID NO: 11, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 11.

21. The method of any one of claims 17-20, wherein the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12.

22. The method of any one of claims 17-20, comprising adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12 to the sample.

23. The method of any one of claims 21-22, wherein the linear dsDNA comprises the sequence of SEQ ID NO: 12, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 12.

24. The method of any one of claims 17-23, wherein the sample comprises no more than 1×10^6 copies of HHV7 genome.

25. A method of detecting Human Herpes Virus 8 (HHV8) in a sample, comprising:

(a) contacting a sample with a pair of primers capable of hybridizing to a target region of HHV8 genome, wherein the target region comprises the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14;

(b) generating amplicons of the target region of HHV8 from the sample, if the sample comprises HHV8; and

(c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HHV8 in the sample.

26. The method of claim 25, wherein each primer in the pair of primers comprises the sequence of SEQ ID NO: 13 or SEQ ID NO: 14, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13 or SEQ ID NO: 14.

27. The method of any one of claims 25-26, wherein determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an

oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 15.

28. The method of claim 27, wherein the oligonucleotide probe comprises the sequence of SEQ ID NO: 15, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 15.

29. The method of any one of claims 25-28, wherein the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16.

30. The method of any one of claims 25-28, comprising adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16 to the sample.

31. The method of any one of claims 29-30, wherein the linear dsDNA comprises the sequence of SEQ ID NO: 16, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 16.

32. The method of any one of claims 25-31, wherein the sample comprises no more than 1×10^6 copies of HHV8 genome.

33. A method of detecting Epstein Barr Virus (EBV) in a sample, comprising:

(a) contacting a sample with a pair of primers capable of hybridizing to a target region of EBV genome, wherein the target region comprises the NA1 gene of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18;

(b) generating amplicons of the target region of EBV from the sample, if the sample comprises EBV; and

(c) determining the presence or amount of the amplicons of the target region as an indication of the presence of EBV in the sample.

34. The method of claim 33, wherein each primer in the pair of primers comprises the sequence of SEQ ID NO: 17 or SEQ ID NO: 18, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17 or SEQ ID NO: 18.

35. The method of any one of claims 33-34, wherein determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 19; optionally wherein the oligonucleotide probe comprises the sequence of SEQ ID NO: 19, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 19.

36. The method of any one of claims 33-35, wherein the sample comprises a linear

double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20.

37. The method of any one of claims 33-35, comprising adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20 to the sample.

38. The method of any one of claims 36-37, wherein the linear dsDNA comprises the sequence of SEQ ID NO: 20, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 20.

39. The method of any one of claims 33-38, wherein the sample comprises no more than 1×10^6 copies of EBV genome.

40. A method of detecting Hepatitis A Virus (HAV) in a sample, comprising:

(a) contacting a sample with a pair of primers capable of hybridizing to a target region of HAV genome, wherein the target region is the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence that exhibits at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22;

(b) generating amplicons of the target region of HAV from the sample, if the sample comprises HAV; and

(c) determining the presence or amount of the amplicons of the target region as an indication of the presence of HAV in the sample.

41. The method of claim 40, wherein (a) contacting the sample with a pair of primers is in the presence of a reverse transcriptase.

42. The method of claim 40, wherein each primer in the pair of primers comprises the sequence of SEQ ID NO: 21 or SEQ ID NO: 22, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21 or SEQ ID NO: 22.

43. The method of any one of claims 40-41, wherein determining the presence or amount of the amplicons of the target region comprises contacting the amplicons with an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 23; optionally wherein the oligonucleotide probe comprises the sequence of SEQ ID NO: 23, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 23.

44. The method of any one of claims 40-43, wherein the sample comprises a linear double-stranded DNA (dsDNA) comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24.

45. The method of any one of claims 40-43, comprising adding a linear dsDNA comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24 to

the sample.

46. The method of any one of claims 44-45, wherein the linear dsDNA comprises the sequence of SEQ ID NO: 24, or a sequence differing by one, two or three nucleotide mismatches relative to SEQ ID NO: 24.

