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 of at least five bacterial genera, comprising:

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 bases in a single reaction mixture, said reaction mixture comprising a single primer pair having a forward primer and a reverse primer and a single detectable probe, wherein the forward primer and the reverse primer each hybridizes to a separate conserved region of 16s rRNA polynucleotide, and the primer pair flanks the amplicon, wherein the amplicon comprises a conserved sequence to which the detectable probe hybridizes, and the conserved sequence is identical among the at least five bacterial genera; and

detecting hybridization to the amplicon by the probe, wherein the hybridization by the probe yields a detectable signal indicative of bacterial contamination of the platelet sample by any of the at least five bacterial genera.

[0006b] In one aspect, there is provided a system for detecting bacterial contamination of a platelet sample by any of at least five bacterial genera, comprising:

a computer configured to receive a customer request to perform a detection reaction on a sample;

an amplification system that performs a nucleic acid amplification reaction on the sample or a portion thereof in response to the customer request, wherein the amplification reaction yields a detectable amount of an amplicon of no more than about 800 bases of a 16S rRNA polynucleotide using a single primer pair and single probe, and further wherein amplification of 1pg-5pg of DNA from any one of the at least five genera has a C_T of less than 30, wherein the at least five bacterial genera are selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Escherichia*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Propionibacterium*, *Pseudomonas, Serratia*, *Bacillus*, *Yersinia, Micrococcus*, and *Enterobacter*; and

a report generator that sends a report to a recipient, wherein the report contains results for detection of a signal intensity produced by the probe.

[0006c] In one aspect, there is provided a computer-readable medium comprising codes that, upon execution by one or more processors, implement a method of detecting bacterial contamination of a platelet sample by any of at least five bacterial genera, the method comprising:

receiving a customer request to perform a detection reaction on a sample;

performing a nucleic acid amplification reaction on the sample or a portion thereof in response to the customer request, wherein the amplification reaction yields a detectable amount of an amplicon of no more than about 800 bases of 16S rRNA using a single primer pair and single probe, and further wherein amplification of 1pg-5pg of DNA from any one of the at least five genera has a C_T of less than 30, wherein the at least five bacterial genera are selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Escherichia*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Propionibacterium*, *Pseudomonas, Serratia*, *Bacillus*, *Yersinia, Micrococcus*, and *Enterobacter*; and

generating a report that contains results for detection of a signal intensity produced by the probe.

[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,

CLAIMS

WHAT IS CLAIMED IS:

1. A method of detecting bacterial contamination of a platelet sample by any of at least five bacterial genera, comprising:

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 bases in a single reaction mixture, said reaction mixture comprising a single primer pair having a forward primer and a reverse primer and a single detectable probe, wherein the forward primer and the reverse primer each hybridizes to a separate conserved region of 16s rRNA polynucleotide, and the primer pair flanks the amplicon, wherein the amplicon comprises a conserved sequence to which the detectable probe hybridizes, and the conserved sequence is identical among the at least five bacterial genera; and

detecting hybridization to the amplicon by the probe, wherein the hybridization by the probe yields a detectable signal indicative of bacterial contamination of the platelet sample by any of the at least five bacterial genera.

2. The method of claim 1, wherein the sample or 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 sample, detecting hybridization to said amplicon by said probe occurs, wherein said hybridization yields a detectable signal indicative of bacterial contamination of the platelet sample.

4. The method of claim 1, wherein amplification of about 1pg-5pg of DNA from any one of the genera has a cycle threshold value (C_T) of less than 30.

5. The method of claim 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 detection yields a detectable signal indicative of bacterial contamination of a 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 any one of the plurality of bacterial genera.

9. The method of any of claims 1-3, wherein the forward primer and the reverse primer each hybridizes to a separate conserved region of 16s rRNA polynucleotide, and wherein the conserved region is selected from the group consisting of from 9 to 28, from 32 to 48, from 522 to 545, from 888 to 903, from 916 to 937, from 939 to 973, from 975 to 994, from 957 to 981, from 1093 to 1125, from 1184 to 1206, from 1231 to 1252, from 1378 to 1396, from 1398 to 1422, or from 1496 to 1516, of 16S rRNA of *Staphylococcus aureus* (GenBank accession Number NC_007622) or the corresponding region in a genome of any one of the at least five bacterial genera.

10. The method of any of claims 1-4, characterized by one or more of the following:

(a) wherein the detecting of bacterial contamination is completed prior to transfusion into a recipient;

(b) wherein the amplification reaction yields a detectable amount of amplicon of no more than 300 bases, or no more than 150 bases;

(c) wherein the amplification reaction yields a detectable amount of amplicon of 100-200 bases;

(d) wherein the single primer pair comprises a first primer comprising at least 10 contiguous nucleotides of a sequence as set forth in SEQ ID NO: 4, 7, or 9 and a second primer comprising at least 10 nucleotides of a sequence as set forth in SEQ ID NO: 5, 6, 8, or 10;

(e) wherein the probe comprises at least 10 contiguous nucleotides of a sequence as set forth in Table 3, or a complement thereof;

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

(g) 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;

(h) 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.

11. The method of any of claims 1-10, wherein all of the bacterial genera are Gram-positive bacteria.

12. The method of any of claims 1-10, wherein all of the bacterial genera are Gram-negative bacteria.

13. The method of claim 1, wherein the at least five bacterial genera comprise a plurality of Gram-positive bacterial species and a plurality of Gram-negative bacterial species.

