

The present disclosure may provide a method for detecting *C. difficile* in a sample, the method comprising performing an amplifying step comprising contacting the sample with a set of *tcdB* primers to produce an amplification product if *C. difficile* is present in the sample; performing a hybridizing step comprising contacting the amplification product

5 with one or more detectable *tcdB* probes; and detecting the presence or absence of the amplified product, wherein the presence of the amplified product is indicative of the presence of *C. difficile* in the sample and wherein the absence of the amplified product is indicative of the absence of *C. difficile* in the sample. In one embodiment, each primer of the set of *tcdB* primers comprises or consists of a sequence of nucleotides selected from the

10 group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, and 6, or a complement thereof; and wherein the one or more detectable *tcdB* probes comprise or consists of a sequence of nucleotides selected from the group consisting SEQ ID NOs: 7, 8, and 9, or a complement thereof. Hence, in one aspect, there is provided a method of detecting *C. difficile* in a sample, the method comprising: performing multiple cycling steps, wherein each cycling step

15 comprises: an amplifying step comprising contacting the sample with a set of *tcdB* primers to produce an amplification product if a *tcdB* nucleic acid is present in the sample; and ~~performing a hybridizing step comprising contacting the amplification product if produced with one or more a detectable *tcdB* probes; and detecting the presence or absence of the~~ amplification amplified-product, wherein the presence of the amplification amplified

20 product is indicative of the presence of *C. difficile* in the sample and wherein the absence of the amplification amplified-product is indicative of the absence of *C. difficile* in the sample; wherein each primer of the set of *tcdB* primers comprises a first oligonucleotide primer comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3, 4, 5 and 6, or a complement thereof and a second oligonucleotide primer comprising a sequence

25 selected from the group consisting of SEQ ID NOs: 4, 5 and 6; and wherein the one or more detectable *tcdB* probes comprises a sequence selected from the group consisting SEQ ID NOs: 7, 8, and 9, or a complement thereof. In some embodiments, a hybridizing step includes contacting the amplification product with a probe that is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety. The method may

30 further include detecting the presence or absence of fluorescence resonance energy transfer

(FRET) between the donor fluorescent moiety and the acceptor fluorescent moiety of the probe. The presence or absence of fluorescence is indicative of the presence or absence of *C. difficile* in the sample.

- 5 In one embodiment, amplification can employ a polymerase enzyme having 5' to 3' exonuclease activity. In certain embodiments, the first and second fluorescent moieties may be within no more than 5 nucleotides of each other along the length of the probe. In another embodiment, the *tcdB* probe includes a nucleic acid sequence that permits secondary structure formation. Such secondary structure formation generally results in spatial
- 10 proximity between the first and second fluorescent moiety. In certain embodiments of this method, the second fluorescent moiety on the probe can be a quencher.

The kit can also include at least one of nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase. The kit can also include a package insert and instructions for using the primers, probes, and fluorophoric moieties to detect the presence or absence of *C. difficile* in a sample.

5 The present disclosure may also provide for an oligonucleotide comprising or consisting of a sequence of nucleotides selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, and 9 or a complement thereof, which oligonucleotide has 100 or fewer nucleotides. In another aspect, there is provided an set of oligonucleotides comprising a first oligonucleotide primer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: ~~1, 2, or~~  
10 ~~3, 4, 5, 6, 7, 8, and 9~~ or a complement thereof and a second oligonucleotide primer comprising a sequence selected from the group consisting of SEQ ID NOs: 4 or 5, or a complement thereof. In certain embodiments, the oligonucleotide has 40 or fewer nucleotides. The present disclosure may also provide for an oligonucleotide that includes a nucleic acid having at least 70% sequence identity (e.g., at least 75%, 80%, 85%, 90% or 95%, etc.) to one  
15 of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, and 9, or a complement thereof, which oligonucleotide has 100 or fewer, more specifically 40 or fewer nucleotides. In certain embodiments the oligonucleotides have 90% sequence identity to one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, and 9, or a complement thereof. Generally, these oligonucleotides may be primer nucleic acids, probe nucleic acids, or the like in these embodiments. In certain of these embodiments, the  
20 oligonucleotides have 40 or fewer nucleotides (e.g., 35 or fewer nucleotides, 30 or fewer nucleotides, etc.). In some embodiments, the oligonucleotides comprise at least one modified nucleotide, e.g., to alter nucleic acid hybridization stability relative to unmodified nucleotides. Optionally, the oligonucleotides comprise at least one label and/or at least one quencher moiety. In some embodiments, the oligonucleotides include at least one conservatively modified  
25 variation.