47. The method of any one of claims 40-46, wherein the sample comprises no more than 1×10^6 copies of HAV genome.

48. A method of detecting one or more of Human Parvovirus B19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), and Hepatitis A Virus (HAV) in a sample, comprising:

a) contacting the sample with a plurality of pairs of primers, wherein the plurality of pairs of primers comprises:

a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2;

a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6;

a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10;

a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14;

a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or

a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22;

b) generating amplicons of the NS1 gene region of B19, amplicons of the U31 gene region of HHV6, amplicons of the U57 gene region of HHV7, amplicons of the BSLF1 gene region of HHV8, amplicons of the NA1 gene region of EBV, amplicons of the 5'-UTR region of HAV, or any combination thereof, if the sample comprises one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV; and

c) determining the presence or amount of one or more amplicons as an indication of the presence of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV in the sample.

49. The method of claim 48, wherein

the pair of primers capable of hybridizing to the NS1 gene region of B19 comprises (a) a primer having the sequence of SEQ ID NO: 1 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1 and (b) a primer having the sequence of SEQ ID NO: 2 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 2;

the pair of primers capable of hybridizing to the U31 gene region of HHV6 comprises (a) a primer having the sequence of SEQ ID NO: 5 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5 and (b) a primer having the sequence of SEQ ID NO: 6 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 6;

the pair of primers capable of hybridizing to the U57 gene region of HHV7 comprises (a) a primer having the sequence of SEQ ID NO: 9 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9 and (b) a primer having the sequence of SEQ ID NO: 10 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 10;

the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 comprises (a) a primer having the sequence of SEQ ID NO: 13 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13 and (b) a primer having the sequence of SEQ ID NO: 14 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 14;

the pair of primers capable of hybridizing to the NA1 gene region of EBV comprises (a) a primer having the sequence of SEQ ID NO: 17 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17 and (b) a primer having the sequence of SEQ ID NO: 18 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 18; and/or

the pair of primers capable of hybridizing to the 5'-UTR region of HAV comprises (a) a primer having the sequence of SEQ ID NO: 21 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21 and (b) a primer having the sequence of SEQ ID NO: 22 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 22.

50. The method of any one of claims 48-49, wherein determining the presence or

amount of the one or more amplicons comprises contacting the amplicons with one or more oligonucleotide probes, wherein the one or more oligonucleotide probes comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23.

51. The method of claim 50, wherein each of the one or more oligonucleotide probes comprises the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23; or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3, 7, 11, 15, 19, or 23.

52. The method of any one of claims 48-51, wherein the sample comprises one or more linear dsDNAs each comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24.

53. The method of claim 52, wherein each of the one or more linear dsDNAs comprises the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24; or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 4, 8, 12, 16, 20, or 24.

54. The method of any one of claims 3-53, wherein the oligonucleotide probe comprises a fluorescence emitter moiety, a fluorescence quencher moiety, or both.

55. The method of any one of claims 3-53, wherein each oligonucleotide probe is flanked by complementary sequences at the 5' end and 3' end; and optionally wherein one of the complementary sequences comprises a fluorescence emitter moiety and the other complementary sequence comprises a fluorescence quencher moiety.

56. The method of any one of claims 5-55, wherein the linear dsDNA is present in the sample in an amount of no more than 100 copies.

57. The method of any one of claims 1-56, wherein (c) determining the presence or amount of the amplicons comprises using a reference standard curve; optionally wherein the reference standard curve is generated based on serial dilutions of the linear dsDNA; and optionally wherein the serial dilutions of the linear dsDNA comprise a logarithmic dilution series from about 10 copies of the linear dsDNA to about 1×10^8 copies of the linear dsDNA in reference samples.

58. The method of any one of claims 48-57, wherein the sample comprises nucleic acids of one or more of B19, HHV6, HHV7, HHV8, EBV, and HAV.

59. The method of any one of claims 1-58, wherein the sample is or is derived from a biological sample or an environmental sample.

60. The method of claim 59, wherein the biological sample is obtained from a tissue sample, saliva, blood, plasma, sera, stool, urine, sputum, mucous, lymph, synovial fluid, cerebrospinal fluid, ascites, pleural effusion, seroma, pus, swab of skin or a mucosal membrane surface, cultures thereof, or any combination thereof.

61. The method of claim 59, wherein the biological sample comprises genetically

modified cells.

62. The method of any one of claims 1-58, wherein the sample is a food sample, a beverage sample, a paper surface, a fabric surface, a metal surface, a wood surface, a plastic surface, a soil sample, a fresh water sample, a waste water sample, a saline water sample, a gas sample, cultures thereof, or any combination thereof.

