

Claims:

1. A method for detecting and/or quantifying the presence of a target nucleic acid sequence of a microorganism in a sample obtained from a subject, comprising amplifying the target sequence in a CpG island of the nucleic acid of the microorganism, irrespective of the methylation status of the CpG island.

2. The method of claim 1, wherein the target sequence is within the 5' end of the nucleic acid of the CpG island of the nucleic acid of the microorganism.

3. The method of claim 1 or 2, wherein the method is a polymerase chain reaction (PCR);
optionally wherein the polymerase chain reaction is selected from the group consisting of quantitative polymerase chain reaction, digital polymerase chain reaction, real-time polymerase chain reaction, and traditional polymerase chain reaction.

4. The method of any one of claims 1 to 3, wherein the sample obtained from the subject is a blood plasma sample, a blood serum sample or a whole blood sample;

optionally wherein the sample obtained from the subject comprises cfDNAs (cell free DNAs).

5. The method of any one of claims 1 to 4, wherein the microorganism is a virus, a bacterium, a fungus or a parasite;

optionally wherein the virus is Epstein-Barr virus (EBV);

optionally wherein the bacterium is a *Mycobacterium*; and

optionally wherein the *Mycobacterium* is *Mycobacterium tuberculosis*.

6. The method of claim 5, wherein the target sequence is in a CpG island of the *Bam*HI-W region of EBV;

optionally wherein the CpG island of the *Bam*HI-W region of EBV is selected from the group consisting of:

CTCCTCTCCAACCTTCGCTCCACCCTAGACCCCAGCTTCTGGCCTCCCCGGGTCCA
CCAGGCCAGCCGGAGGGACCCCGGCAGCCCGGGCGAGTCGCCTTCCCTCTCCCC
TGGCCTCTCCTTCCCGCCTCCCACCCGAGCCCCCTCAGCTTGCCTCCCCACCGGGT

CCATCAGGCCGGCCGGAGGGACCCCGGCGGCCCGGTGTCA (SEQ ID NO.: 6),
 AGGCCATGCGCGCCCTGTCACCAGGCCTGCCAAAGAGCCAGATCTAAGGCCGGG
 AGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTCTGGTAGTGATTTGGA
 CCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAACTCTAAAAATCAAA
 ACTTTAGAGGGCGAATGGGCGCCATTTTGTCCCCACGCGCGCATAATGGCGGACCT
 AGGCCTAAAACCCCCAGGAAGCGGGTCTATGGTTGGCTGCGCTGCTGCTATCTTT
 AGAGGGGAAAAGAGGAATAAGCCCCAGACAGGGGAGTGGGCTTGTTTGTGACT
 TCACCAAAGGTCAGGGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTC
 CAGCGCGTTTACGTAAGC (SEQ ID NO.: 7),

CTGGTATAAAGTGGTCCTGCAGCTATTTCTGGTCGCATCAGAGCGCCAGGAGTCC
 ACACAAATGTAAGAGGGGGTCTTCTACCTCTCCCTAGCCCTCCGCCCCCTCCAAG
 GACTCGGGCCCAGTTTCTAACTTTTCCCCTTCCCTCCCTCGTCTTGCCCTGCGCCC
 GGGGCCACCTTCATCACCGTCGCTGACTCCGCCATCCAAGCCTAGGGGAGACCG
 AAGTGAAGGCCCTGGACCAACCCGGCCCCGGGCCCCCGGTATCGGGCCAGAGGT
 AAGTGGACTTTAATTTTTTCTGCTAAGCCCAACACTCCACCACACCCAGGCACAC
 ACTACACACACCCACCCGTCTCAGGGTCCCCTCGGA (SEQ ID NO.: 8), and

CGAGGAGGCGCCCGGAGTGGGGCCGGTCGGCTGGGCTGGCCGAGCCCGGGTCTG
 GGAGGTCTGGGGTGGCGAGCCTGCTGTCTCAGGAGGGGCCTGGCTCCGCCGGGT
 GGCCCTGGGGTAAGTCTGGGAGGCAGAGGGTCGGCCTAGGCCCGGGGAAGTGG
 AGGGGGATCGCCCGGGTCTCTGTTGGCAGAGTCCGGGCGATCCTCTGAGACCCTC
 CGGGCCCGGACGGTCGCCCTCAGCCCCCAGACAGACCCAGGGTCTCCAGGCA
 GGGTCCGGCATCTTCAGGGGCAGCAGGCTCACCACCACAGGCCCCCAGACCCG
 GGTCTCGGCCAGCCGAGCCGACCGGCCCGCGCCTGGCGCCTCCTCGGGGCCAG
 CCGCCGGGGTTGGTTCTGCCCTCTCTCTGTCTTCAGAGGAACCAGGGACCTCG
 GGCACCCAGAGCCCCTCGGGCCCGCCTCCAGGCGCCCTCCTGGTCTCCGCTCCC
 CTCTGAGCCCCGTAAACCCAAAGAATGTCTGAGGGGAGCCACCCTCGG (SEQ ID
 NO.: 9);

optionally wherein the target sequence comprises the sequence selected from the group
 consisting of:

AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC
 TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA
 CTCTAAAAATCAAACTTTAGAGGGCGAATGGGCG (SEQ ID NO.: 1),

GGAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCA
GGGCCCAAGGGGGTTTCGCGTTGCTAGGCCACCTTCTCAGTCCAGCGCGTTTACGT
AA (SEQ ID NO.: 10),

AGGAAGCGGGTCTATGGTTGGCTGCGCTGCTGCTATCTTTAGAGGGGAAAAGAG
GAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCAG
GGGCCAAGGGGGTTTCGCGTTGCTAGGCCACCTTCTCAGTC (SEQ ID NO.:11), a
complementary sequence, a fragment and a variant thereof;

optionally wherein amplifying the target sequence comprises the use of a pair of
oligonucleotide primers, wherein the first oligonucleotide primer comprises a sequence
selected from the group consisting of: 5'-AGATCTAAGGCCGGGAGAGG-3' (SEQ ID
NO.:2), 5'-GGAATAAGCCCCCAGACAGG-3' (SEQ ID NO.:12), 5'-
AGGAAGCGGGTCTATGGTTG-3' (SEQ ID NO.:13), a fragment and a variant thereof, and
the second oligonucleotide primer comprises a sequence selected from the group consisting
of 5'-CGCCCATTCGCTCTAAAGT-3' (SEQ ID NO.:3), 5'-
TTACGTAAACGCGCTGGACT-3' (SEQ ID NO.:14), 5'-
GACTGAGAAGGTGGCCTAGC-3' (SEQ ID NO.:15), a fragment and a variant thereof.

7. The method of claim 5 or 6, wherein amplifying the target sequence further comprises the
use of a probe capable of binding to the target sequence;

optionally wherein the probe comprises a sequence selected from the group consisting of 5'-
CTCTGGTAGTGATTTGGACCCGAAATCTG-3' (SEQ ID NO.: 16), 5'-
CCACCTTCTCAGTCCAGCGCGTTT-3' (SEQ ID NO.: 17), 5'-
GTGACTTCACCAAAGGTCAGGGCCC-3' (SEQ ID NO.: 18), 5'-
GGTGGTAAGCGGTTTACCTTCAGGG-3' (SEQ ID NO.: 19), a fragment and variant
thereof.

8. The method of any one of claims 5 to 7, further comprising calculating the copy number of
*Bam*HI-W region in the amplified product, wherein the copy number is calculated using the
following formula:

$$\text{Copy Number of } BamHI - W = \frac{\text{DNA Quantity (ng)} \times \text{Avogadro's Number}}{88390.29 \text{ (Da)} \times 10^9}$$

9. The method of any one of claims 5 to 8, wherein the method is capable of detecting the lowest concentration of EBV of 100 IU (International Unit)/ml of sample, or 10 IU/ml of sample, or 1 IU/ml of sample.

10. A method for detecting and/or quantifying the presence of a target nucleic acid sequence of Epstein-Barr virus (EBV) in a sample obtained from a subject, comprising amplifying a target sequence in the *Bam*HI-W region of EBV, wherein the target sequence comprises the sequence of

AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC
TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA
CTCTAAAAATCAAACTTTAGAGGCGAATGGGCG (SEQ ID NO.: 1),

wherein amplifying the target sequence comprises the use of a pair of oligonucleotide primers and a probe, wherein the first oligonucleotide primer comprises the sequence of 5'-

AGATCTAAGGCCGGGAGAGG-3' (SEQ ID NO.: 2), and the second oligonucleotide primer comprises the sequence of 5'-CGCCCATTCGCCTCTAAAGT-3' (SEQ ID NO.:3), and wherein the probe comprises the sequence of 5'-(6-FAM)CTCTGGTAGTGATTTGGACCCGAAATCTG(TAMRA)-3' (SEQ ID NO.: 4),

and wherein the method is a quantitative polymerase chain reaction (qPCR).