14. The method of 4, wherein the at least five bacterial genera are selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Escherichia*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Propionibacterium*, *Pseudomonas*, *Serratia*, *Bacillus*, *Yersinia*, *Micrococcus*, and *Enterobacter*.

15. The method of claim 14, wherein at least six bacterial genera are selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Escherichia*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Propionibacterium*, *Pseudomonas*, *Serratia*, *Bacillus*, *Yersinia*, *Micrococcus*, and *Enterobacter*.

16. The method of claim 14, wherein the at least five bacterial genera includes bacterial species 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*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus aureus Mu50*, *Yersinia enterocolitica*, *Staphylococcus simulans*, *Micrococcus luteus*, and *Enterobacter aerogenes*.

17. The method of any of claims 1-4, comprising detecting bacterial contamination includes detecting at least ten bacterial species in a sample, or at least fifteen bacterial species in a sample.

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

19. The method of claim 18, further comprising determining the amount of bacterial contamination based on the probe signal.

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

21. The method of claim 20, further comprising determining the abundance of the plurality of bacterial species based on the probe signal.

22. A composition for amplification and detection of a portion of a 16S rRNA polynucleotide, said portion being less than about 800 nucleotides in length, comprising:

a first primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 9;

a second primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 10; and,

a probe comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 16, or the complement thereof.

23. The composition of claim 22, wherein the composition is in a container.

24. A composition for amplification and detection of a portion of a 16S rRNA polynucleotide, said portion being less than about 800 nucleotides in length, comprising:

primers that, in an amplification reaction with a target 16S rRNA polynucleotide, amplify an amplicon of at least 50 nucleotides in length, the amplicon having 90% sequence identity with any of SEQ ID NO: 1-3 when optimally aligned; and

a probe that specifically hybridizes to either strand of the amplicon.

25. The composition of claim 24, wherein the composition is in a container.

26. The composition of claim 24, wherein, in an amplification reaction with a target 16S rRNA polynucleotide, amplification of about 1pg-5pg of DNA from any one of a plurality of target species by the primers and probes has a cycle threshold value (C_T) of less than 30.

27. A reaction mixture for amplification and detection of a portion of a 16S rRNA polynucleotide, said portion being less than about 800 nucleotides in length, comprising

sample nucleic acid;

primers that, in an amplification reaction with a target 16S rRNA polynucleotide, amplify an amplicon of at least 50 nucleotides in length, the amplicon having 90% sequence identity with any of SEQ ID NO: 1-3 when optimally aligned;

a probe that specifically hybridizes to either strand of the amplicon; and

a polymerase;

wherein the reaction mixture is in a reaction site.

28. The reaction mixture of claim 27, wherein the reaction site is a droplet, a well, a plate, a tube, a chamber, a flow cell, or a chip.

29. The reaction mixture of claim 27, wherein, in an amplification reaction with a target 16S rRNA polynucleotide, amplification of about 1pg-5pg of DNA from any one of a plurality of target species by the primers and probes has a cycle threshold value (C_T) of less than 30.

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

a first primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 9;

a second primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 10;

a probe comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 16, or the complement thereof.

31. A method of using the kit of claim 30, the method comprising:

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 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.

32. A system for detecting bacterial contamination of a platelet sample by any of at least five bacterial genera, comprising:

a computer configured to receive a customer request to perform a detection reaction on a sample;

an amplification system that performs a nucleic acid amplification reaction on the sample or a portion thereof in response to the customer request, wherein the amplification reaction yields a detectable amount of an amplicon of no more than about 800 bases of a 16S rRNA polynucleotide using a single primer pair and single probe, and further wherein amplification of 1pg-5pg of DNA from any one of the at least five genera has a C_T of less than 30, wherein the at least five bacterial genera are selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Escherichia*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Propionibacterium*, *Pseudomonas*, *Serratia*, *Bacillus*, *Yersinia*, *Micrococcus*, and *Enterobacter*; and

a report generator that sends a report to a recipient, wherein the report contains results for detection of a signal intensity produced by the probe.

33. The system of claim 32, wherein the report generator identifies the sample as contaminated or not contaminated based on the signal intensity produced by the probe.

34. The system of claim 32, wherein the recipient is the customer.

35. A computer-readable medium comprising codes that, upon execution by one or more processors, implement a method of detecting bacterial contamination of a platelet sample by any of at least five bacterial genera, the method comprising:

receiving a customer request to perform a detection reaction on a sample;

performing a nucleic acid amplification reaction on the sample or a portion thereof in response to the customer request, wherein the amplification reaction yields a detectable amount of an amplicon of no more than about 800 bases of 16S rRNA using a single primer pair and single probe, and further wherein amplification of 1pg-5pg of DNA from any one of the at least five genera has a C_T of less than 30, wherein the at least five bacterial genera are selected from the group consisting of *Staphylococcus*, *Streptococcus*, *Escherichia*, *Citrobacter*, *Clostridium*, *Enterococcus*, *Klebsiella*,

Lactobacillus, *Listeria*, *Propionibacterium*, *Pseudomonas, Serratia*, *Bacillus*, *Yersinia, Micrococcus*, and *Enterobacter*; and

generating a report that contains results for detection of a signal intensity produced by the probe.