

detection may not be complete. This is particularly true for contamination by bacteria that are relatively slow growing. For example, the mean detection times for *Bacillus sp.*, *Staphylococcal sp.*, *Streptococcal sp.*, *Micrococcus luteus*, *Kocuria varians*, *Corynebacterium sp.*, and *Propionibacterium sp.* have been estimated as 24 hours, 27 hours, 34 hours, 47 hours, 56 hours, 87 hours, and 97 hours, respectively, using standard culture methods.

[0004] One commercially available test is referred to as the BacT/ALERT test (bioMérieux, Inc., Durham, N.C.). Bacterial detection is based on the evolution of carbon dioxide by proliferating bacteria. A carbon-dioxide-sensitive liquid emulsion sensor at the bottom of the culture bottle changes color and is detected through alteration of light reflected on the sensor. Another method for bacterial detection involves measuring the oxygen content in a platelet preparation sample. An example is the Pall eBDS test (Pall Corporation, Port Washington, N.Y.). The approach to detection measures the oxygen content of air within the sample pouch as a surrogate marker for bacteria. An oxygen analyzer is used to measure the percent of oxygen in the headspace gas of the pouch or bag having the platelets. If bacteria are present in the platelet sample collected, an increasing amount of oxygen is consumed through the metabolic activity and proliferation of the bacteria in the sample during incubation, resulting in a measurable decrease in oxygen content of the plasma as well as the air within the sample pouch. While the non-specific measure of bacterial growth permits detection of many kinds of bacteria, the sensitivity is relatively low and requires long incubation times.

[0005] Alternative methods to the standard culture-based screens include bacterial antigen detection and nucleic acid-based screening. A major limitation on antigen-based methods is that they cannot be applied directly for testing of samples where the spectrum of bacterial pathogens is unknown. Detection of common bacterial nucleic acid sequences, such as 16S rRNA has been proposed to achieve a broader spectrum of detection, but such methods have so far been deemed less sensitive and specific than current culture methods and not appropriate for early testing of platelet samples.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides methods, compositions, reaction mixtures, kits, and systems for detecting contamination of biological samples, such as platelet samples, with both a broad range and high specificity and sensitivity of detection. Detection of contamination according to the disclosure is also significantly more rapid than culture-based screening methodologies.

[0006a] In one aspect, there is provided a method of detecting bacterial contamination of a platelet sample by any one of a plurality of bacteria species from different genera, comprising: subjecting the platelet sample or a portion thereof to a nucleic acid amplification reaction under conditions to yield a detectable amount of an amplicon of no more than about 300 bases in a single reaction mixture, said single reaction mixture comprising a single primer pair having a first primer and a second primer and a single detectable probe, wherein the first primer and the second primer each hybridizes to a separate conserved region of 16S rRNA polynucleotide, and the single primer pair flanks the amplicon, wherein the amplicon comprises a conserved sequence to which the single detectable probe hybridizes, and the separate conserved region is identical among the any one of the plurality of

bacteria species from different genera, wherein the any one of the plurality of bacterial species from the different genera is selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus aureus* Mu3; *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Escherichia coli*, *Citrobacter koseri*, *Clostridium perfringens*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Listeria monocytogenes*, *Propionibacterium granulosum*, *Propionibacterium species*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus aureus* Mu50, *Yersinia enterocolitica*, *Staphylococcus simulans*, *Micrococcus luteus*, and *Enterobacter aerogenes*,

wherein (1) the first primer comprises a sequence as set forth in SEQ ID NO: 4, or a complement thereof, and the second primer comprises a sequence as set forth in SEQ ID NO: 5 or 6, or a complement thereof; or (2) the first primer comprises a sequence as set forth in SEQ ID NO: 7, or a complement thereof, and the second primer comprises a sequence as set forth in SEQ ID NO: 8, or a complement thereof; or (3) the first primer comprises a sequence as set forth in SEQ ID NO: 9, or a complement thereof, and the second primer comprises a sequence as set forth in SEQ ID NO: 10, or a complement thereof; and detecting hybridization to the amplicon by the single detectable probe, wherein the hybridization by the single detectable probe yields a detectable signal indicative of bacterial contamination of the platelet sample by the any one of the plurality of bacteria species from the different genera.