11. The method of any one of claims 5 to 10, wherein the method further comprises amplifying a control comprising a target sequence of

TTAGCAGCGACGAAGATCATGCGCTCACGCTCTCGGTGTCCTCATTCATCAGTTA
TTCACAACGCTATGCTGTAACCTCGACCTGACAAGACTGTACCTATGAGAAGGCA
CTTGCTACCTTATGCAAGCGTCAGCCCGCGGTATCGCTTGG (SEQ ID NO.: 80), a complementary sequence, a fragment or a variant thereof;

optionally wherein amplifying the control comprises the use of a pair of oligonucleotide primers, wherein the first oligonucleotide primer comprises the sequence of 5'-

CGCTCTCGGTGTCCTCATTC-3' (SEQ ID NO.: 81), a complementary sequence, a fragment or a variant thereof, and the second oligonucleotide primer comprises the sequence of 5'-GGCTGACGCTTGCATAAGGT-3' (SEQ ID NO.: 82), a complementary sequence, a fragment or a variant thereof;

optionally wherein amplifying the control further comprises the use of a probe capable of binding to the target sequence of the control, wherein the probe comprises the sequence of 5'-VIC-CACAACGCTATGCTGTAACCTCGACCTGAC-TAMRA-3' (SEQ ID NO.: 83).

12. A method of detecting a disease associated with microorganism infection, or risk of developing a disease associated with microorganism infection in a subject, comprising detecting and/or quantifying the presence of a nucleic acid sequence of the microorganism using the method of any one of claims 1 to 11 in a sample obtained from the subject, wherein the presence of the nucleic acid sequence of the microorganism in the sample indicates that the subject has a disease associated with microorganism infection or is at risk of developing a disease associated with microorganism infection;

optionally wherein the disease associated with microorganism infection is selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis, anorexia nervosa, anxiety disorder, asthma, atherosclerosis, autoimmune diseases, cancers, chronic obstructive pulmonary disease, Crohn's disease, coronary heart disease, dementia, diabetes mellitus type 1, diabetes mellitus type 2, dilated cardiomyopathy, epilepsy, Guillain-Barré syndrome, irritable bowel syndrome, lupus, multiple sclerosis, myocardial infarction, Parkinson's disease, psoriasis, rheumatoid arthritis, sarcoidosis, schizophrenia, stroke, thromboangiitis obliterans, tourette syndrome and vasculitis;

optionally wherein the disease associated with microorganism infection is cancer;

optionally wherein the cancer is EBV-associated cancer;

optionally wherein the EBV-associated cancer is selected from the group consisting of nasopharyngeal carcinoma (NPC), gastric cancer, Hodgkin's lymphoma and Burkitt's lymphoma; and

optionally wherein the EBV-associated cancer is NPC.

13. A method of detecting and treating a disease associated with microorganism infection, comprising:

(i) detecting and/or quantifying the presence of a nucleic acid sequence of the microorganism using the method of any one of claims 1 to 11 in a sample obtained from the subject, wherein the presence of the nucleic acid sequence of the microorganism in the sample indicates that the subject has a disease associated with microorganism;

(ii) administering to the subject a medicament suitable for the treatment of the disease associated with the microorganism,

optionally wherein the disease associated with microorganism infection is selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis, anorexia nervosa, anxiety disorder, asthma, atherosclerosis, autoimmune diseases, cancers, chronic obstructive pulmonary disease, Crohn's disease, coronary heart disease, dementia, diabetes mellitus type 1, diabetes mellitus type 2, dilated cardiomyopathy, epilepsy, Guillain–Barré syndrome, irritable bowel syndrome, lupus, multiple sclerosis, myocardial infarction, Parkinson's disease, psoriasis, rheumatoid arthritis, sarcoidosis, schizophrenia, stroke, thromboangiitis obliterans, tourette syndrome and vasculitis;

optionally wherein the disease associated with microorganism infection is cancer;

optionally wherein the cancer is EBV-associated cancer;

optionally wherein the EBV-associated cancer is selected from the group consisting of nasopharyngeal carcinoma (NPC), gastric cancer, Hodgkin's lymphoma and Burkitt's lymphoma; and

optionally wherein the EBV-associated cancer is NPC.

