determine bacteria is present. The problem with this approach apart from being time consuming, is there is no opportunity to quantify the patient's initial pathogen level. Following the initial growth phase, it is close to impossible to accurately calculate the amount of bacterial cells/ml of blood. Ultimately, the number of colonies are counted on a plate to determine CFU, however this is highly inaccurate.

[0007] Additionally, the various molecular methods for detecting bacteria in samples taken from septic patients (e.g., those commercially available from T2 Biosystems and AusDiagnostics) generally involve no specific quantification of the pathogen detected as the methods only register the presence of the pathogen following high levels of amplification making any assumptions on initial CFU very innaccurate.

[0008] Hence, there is a recognized need for rapid and reliable techniques for accurate quantification of bacteria in a sample.

SUMMARY OF INVENTION

[0008a] In one aspect, provided herein is a method of determining the quantity or concentration of a bacterium in a sample, said method including the steps of: (a) amplifying a target nucleic acid of the bacterium from genetic material obtained from the sample to form an amplification product, wherein the target nucleic acid comprises at least a portion of a bacterial 16S rRNA gene and wherein the target nucleic acid comprises one or a plurality of single nucleotide polymorphisms (SNPs) in the bacterial 16S rRNA gene, and wherein the one or plurality of SNPs correspond to at least one of positions 273, 378, 408, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1; (b) identifying the bacterium in the sample and measuring a quantity or concentration of the amplification product in a single amplification step, wherein the step of identifying the bacterium comprises analysing the amplification product for the presence or absence of one or a plurality of SNPs; (c) calculating a quantity or concentration of the target nucleic acid in the sample by comparing the quantity or concentration of the amplification product with a reference level thereof; and (d) determining a copy number of the bacterial 16S rRNA gene in the sample from the quantity or concentration of the target nucleic acid therein, the copy number being a function of or correlated to the quantity of the bacterium in the sample, wherein the step of analysing the amplification product for the presence or the absence of the at least one SNP comprises the use of high resolution melt analysis.

[0008b] In another aspect, provided herein is a method of determining a prognosis for an infection by a bacterium in a subject, including the step of quantifying the bacterium in a biological sample from the subject according to the method as described herein to thereby evaluate the prognosis of the infection in the subject.

[0008c] In another aspect, provided herein is a method of evaluating treatment efficacy of an

infection by a bacterium in a subject including: quantifying the bacterium in a biological sample from the subject according to the method as described herein; and determining whether or not the treatment is efficacious according to whether said quantity of the bacterium in the subject's biological sample is reduced or absent.

[0008d] In another aspect, provided herein is a kit or assay when used according to the method as described herein, said kit or assay comprising one or more reagents for performing the method as described herein and instructions for use.

[0009] In various aspects, the present invention is predicated in part on high conservation of the 16S (Svedberg unit) ribosomal RNA (16S rRNA) gene between prokaryotes, including bacteria and the multiple single nucleotide polymorphisms (SNPs) therein that may be useful in the identification and quantification of bacteria in a sample based on the bacteria's copy number of the 16S rRNA gene therein.

[0010] Generally speaking, prokaryotes, including bacteria, contain 16S rRNA, which is a component of the 30S small subunit of the prokaryotic ribosome. The 16S rRNA is approximately 1,500 nucleotides in length and encoded by the 16S rRNA gene (also referred to as 16S rDNA), which is generally part of a co-transcribed operon also containing the 23S and 5S rRNA genes. Although the DNA sequence of the 16S rRNA genes (and thus the RNA sequence of the 16S rRNA molecules) is highly conserved between prokaryotes, there are regions of variation (Weisberg W.G., *et al.*, 1991). Relevant to the present invention, the gene copy numbers of the 16S rRNA gene per genome are typically species or strain-specific and consistent therein, but may vary from 1 up to 15 or more copies of this gene between bacterial species and strains (Klappenbach JA, *et al*., 2001). Exemplary bacterial 16S rRNA gene sequences are set forth in SEQ ID NOs:1-15 and 38-47.