63. The method of any one of claims 1-62, wherein the sample comprises DNA or RNA.

64. The method of any one of claims 1-63, wherein the amplification is carried out using a method selected from the group consisting of polymerase chain reaction (PCR), ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), replicase-mediated amplification, Immuno-amplification, nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), rolling circle amplification, transcription-mediated amplification (TMA), or a combination thereof.

65. The method of claim 64, wherein the PCR is quantitative real-time PCR (qPCR), optionally wherein the qPCR is or comprises reverse-transcription qPCR (RT-qPCR).

66. A composition for detecting Human Parvovirus B19 (B19) in a sample, comprising: a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO:2.

67. The composition of claim 66, further comprising an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 3.

68. A composition for detecting Human Herpes Virus 6 (HHV6) in a sample, comprising: a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

69. The composition of claim 68, further comprising an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 7.

70. A composition for detecting Human Herpes Virus 7 (HHV7) in a sample, comprising: a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10.

71. The composition of claim 70, further comprising an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 11.

72. A composition for detecting Human Herpes Virus 8 (HHV8) in a sample, comprising: a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8,

wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14.

73. The composition of claim 72, further comprising an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 15.

74. A composition for detecting Epstein Barr Virus (EBV) in a sample, comprising: a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18.

75. The composition of claim 74, further comprising an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 19.

76. A composition for detecting Hepatitis A Virus (HAV) in a sample, comprising: a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein each primer in the pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

77. The composition of claim 76, further comprising an oligonucleotide probe comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 23.

78. A composition for detecting one or more of Human Parvovirus B19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), and Hepatitis A Virus (HAV) in a sample, comprising:

a pair of primers capable of hybridizing to the NS1 gene region of B19, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 1 or SEQ ID NO: 2;

a pair of primers capable of hybridizing to the U31 gene region of HHV6, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 5 or SEQ ID NO: 6;

a pair of primers capable of hybridizing to the U57 gene region of HHV7, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 9 or SEQ ID NO: 10;

a pair of primers capable of hybridizing to the BSLF1 gene region of HHV8, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 13 or SEQ ID NO: 14;

a pair of primers capable of hybridizing to the NA1 gene region of EBV, wherein each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 17 or SEQ ID NO: 18; and/or

a pair of primers capable of hybridizing to the 5'-UTR region of HAV, wherein

each primer in the at least one pair of primers comprises a sequence having at least about 85% identity to the sequence of SEQ ID NO: 21 or SEQ ID NO: 22.

79. The composition of claim 78, wherein

the pair of primers capable of hybridizing to the NS1 gene region of B19 comprises (a) a primer comprising the sequence of SEQ ID NO: 1 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 1, and (b) a primer comprising the sequence of SEQ ID NO: 2 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 2;

the pair of primers capable of hybridizing to the U31 gene region of HHV6 comprises (a) a primer comprising the sequence of SEQ ID NO: 5 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 5, and (b) a primer comprising the sequence of SEQ ID NO: 6 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 6;

the pair of primers capable of hybridizing to the U57 gene region of HHV7 comprises (a) a primer comprising the sequence of SEQ ID NO: 9 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 9, and (b) a primer comprising the sequence of SEQ ID NO: 10 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 10;

the pair of primers capable of hybridizing to the BSLF1 gene region of HHV8 comprises (a) a primer comprising the sequence of SEQ ID NO: 13 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 13, and (b) a primer comprising the sequence of SEQ ID NO: 14 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 14;

the pair of primers capable of hybridizing to the NA1 gene region of EBV comprises (a) a primer comprising the sequence of SEQ ID NO: 17 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 17, and (b) a primer comprising the sequence of SEQ ID NO: 18 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 18; and

the pair of primers capable of hybridizing to the 5'-UTR region of HAV comprises (a) a primer comprising the sequence of SEQ ID NO: 21 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 21, and (b) a primer comprising the sequence of SEQ ID NO: 22 or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 22.

80. The composition of any one of claims 78-79, further comprising one or more oligonucleotide probes each comprising a sequence having at least about 85% identity to the

sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23; optionally wherein each of the one or more oligonucleotide probes comprises the sequence of SEQ ID NO: 3, 7, 11, 15, 19, or 23, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 3, 7, 11, 15, 19, or 23.