[0007] In one aspect, the disclosure provides a method of detecting bacterial contamination of a sample by any of a plurality of bacterial species, such as at least eight bacterial species. In one embodiment, the method comprises subjecting the sample or a portion thereof to a nucleic acid amplification reaction under conditions to yield a detectable amount of an amplicon of no more than about 800, 700, 600, 500, 400, 300, 200, 150, or 100 bases in a single reaction mixture, said reaction mixture comprising a single primer pair and a single detectable probe, wherein the primer pair flanks the amplicon, the amplicon comprises a conserved sequence to which the detectable probe hybridizes,

[0015] In one aspect, the disclosure provides a method for using a kit of the disclosure. In one embodiment, the method comprises performing a nucleic acid amplification reaction on a sample or a portion thereof with a single primer pair to yield a detectable amount of an amplicon of no more than about 800, 700, 600, 500, 400, 300, 200, 150, or 100 bases of a 16S rRNA polynucleotide, wherein amplification of about 1pg-5pg of DNA from any one of a plurality of bacterial species from different genera has a cycle threshold value (C_T) of less than 30; and detecting the amplicon with one or more detectable probes, wherein each of the one or more detectable probes specifically hybridizes to a conserved sequence, and the conserved sequence is identical among a plurality of bacterial species from different genera.

[0015a] In one aspect, the disclosure provides a method of using the kit as disclosed herein, the method comprising: performing a nucleic acid amplification reaction on the platelet sample or a portion thereof with the first primer and the second primer to yield a detectable amount of an amplicon of no more than 300 bases of a 16S rRNA polynucleotide, wherein amplification of about 1pg-5pg of DNA from any one of a plurality of bacterial species from different genera has a cycle threshold value (C_T) of less than 30, wherein the any one of the plurality of bacterial species from the different genera is selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus aureus* Mu3; *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Escherichia coli*, *Citrobacter koseri*, *Clostridium perfringens*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Listeria monocytogenes*, *Propionibacterium granulosum*, *Propionibacterium species*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus aureus* Mu50, *Yersinia enterocolitica*, *Staphylococcus simulans*, *Micrococcus luteus*, and *Enterobacter aerogenes*; and detecting the amplicon with the probe, wherein the probe specifically hybridizes to a conserved sequence, and the conserved sequence is identical among the plurality of bacterial species from the different genera.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of detecting bacterial contamination of a platelet sample by any one of a plurality of bacteria species from different genera, comprising:

subjecting the platelet sample or a portion thereof to a nucleic acid amplification reaction under conditions to yield a detectable amount of an amplicon of no more than about 300 bases in a single reaction mixture, said single reaction mixture comprising a single primer pair having a first primer and a second primer and a single detectable probe, wherein the first primer and the second primer each hybridizes to a separate conserved region of 16S rRNA polynucleotide, and the single primer pair flanks the amplicon, wherein the amplicon comprises a conserved sequence to which the single detectable probe hybridizes, and the separate conserved region is identical among the any one of the plurality of bacteria species from different genera,

wherein the any one of the plurality of bacterial species from the different genera is selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus aureus* Mu3; *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Escherichia coli*, *Citrobacter koseri*, *Clostridium perfringens*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Listeria monocytogenes*, *Propionibacterium granulosum*, *Propionibacterium species*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus aureus* Mu50, *Yersinia enterocolitica*, *Staphylococcus simulans*, *Micrococcus luteus*, and *Enterobacter aerogenes*,

wherein (1) the first primer comprises a sequence as set forth in SEQ ID NO: 4, or a complement thereof, and the second primer comprises a sequence as set forth in SEQ ID NO: 5 or 6, or a complement thereof; or (2) the first primer comprises a sequence as set forth in SEQ ID NO: 7, or a complement thereof, and the second primer comprises a sequence as set forth in SEQ ID NO: 8, or a complement thereof; or (3) the first primer comprises a sequence as set forth in SEQ ID NO: 9, or a complement thereof, and the second primer comprises a sequence as set forth in SEQ ID NO: 10, or a complement thereof; and

detecting hybridization to the amplicon by the single detectable probe, wherein the hybridization by the single detectable probe yields a detectable signal indicative of bacterial contamination of the platelet sample by the any one of the plurality of bacteria species from the different genera.

2. The method of claim 1, wherein the platelet sample or the portion thereof has not been cultured at a temperature above 35°C prior to amplification.
3. The method of claim 1, wherein
within about 24 hours after obtaining said platelet sample, detecting hybridization to said amplicon by said single detectable probe occurs, wherein said hybridization yields the detectable signal indicative of the bacterial contamination of the platelet sample.
4. The method of claim 1, wherein the nucleic acid amplification reaction of about 1pg-5pg of DNA from the any one of the plurality of bacteria species from the different genera has a cycle threshold value (C_T) of less than 30.
5. The method of any of claims 1-4, wherein the platelet sample is a platelet concentrate isolated by aphaeresis.
6. The method of claim 5, wherein nucleic acids isolated from less than 5mL of the platelet concentrate are subjected to the nucleic acid amplification reaction.
7. The method of claim 3, wherein the detecting hybridization yields the detectable signal indicative of the bacterial contamination of the platelet sample having a bacterial load of about 1.0 colony forming unit per mL (CFU/mL).
8. The method of claim 4, wherein the C_T for a negative control sample is at least 5 cycles higher than the C_T for a sample containing 1pg-5pg of DNA from the any one of the plurality of bacteria species from the different genera.
9. The method of any of claims 1-4, characterized by one or more of the following:
 - (a) wherein the detecting of the bacterial contamination is completed prior to transfusion into a recipient;
 - (b) wherein the nucleic acid amplification reaction yields a detectable amount of the amplicon of no more than 150 bases;
 - (c) wherein the nucleic acid amplification reaction yields a detectable amount of the amplicon of 100-200 bases;
 - (d) wherein the single detectable probe comprises at least 10 contiguous nucleotides of a sequence as set forth in Table 3, or a complement thereof;