14. A method of predicting the treatment outcome of a disease associated with microorganism infection in a patient, comprising:

(i) quantifying the nucleic acid sequence of the microorganism in a sample collected from the patient before treatment or before a treatment step, and quantifying the nucleic acid sequence of the microorganism in a sample collected from the same patient after treatment or after a treatment step;

(ii) comparing the amount of the nucleic acid sequence of the microorganism in the sample before and after treatment or a treatment step, wherein a decrease in the amount of the nucleic acid sequence of the microorganism in the sample after treatment or a treatment step indicates that treatment outcome of the disease associated with microorganism infection in the patient is positive,

wherein the quantifying of the nucleic acid sequence of the microorganism in the sample is performed according to the method of any one of claims 1-11,

optionally wherein the disease associated with microorganism infection is selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis, anorexia nervosa, anxiety disorder, asthma, atherosclerosis, autoimmune diseases, cancers, chronic obstructive pulmonary disease, Crohn's disease, coronary heart disease, dementia, diabetes mellitus type 1, diabetes mellitus type 2, dilated cardiomyopathy, epilepsy, Guillain–Barré syndrome, irritable bowel syndrome, lupus, multiple sclerosis, myocardial infarction, Parkinson's disease, psoriasis, rheumatoid arthritis, sarcoidosis, schizophrenia, stroke, thromboangiitis obliterans, tourette syndrome and vasculitis;

optionally wherein the disease associated with microorganism infection is cancer;

optionally wherein the cancer is EBV-associated cancer;

optionally wherein the EBV-associated cancer is selected from the group consisting of nasopharyngeal carcinoma (NPC), gastric cancer, Hodgkin's lymphoma and Burkitt's lymphoma; and

optionally wherein the EBV-associated cancer is NPC.

15. A kit for detecting and/or quantifying the nucleic acid sequence of a microorganism in a sample obtained from a subject, comprising a pair of oligonucleotide primers specific for the amplification of a target sequence in a CpG island of the nucleic acid of the microorganism.

16. The kit of claim 15, wherein the target sequence is within the 5' end of the CpG island of the nucleic acid of the microorganism.

17. The kit of claim 15 or 16, wherein the sample obtained from the subject is a blood plasma sample, a blood serum sample or a whole blood sample;

optionally wherein the sample obtained from the subject comprises cfDNAs (cell free DNAs).

18. The kit of any one of claims 15 to 17, wherein the microorganism is a virus, a bacterium, a fungus or a parasite;

optionally wherein the virus is Epstein-Barr virus (EBV).

19. The kit of claim 18, wherein the target sequence is in a CpG island of the *Bam*HI-W region of EBV;

optionally wherein the target sequence comprises the sequence selected from the group consisting of:

AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC
TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA
CTCTAAAAATCAAACTTTAGAGGCGAATGGGCG (SEQ ID NO.: 1),

GGAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCA
GGGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTCCAGCGCGTTTACGT
AA (SEQ ID NO.: 10),

AGGAAGCGGGTCTATGGTTGGCTGCGCTGCTGCTATCTTTAGAGGGGAAAAGAG
GAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCAG
GGCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTC (SEQ ID NO.:11), a
complementary sequence, a fragment and a variant thereof;

optionally wherein the first oligonucleotide primer comprises a sequence selected from the group consisting of: 5'-AGATCTAAGGCCGGGAGAGG-3' (SEQ ID NO.: 2), 5'-

GGAATAAGCCCCCAGACAGG-3' (SEQ ID NO.:12), 5'-

AGGAAGCGGGTCTATGGTTG-3' (SEQ ID NO.:13), a fragment and a variant thereof,

and the second oligonucleotide primer comprises a sequence selected from the group

consisting of 5'-CGCCCATTCGCCTCTAAAGT-3' (SEQ ID NO.:3), 5'-

TTACGTAAACGCGCTGGACT-3' (SEQ ID NO.:14), 5'-

GACTGAGAAGGTGGCCTAGC-3' (SEQ ID NO.:15), a fragment and a variant thereof.

20. The kit of any one of claims 15 to 19, further comprises a probe capable of binding to the target sequence;

optionally wherein the probe comprises a sequence selected from the group consisting of 5'-

CTCTGGTAGTGATTTGGACCCGAAATCTG-3' (SEQ ID NO.: 16), 5'-

CCACCTTCTCAGTCCAGCGCGTTT-3' (SEQ ID NO.: 17), 5'-

GTGACTTCACCAAAGGTCAGGGCCC-3' (SEQ ID NO.: 18), 5'-

GGTGGTAAGCGGTTACCTTCAGGG-3' (SEQ ID NO.: 19), a fragment and variant thereof.