The present disclosure may provide for a pair of oligonucleotide primers, wherein a first oligonucleotide primer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3, or a complement thereof and wherein a second oligonucleotide primer comprises or consists of a sequence selected from the group consisting of SEQ ID NOs:  
30 4, 5, and 6, or a complement thereof. In certain of these

**WHAT IS CLAIMED:**

1. A method of detecting *C. difficile* in a sample, the method comprising:

- performing multiple cycling steps, wherein each cycling step comprises:
  - an amplifying step comprising contacting the sample with a set of *tcdB* primers to produce an amplification product if a *tcdB* nucleic acid is present in the sample; and
  - ~~performing~~ a hybridizing step comprising contacting the amplification product if produced with ~~one or more~~ detectable *tcdB* probes; and
- detecting the presence or absence of the ~~amplified~~ amplification product, wherein the presence of the ~~amplified~~ amplification product is indicative of the presence of *C. difficile* in the sample and wherein the absence of the ~~amplified~~ amplification product is indicative of the absence of *C. difficile* in the sample;

wherein ~~each primer~~ of the set of *tcdB* primers comprises a first oligonucleotide primer comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3, 4, 5 and 6, or a complement thereof and a second oligonucleotide primer comprising a sequence selected from the group consisting of SEQ ID NOs: 4, 5, and 6; and wherein the ~~one or more~~ detectable *tcdB* probes comprises a sequence selected from the group consisting of SEQ ID NOs: 7, 8, and 9, or a complement thereof.

2. The method of claim 1, wherein:

- the hybridizing step comprises contacting the amplification product with a probe that is labeled with a donor fluorescent moiety and a corresponding acceptor fluorescent moiety; and
- the detecting step comprises detecting the presence or absence of fluorescence resonance energy transfer (FRET) between the donor fluorescent moiety and the acceptor fluorescent moiety of the probe, wherein the presence or absence of fluorescence is indicative of the presence or absence of *C. difficile* in the sample.

3. The method of any one of claims 1 or 2, wherein said amplification employs a polymerase enzyme having 5' to 3' exonuclease activity.
4. The method of any one of claims 2 or 3, wherein said donor fluorescent moiety and acceptor fluorescent moiety~~first and second fluorescent moieties~~ are within no more than 5 nucleotides of each other on said probe.
5. The method of any one of claims 2 to 4, wherein said ~~second~~acceptor fluorescent moiety is a quencher.
6. The method of any one of claims 1 to 5, wherein the *tcdB* probe comprises a nucleic acid sequence that permits secondary structure formation, wherein the secondary structure formation results in spatial proximity between the ~~first and the second~~donor fluorescent moiety and the acceptor fluorescent moiety.
7. A kit for detecting a nucleic acid of *C. difficile* comprising:
  - a first oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 1, 2, and 3, or a complement thereof;
  - a second oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 4, 5, and 6, or a complement thereof; and
  - a third detectably labeled oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 7, 8, and 9, or a complement thereof.
8. The kit of claim 7, wherein the third detectably labeled oligonucleotide comprises a donor fluorescent moiety and a corresponding acceptor fluorescent moiety.
9. The kit of claim 8, wherein the acceptor fluorescent moiety is a quencher.
10. The kit of any one of claims 7 to 9, further comprising at least one of the following components: nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase.

11. A set of oligonucleotides comprising a first oligonucleotide primer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, or 3, or a complement thereof and a second oligonucleotide primer comprising a sequence selected from the group consisting of SEQ ID NOs: 4, or 5, 6, 7, 8, and 9, or a complement thereof.
12. The set of oligonucleotides of claim 11, wherein at least one of the oligonucleotides comprises at least one modified nucleotide.
13. The set of oligonucleotides of any one of claims 11 or 12, wherein at least one of the oligonucleotides comprises at least one conservatively modified variation.
14. ~~The oligonucleotide of any one of claims 11 to 13, wherein the oligonucleotide has 40 or fewer nucleotides.~~
14. The set of oligonucleotides of any one of claims 11 to 13, further comprising one or more detectable label a third detectably labeled oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NOs: 7, 8, and 9, or a complement thereof.
15. The set of oligonucleotides of any one of claims 11 to 14, wherein the oligonucleotides have 40 or fewer nucleotides.
16. The set of oligonucleotide of any one of claims 11 to 15, wherein at least one of the oligonucleotide comprises at least one labeling moiety and/or at least one quencher moiety.