81. The composition of claim 80, wherein at least one of the one or more oligonucleotide probes comprises a fluorescence emitter moiety, a fluorescence quencher moiety, or both.

82. The composition of any one of claims 78-81, further comprising one or more linear dsDNAs each comprising a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24; and optionally wherein each of the one or more linear dsDNAs comprises the sequence of SEQ ID NO: 4, 8, 12, 16, 20, or 24, or a sequence differing by one or two nucleotide mismatches relative to SEQ ID NO: 4, 8, 12, 16, 20, or 24.

83. A composition, comprising one or more compositions of any one of claims 66-82 and a DNA polymerase.

84. The composition of claim 83, comprising a reverse transcriptase.

85. The composition of any one of claims 83-84, comprising one or more of a buffer and $MgCl_2$.

86. The composition of any one of claims 83-85, comprising nucleic acids from Human Parvovirus B19 (B19), Human Herpes Virus 6 (HHV6), Human Herpes Virus 7 (HHV7), Human Herpes Virus 8 (HHV8), Epstein Barr Virus (EBV), Hepatitis A Virus (HAV), or any combination thereof.

87. The composition of claim 86, wherein the nucleic acids comprise a B19 DNA, an HHV6 DNA, an HHV7 DNA, an HHV8 DNA, an EBV DNA, an HAV RNA, cDNA of an HAV RNA, or any combination thereof.

88. The composition of any one of claims 83-87, wherein the composition is an amplification reaction mixture.

89. An oligonucleotide probe or primer up to about 100 nucleotides in length which is capable of hybridizing to the NS1 gene region of Human Parvovirus B19 (B19), wherein the probe or primer comprises the sequence of any one of SEQ ID NOs: 1-3, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 1-3.

90. An oligonucleotide probe or primer up to about 100 nucleotides in length which is capable of hybridizing to the U31 gene region of Human Herpes Virus 6 (HHV6), wherein the probe or primer comprises the sequence of any one of SEQ ID NOs: 5-7, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 5-7.

91. An oligonucleotide probe or primer up to about 100 nucleotides in length which

is capable of hybridizing to the U57 gene region of Human Herpes Virus 7 (HHV7), wherein the probe or primer comprises the sequence of any one of SEQ ID NOs: 9-11, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 9-11.

92. An oligonucleotide probe or primer up to about 100 nucleotides in length which is capable of hybridizing to the BSLF1 gene region of Human Herpes Virus 8 (HHV8), wherein the probe or primer comprises the sequence of any one of SEQ ID NOs: 13-15, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 13-15.

93. An oligonucleotide probe or primer up to about 100 nucleotides in length which is capable of hybridizing to the NA1 gene region of Epstein Barr Virus (EBV), wherein the probe or primer comprises the sequence of any one of SEQ ID NOs: 17-19, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 17-19.

94. An oligonucleotide probe or primer up to about 100 nucleotides in length which is capable of hybridizing to the 5'-UTR of Hepatitis A Virus (HAV), wherein the probe or primer comprises the sequence of any one of SEQ ID NOs: 21-23, or a sequence having at least about 85% identity to any one of SEQ ID NOs: 21-23.

95. A composition, comprising two or more of the oligonucleotide probe or primer of any one of claims 89-94.

96. A linear double-stranded DNA (dsDNA) up to about 300 bp in length, wherein the linear dsDNA comprises:

the sequence of SEQ ID NO: 4 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 4;

the sequence of SEQ ID NO: 8 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 8;

the sequence of SEQ ID NO: 12 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 12;

the sequence of SEQ ID NO: 16 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 16;

the sequence of SEQ ID NO: 20 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 20; or

the sequence of SEQ ID NO: 24 or a sequence having at least about 85% identity to the sequence of SEQ ID NO: 24.

97. A composition comprising one or more of the linear dsDNA of claim 96.

98. A composition comprising two or more of the oligonucleotide probe or primer of any one of claims 89-94, and one or more of the linear dsDNA of claim 96.

