due to the presence of cross-reacting antibodies. Microscopy techniques may be limited by sample staining and fixing and the use of strong illumination, which may destroy or distort cellular features of the microorganisms to be detected.

[0005] Molecular diagnostic procedures such as PCR-based techniques have been introduced for the detection of microorganism infections in recent years. However, the currently available PCR-based techniques have some limitations in the quantitative measurement of the bacterial/viral load in patients' samples, as well as problems of false positive results. Thus, what is needed is an improved method of detecting and/or quantifying of microorganisms with better sensitivity and specificity.

SUMMARY

[0005a] In one aspect, there is provided a method for detecting and/or quantifying the presence of a nucleic acid sequence of Epstein-Barr virus (EBV) in a sample obtained from a subject, wherein the sample is a blood plasma or blood serum sample; wherein the sample comprises cell free DNAs (cfDNAs); and wherein said method comprises amplifying a target sequence in a CpG island of the BamHI-W region of EBV, irrespective of the methylation status of the CpG island.

[0005b] In another aspect, there is provided a method for detecting and/or quantifying the presence of a nucleic acid sequence of Epstein-Barr virus (EBV) in a sample obtained from a subject, wherein the sample is a blood plasma or blood serum sample; wherein the sample comprises cell free DNAs (cfDNAs); and wherein said method comprises amplifying a target sequence in the BamHI-W region of EBV, wherein the target sequence comprises the sequence of AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAG GTAATCTCTGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGA CTTTAAAACTCTAAAAATCAAAACTTTAGAGGCGAATGGGCG (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)CTCTGGTAGTGATTTGGAC CCGAAATCTG(TAMRA)-3' (SEQ ID NO.: 4), and wherein the method is a quantitative polymerase chain reaction (qPCR).

[0005c] In yet another aspect, there is provided a kit for detecting and/or quantifying a nucleic acid sequence of EBV in a sample obtained from a subject, wherein the sample is a blood plasma or blood serum sample; wherein the sample comprises cell free DNAs (cfDNAs); and wherein said method comprises a pair of oligonucleotide primers specific for the amplification of a target sequence in a CpG island of the BamHI-W region of EBV.

[0006] In one aspect, there is provided 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.

[0007] In another aspect, there is provided 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 *BamHI-W* region of EBV, wherein the target sequence comprises the sequence of AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA CTCTAAAAATCAAAACTTTAGAGGCGAATGGGCG (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).

[0008] In yet another aspect, there is provided 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 the present disclosure 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.

[0009] In another aspect, there is provided 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 the present disclosure 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; and (ii) administering to the subject a medicament suitable for the treatment of the disease associated with the microorganism.

[0010] In yet another aspect, there is provided 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 the present disclosure.

[0011] In a further aspect, there is provide 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

Claims:

1. A method for detecting and/or quantifying the presence of a nucleic acid sequence of Epstein-Barr virus (EBV) in a sample obtained from a subject, wherein the sample is a blood plasma or blood serum sample; wherein the sample comprises cell free DNAs (cfDNAs); and wherein said method comprises amplifying a target sequence in a CpG island of the *Bam*HI-W region of EBV, 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 *Bam*HI-W region of EBV.

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 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
ACTTTAGAGGCGAATGGGCGCCATTTTGTCCCCACGCGCGCATAATGGCGGACCT
AGGCCTAAAACCCCCAGGAAGCGGGTCTATGGTTGGCTGCGCTGCTGCTATCTTT
AGAGGGGAAAAGAGGAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACT
TCACCAAAGGTCAGGGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTC
CAGCGCGTTTACGTAAGC (SEQ ID NO.: 7),

CTGGTATAAAGTGGTCCTGCAGCTATTTCTGGTCGCATCAGAGCGCCAGGAGTCC
ACACAAATGTAAGAGGGGGTCTTCTACCTCTCCCTAGCCCTCCGCCCCCTCCAAG
GACTCGGGCCCAGTTTCTAACTTTTCCCCTTCCCTCCCTCGTCTTGCCCTGCGCCC
GGGGCCACCTTCATCACCGTCGCTGACTCCGCCATCCAAGCCTAGGGGAGACCG
AAGTGAAGGCCCTGGACCAACCCGGCCCGGGCCCCCCGGTATCGGGCCAGAGGT

AAGTGGACTTTAATTTTTTCTGCTAAGCCCAACACTCCACCACACCCAGGCACAC
ACTACACACACCCACCCGTCTCAGGGTCCCCTCGGA (SEQ ID NO.: 8), and

CGAGGAGGCGCCCGGAGTGGGGCCGGTCGGCTGGGCTGGCCGAGCCCGGGTCTG
GGAGGTCTGGGGTGGCGAGCCTGCTGTCTCAGGAGGGGCCTGGCTCCGCCGGGT
GGCCCTGGGGTAAGTCTGGGAGGCAGAGGGTCGGCCTAGGCCCGGGGAAGTGG
AGGGGGATCGCCCGGGTCTCTGTTGGCAGAGTCCGGGCGATCCTCTGAGACCCTC
CGGGCCCGGACGGTCGCCCTCAGCCCCCCAGACAGACCCCAGGGTCTCCAGGCA
GGGTCCGGCATCTTCAGGGGCAGCAGGCTCACCACCACAGGCCCCCCAGACCCG
GGTCTCGGCCAGCCGAGCCGACCGGCCCCGCGCCTGGCGCCTCCTCGGGGCCAG
CCGCCGGGGTTGGTTCTGCCCCTCTCTCTGTCCTTCAGAGGAACCAGGGACCTCG
GGCACCCCAGAGCCCCTCGGGCCCGCCTCCAGGCGCCCTCCTGGTCTCCGCTCCC
CTCTGAGCCCCGTTAAACCCAAAGAATGTCTGAGGGGAGCCACCCTCGG (SEQ ID
NO.: 9);