(e) wherein the nucleic acid amplification reaction contains less than 5 pg of starting nucleic acids from the bacterial contamination;

(f) wherein the nucleic acid amplification reaction is performed using a method selected from the group consisting of: polymerase chain reaction, real-time polymerase chain reaction, isothermal amplification, strand displacement amplification, rolling circle amplification, ligase chain reaction, transcription-mediated amplification, solid phase amplification, nucleic acid sequence-based amplification (NASBA), and linear amplification;

(g) wherein the nucleic acid amplification reaction is performed in a well, in a plate, in a tube, in a chamber, in a droplet, in a flow cell, in a slide, in a chip, attached to a solid substrate, attached to a bead, or in an emulsion.

10. The method of any of claims 1-9, wherein all of the any one of the plurality of bacteria species from the different genera are Gram-positive bacteria.

11. The method of any of claims 1-9, wherein all of the any one of the plurality of bacteria species from the different genera are Gram-negative bacteria.

12. The method of claim 1, wherein the any one of the plurality of bacteria species from the different genera comprise a plurality of Gram-positive bacterial species and a plurality of Gram-negative bacterial species.

13. The method of claim 4, wherein at least five of the any one of the plurality of bacteria species from the different genera are selected from the group.

14. The method of claim 13, wherein at least six of the any one of the plurality of bacteria species from the different genera are selected from the group.

15. The method of any of claims 1-4, wherein the detecting of the bacterial contamination includes detecting at least ten bacterial species in the platelet sample, or at least fifteen bacterial species in the platelet sample.

16. The method of any of claims 1-3, wherein the detectable signal has a linear range of detection across at least 5 logs.

17. The method of claim 16, further comprising determining an amount of the bacterial contamination based on the detectable signal.

18. The method of claim 4, wherein the single detectable probe yields a signal that has a linear range of detection across at least 5 logs.

19. The method of claim 18, further comprising determining an abundance of the any one of the plurality of bacterial species from the different genera based on the signal.

20. A composition for amplification and detection of a portion of a 16S rRNA polynucleotide, said portion being no more than 300 nucleotides in length, comprising:

a first primer comprising a sequence as set forth in SEQ ID NO: 9, or a complement thereof;

a second primer comprising a sequence as set forth in SEQ ID NO: 10, or a complement thereof; and

a probe comprising a sequence as set forth in SEQ ID NO: 16, or a complement thereof.

21. The composition of claim 20, wherein the composition is in a container.

22. A kit for detection of bacterial contamination of a platelet sample, said kit comprising:

a first primer comprising a sequence as set forth in SEQ ID NO: 9, or a complement thereof;

a second primer comprising a sequence as set forth in SEQ ID NO: 10, or a complement thereof;

a probe comprising a sequence as set forth in SEQ ID NO: 16, or a complement thereof.

23. A method of using the kit of claim 22, the method comprising:

performing a nucleic acid amplification reaction on the platelet sample or a portion thereof with the first primer and the second primer to yield a detectable amount of an amplicon of no more than 300 bases of a 16S rRNA polynucleotide, wherein amplification of about 1pg-5pg of DNA from any one of a plurality of bacterial species from different genera has a cycle threshold value (C_T) of less than 30,

wherein the any one of the plurality of bacterial species from the different genera is selected from the group consisting of *Staphylococcus aureus*, *Staphylococcus aureus* Mu3; *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Escherichia coli*, *Citrobacter koseri*, *Clostridium perfringens*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Listeria*

monocytogenes, *Propionibacterium granulosum*, *Propionibacterium species*,
Pseudomonas aeruginosa, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus aureus*
Mu50, *Yersinia enterocolitica*, *Staphylococcus simulans*, *Micrococcus luteus*, and
Enterobacter aerogenes; and

detecting the amplicon with the probe, wherein the probe specifically hybridizes
to a conserved sequence, and the conserved sequence is identical among the plurality of
bacterial species from the different genera.