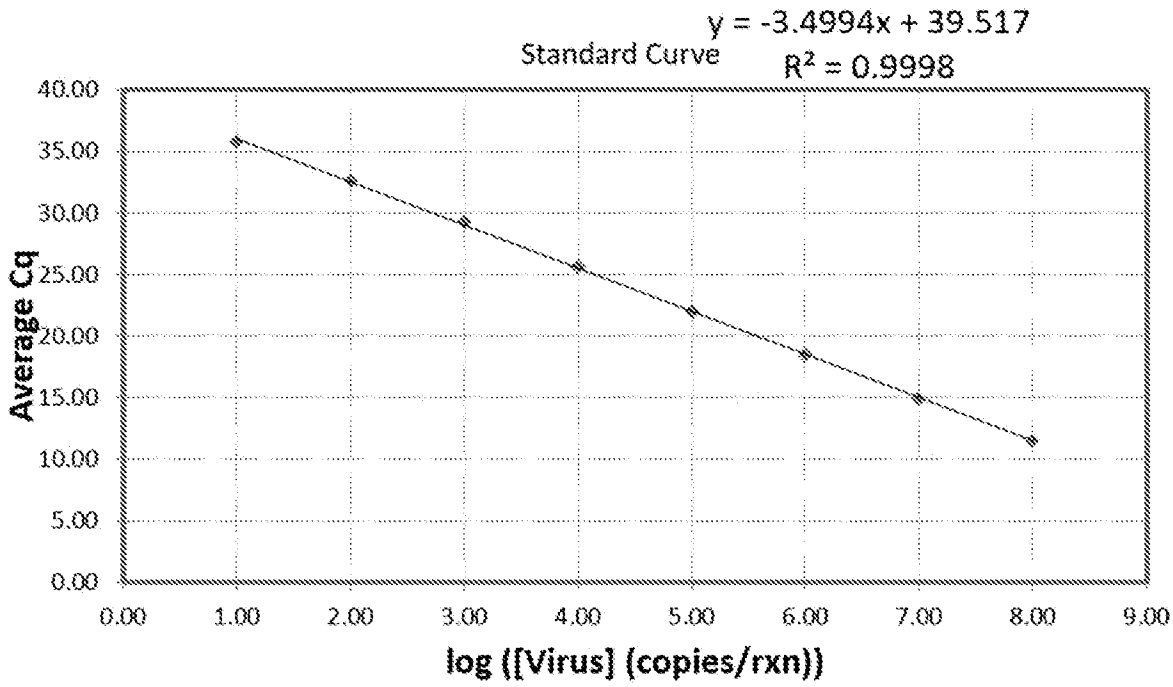
y = -3.4994x + 39.517
Standard Curve
R² = 0.9998
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Average Cq
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
log ([Virus] (copies/rxn))

FIG. 1

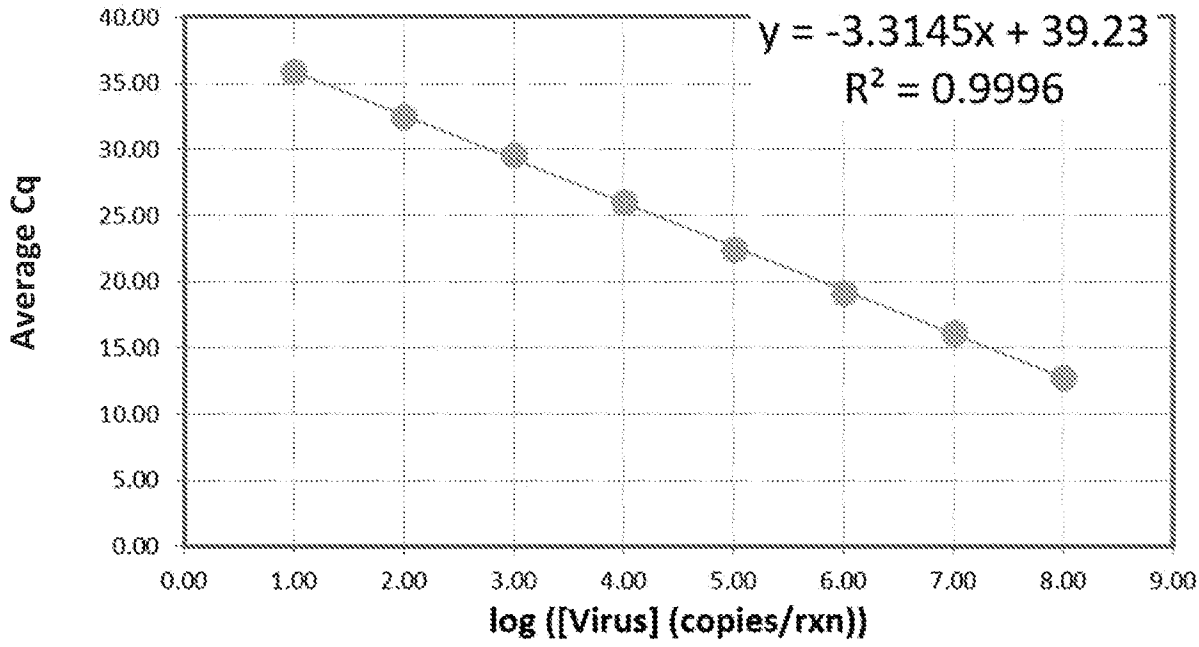
y = -3.3145x + 39.23
R² = 0.9996
Average Cq
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
log ([Virus] (copies/rxn))