[0011] According to a first aspect of the invention, there is provided a method of determining a quantity or concentration of a bacterium in a sample, said method includes the steps of:

(a) amplifying a target nucleic acid of the bacterium from genetic material obtained from

CLAIMS

1. A method of determining the quantity or concentration of a bacterium in a sample, said method including the steps of:

(a) amplifying a target nucleic acid of the bacterium from genetic material obtained from the sample to form an amplification product, wherein the target nucleic acid comprises at least a portion of a bacterial 16S rRNA gene and wherein the target nucleic acid comprises one or a plurality of single nucleotide polymorphisms (SNPs) in the bacterial 16S rRNA gene, and wherein the one or plurality of SNPs correspond to at least one of positions 273, 378, 408, 412, 440, 488, 647, 653, 737, 755, 762 and 776 of the 16S rRNA gene set forth in SEQ ID NO: 1;

(b) identifying the bacterium in the sample and measuring a quantity or concentration of the amplification product in a single amplification step, wherein the step of identifying the bacterium comprises analysing the amplification product for the presence or absence of one or a plurality of SNPs;

(c) calculating a quantity or concentration of the target nucleic acid in the sample by comparing the quantity or concentration of the amplification product with a reference level thereof; and

(d) determining a copy number of the bacterial 16S rRNA gene in the sample from the quantity or concentration of the target nucleic acid therein, the copy number being a function of or correlated to the quantity of the bacterium in the sample,

wherein the step of analysing the amplification product for the presence or the absence of the at least one SNP comprises the use of high resolution melt analysis.

2. The method of claim 1, further including the step of generating the reference level from one or a plurality of control samples.

3. The method of claim 2, wherein the step of generating the reference level comprises amplifying the target nucleic acid from the one or plurality of control samples, wherein the control samples comprise genetic material of a known quantity or concentration.

4. The method of claim 3, wherein amplifying the target nucleic acid from the one or plurality of control samples is performed substantially simultaneously or in parallel with step (a).

5. The method of any one of claims 2 to 4, wherein the one or plurality of control samples

further comprise genetic material from one or a plurality of further bacteria.

6. The method of any one of the preceding claims, wherein amplifying the target nucleic acid from genetic material of the sample and/or the one or plurality of control samples is conducted with a pair of primers that comprise at least one of SEQ ID NOs: 16-37 and 48-51.

7. The method of any one of the preceding claims, wherein the copy number of the bacterial 16S rRNA gene is determined using the formula:

$$copy\ number = \frac{X\ \text{ng} * 6.0221\text{x}10^{23}\ \text{molecules/mole}}{(\text{N} * 660\text{g/mole}) * 1\text{x}10^{9}\text{ng/g}}$$

wherein X is the quantity of the amplification product and N is the length in nucleotides of the target nucleic acid.

8. The method of any one of the preceding claims, wherein the sample is a biological sample, such as sputum, blood, cerebrospinal fluid or urine, to be taken from a subject.

9. The method of claim 8, wherein the subject has an infection by the bacterium.

10. A method of determining a prognosis for an infection by a bacterium in a subject, including the step of quantifying the bacterium in a biological sample from the subject according to the method of any one of claims 1 to 9 to thereby evaluate the prognosis of the infection in the subject.

11. A method of evaluating treatment efficacy of an infection by a bacterium in a subject including:

quantifying the bacterium in a biological sample from the subject according to the method of any one of claims 1 to 9; and

determining whether or not the treatment is efficacious according to whether said quantity of the bacterium in the subject's biological sample is reduced or absent.

12. The method of any one of claims 10 to 11, wherein the subject has sepsis.

13. A kit or assay when used according to the method defined in any one of claims 1 to 12, said kit or assay comprising one or more reagents for performing the method according to any one of claims 1 to 12 and instructions for use.