optionally wherein the target sequence comprises the sequence selected from the group consisting of:
AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC
TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA
CTCTAAAAATCAAAACTTTAGAGGCGAATGGGCG (SEQ ID NO.: 1),
GGAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCA
GGGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTCCAGCGCGTTTACGT
AA (SEQ ID NO.: 10),
AGGAAGCGGGTCTATGGTTGGCTGCGCTGCTGCTATCTTTAGAGGGGAAAAGAG
GAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCAG
GGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTC (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’-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.

5. The method of claim 4, 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’-GGTGGTAAGCGGTTCACCTTCAGGG-3’ (SEQ ID NO.: 19), a fragment and variant thereof.

6. The method claim 4 or 5, 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:

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

7. The method of any one of claims 4 to 6, 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.

8. A method for detecting and/or quantifying the presence of a nucleic acid sequence of Epstein-Barr virus (EBV) in a sample obtained from a subject, wherein the sample is a blood plasma or blood serum sample; wherein the sample comprises cell free DNAs (cfDNAs); and wherein said method comprises amplifying a target sequence in the *Bam*HI-W region of EBV, wherein the target sequence comprises the sequence of AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA CTCTAAAAATCAAAACTTTAGAGGCGAATGGGCG (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).

9. The method of any one of claims 4 to 8, wherein the method further comprises amplifying a control comprising a target sequence of TTAGCAGCGACGAAGATCATGCGCTCACGCTCTCGGTGTCCTCATTCATCAGTTATT**C**ACAACGCTATGCTGTAACTCGACCTGACAAGACTGTACCTATGAGAAGGCACTTGCTACCTTATGCAAGCGTCAGCCCGCGGTATCGCTTGG (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-CACAACGCTATGCTGTAACTCGACCTGAC-TAMRA-3’ (SEQ ID NO.: 83).

10. A method of detecting a disease associated with Epstein-Barr virus (EBV) infection, or risk of developing a disease associated with EBV infection in a subject, comprising detecting and/or quantifying the presence of a nucleic acid sequence of EBV using the method of any one of claims 1 to 9 in a sample obtained from the subject, wherein the presence of the nucleic acid sequence of EBV in the sample indicates that the subject has a disease associated with EBV infection or is at risk of developing a disease associated with EBV infection;

optionally wherein the disease associated with EBV infection is EBV-associated cancer or Guillain–Barré syndrome;

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.

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

(i) quantifying a nucleic acid sequence of EBV in a sample collected from the patient before treatment or before a treatment step, and quantifying the nucleic acid sequence of EBV 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 EBV in the sample before and after treatment or a treatment step, wherein a decrease in the amount of the nucleic acid sequence of EBV in the sample after treatment or a treatment step indicates that treatment outcome of the disease associated with EBV infection in the patient is positive,

wherein the quantifying of the nucleic acid sequence of EBV in the sample is performed according to the method of any one of claims 1-9,

optionally wherein the disease associated with EBVinfection is EBV-associated cancer or Guillain–Barré syndrome;

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.

12. A kit for detecting and/or quantifying a nucleic acid sequence of EBV in a sample obtained from a subject, wherein the sample is a blood plasma or blood serum sample; wherein the sample comprises cell free DNAs (cfDNAs); and wherein said method comprises a pair of oligonucleotide primers specific for the amplification of a target sequence in a CpG island of the *Bam*HI-W region of EBV.

13. The kit of claim 12, wherein the target sequence is within the 5’end of the CpG island of the *Bam*HI-W region of EBV.

14. The kit of claim 12 or 13, wherein the target sequence comprises the sequence selected from the group consisting of:
AGATCTAAGGCCGGGAGAGGCAGCCCCAAAGCGGGTGCAGTAACAGGTAATCTC
TGGTAGTGATTTGGACCCGAAATCTGACACTTTAGAGCTCTGGAGGACTTTAAAA

CTCTAAAAATCAAAACTTTAGAGGCGAATGGGCG (SEQ ID NO.: 1), GGAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCA GGGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTCCAGCGCGTTTACGT AA (SEQ ID NO.: 10), AGGAAGCGGGTCTATGGTTGGCTGCGCTGCTGCTATCTTTAGAGGGGAAAAGAG GAATAAGCCCCCAGACAGGGGAGTGGGCTTGTTTGTGACTTCACCAAAGGTCAG GGCCCAAGGGGGTTCGCGTTGCTAGGCCACCTTCTCAGTC (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.

15. The kit of any one of claims 12 to 14, 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'-GGTGGTAAGCGGTTCACCTTCAGGG-3' (SEQ ID NO.: 19), a fragment and variant thereof.