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/056721

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/70
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Kiminari Ito ET AL: "Analysis of Viral Infection by Multiplex Polymerase Chain Reaction Assays in Patients with Liver Dysfunction", Internal Medicine, 1 January 2013 (2013-01-01), pages 201-211, XP055450491, Japan DOI: 10.2169/internalmedicine.52.8206 Retrieved from the Internet: URL:https://www.jstage.jst.go.jp/article/internalmedicine/52/2/52_52.8206/_pdf/-char/en the whole document ----- -/--	1-8, 48-67, 78-89, 95-98

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2023	11/12/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ulbrecht, Matthias

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2023/056721

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2023/056721

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-8, 66, 67, 89(completely); 48-65, 78-88, 95-98(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/056721

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLENAAR-DE BACKER MARIJKE W.A. ET AL: "Validation of new real-time polymerase chain reaction assays for detection of hepatitis A virus RNA and parvovirus B19 DNA : VALIDATION B19V DNA AND HAV RNA NAT", TRANSFUSION, vol. 56, no. 2, 9 September 2015 (2015-09-09), pages 440-448, XP093090338, US ISSN: 0041-1132, DOI: 10.1111/trf.13334 abstract table 1	1-8, 48-67, 78-89, 95-98
X	WO 2005/075686 A1 (ROCHE DIAGNOSTICS GMBH [DE]; HOFFMANN LA ROCHE [DE] ET AL.) 18 August 2005 (2005-08-18) page 4, paragraph 3 - page 5, last paragraph example 4	1-8, 48-67, 78-89, 95-98
X	US 9 598 739 B1 (WRONSKA DANUTA [US] ET AL) 21 March 2017 (2017-03-21) example 6 table 3 sequence 142	1-8, 48-67, 78-89, 95-98
A	YESSICA SANCHEZ-PONCE ET AL: "Simultaneous Detection of Beta and Gamma Human Herpesviruses by Multiplex qPCR Reveals Simple Infection and Coinfection Episodes Increasing Risk for Graft Rejection in Solid Organ Transplantation", VIRUSES, vol. 10, no. 12, 30 November 2018 (2018-11-30), pages 1-19, XP055632687, DOI: 10.3390/v10120730 abstract table 1	1-8, 48-67, 78-89, 95-98

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8, 66, 67, 89(completely); 48-65, 78-88, 95-98(partially)

A method of detecting B19 as defined in claim 1; a method as defined in claim 48 requiring at least the detection of B19; a composition for detecting B19 as defined in claim 66; a composition as defined in claim 78 comprising at least the pair of primers specific for B19; an oligonucleotide probe or primer specific for B19 as defined in claim 89; a composition as defined in claim 95 but comprising at least an oligonucleotide probe or primer specific for B19 as defined in claim 89; a linear dsDNA as defined in claim 96 but limited to SEQ ID No. 4; a composition as defined in claim 97 but limited to SEQ ID No. 4; a composition as defined in claim 98 comprising at least the oligonucleotide probe and primer defined by claim 89 and the linear dsDNA with the aforesaid limitation.

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2. claims: 9-16, 68, 69, 90(completely); 48-65, 78-88, 95-98(partially)

A method of detecting HHV6 as defined in claim 9; a method as defined in claim 48 requiring at least the detection of HHV6; a composition for detecting HHV6 as defined in claim 68; a composition as defined in claim 78 comprising at least the pair of primers specific for HHV6; an oligonucleotide probe or primer specific for HHV6 as defined in claim 90; a composition as defined in claim 95 but comprising at least an oligonucleotide probe or primer specific for HHV6 as defined in claim 90; a linear dsDNA as defined in claim 96 but limited to SEQ ID No. 8; a composition as defined in claim 97 but limited to SEQ ID No. 4; a composition as defined in claim 98 comprising at least the oligonucleotide probe and primer defined by claim 90 and the linear dsDNA with the aforesaid limitation.

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3. claims: 17-24, 70, 71, 91(completely); 48-65, 78-88, 95-98(partially)

A method of detecting HHV7 as defined in claim 17; a method as defined in claim 48 requiring at least the detection of HHV7; a composition for detecting HHV7 as defined in claim 70; a composition as defined in claim 78 comprising at least the pair of primers specific for HHV7; an oligonucleotide probe or primer specific for HHV7 as defined in claim 91; a composition as defined in claim 95 but comprising at least an oligonucleotide probe or primer specific for HHV7 as defined in claim 91; a linear dsDNA as defined in claim 96 but limited to SEQ ID No. 12; a composition as defined in claim 97 but limited to SEQ ID No. 4; a composition as

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

defined in claim 98 comprising at least the oligonucleotide probe and primer defined by claim 91 and the linear dsDNA with the aforesaid limitation.

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4. claims: 25-32, 72, 73, 92(completely); 48-65, 78-88, 95-98(partially)

A method of detecting HHV8 as defined in claim 25; a method as defined in claim 48 requiring at least the detection of HHV8; a composition for detecting HHV8 as defined in claim 72; a composition as defined in claim 78 comprising at least the pair of primers specific for HHV8; an oligonucleotide probe or primer specific for HHV8 as defined in claim 92; a composition as defined in claim 95 but comprising at least an oligonucleotide probe or primer specific for HHV8 as defined in claim 92; a linear dsDNA as defined in claim 96 but limited to SEQ ID No. 16; a composition as defined in claim 97 but limited to SEQ ID No. 4; a composition as defined in claim 98 comprising at least the oligonucleotide probe and primer defined by claim 92 and the linear dsDNA with the aforesaid limitation.

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5. claims: 33-39, 74, 75, 93(completely); 48-65, 78-88, 95-98(partially)

A method of detecting EBV as defined in claim 33; a method as defined in claim 48 requiring at least the detection of EBV; a composition for detecting EBV as defined in claim 74; a composition as defined in claim 78 comprising at least the pair of primers specific for EBV; an oligonucleotide probe or primer specific for EBV as defined in claim 93; a composition as defined in claim 95 but comprising at least an oligonucleotide probe or primer specific for EBV as defined in claim 93; a linear dsDNA as defined in claim 96 but limited to SEQ ID No. 20; a composition as defined in claim 97 but limited to SEQ ID No. 4; a composition as defined in claim 98 comprising at least the oligonucleotide probe and primer defined by claim 93 and the linear dsDNA with the aforesaid limitation.

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6. claims: 40-47, 76, 77, 94(completely); 48-65, 78-88, 95-98(partially)

A method of detecting HAV as defined in claim 40; a method as defined in claim 48 requiring at least the detection of HAV; a composition for detecting HAV as defined in claim 76; a composition as defined in claim 78 comprising at least the pair of primers specific for HAV; an oligonucleotide probe or primer specific for HAV as defined in claim 94; a composition as defined in claim 95 but comprising at least an oligonucleotide probe or primer specific for HAV as defined in claim 94; a linear dsDNA as defined in claim 96

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

but limited to SEQ ID No. 24; a composition as defined in claim 97 but limited to SEQ ID No. 4; a composition as defined in claim 98 comprising at least the oligonucleotide probe and primer defined by claim 94 and the linear dsDNA with the aforesaid limitation.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2023/056721

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005075686 A1	18-08-2005	AT E485401 T1	15-11-2010
		CA 2559949 A1	18-08-2005
		CA 2791798 A1	18-08-2005
		EP 1716257 A1	02-11-2006
		EP 2251441 A1	17-11-2010
		ES 2354020 T3	09-03-2011
		ES 2422583 T3	12-09-2013
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		JP 5546977 B2	09-07-2014
		JP 2007521814 A	09-08-2007
		JP 2010279370 A	16-12-2010
		US 2007281294 A1	06-12-2007
		WO 2005075686 A1	18-08-2005
US 9598739 B1	21-03-2017	NONE	